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THE SUBCELLULAR DISTRIBUTION OF NADPH-CYTOCHROME P450 REDUCTASE AND ISOENZYMES OF CYTOCHROME P450 IN THE LUNGS OF RATS AND MICE

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Abstract—NADPH-cytochrome P450 reductase was localized by ultrastructural immunocytochemistry in the cytoplasm of Clara cells, ciliated cells and type II pneumocytes of young female Wistar rats and MF1 mice. Immunogold labelling was particularly dense over the apical cytoplasm of Clara cells. CYP1A was only detected in lungs of animals treated with Aroclor 1254 or 3-methylcholanthrene; it was localized in the cytoplasm of Clara cells of rats and in the endothelial cells of both rats and mice. In rat lung the labelling of cytochrome P450 CYP2B was most intense in the apical cytoplasm of Clara cells, but it was also found in both the type I and type II cells of the alveolar epithelium. Similar immunoreactivity was found in the Clara and type II cells of mice, it was also found in the ciliated cells of this species. CYP4B was localized in the cytoplasm of Clara and type II cells of rats. The cell-specific concentration of these isoenzymes, particularly that of CYP2B in the cytoplasm of type I cells, probably contributes to the particular susceptibility of these cells to pneumotoxins.

Key words: immunocytochemistry; ultrastructure; pulmonary; bioactivation; CYP1A; CYP2B; CYP4B

Metabolism by cytochrome P450 isoenzymes constitutes the first step in the detoxification of many harmful agents but certain compounds can be converted into highly reactive intermediate metabolites by this enzyme system. Cells containing high concentrations of these isoenzymes are, therefore, particularly vulnerable to injury [1]. In lung, cell-specific injury follows the administration of 4-ipomeanol [2], butylated hydroxytoluene [3] or *O*,*S*,*S*-trimethylphosphorodithioate [4]. These compounds are all reported to be activated by the pulmonary mixed-function oxygenase system [5, 6]. The cytochrome P450 isoenzymes CYP1A1, CYP2B1 and CYP4B1 have been shown to be present in the lungs of rats [7] and NADPH-cytochrome P450 reductase, which is essential for the activity of these isoenzymes, has been demonstrated in the alveoli and bronchioles of rats [8–10].

CYP1A1 has been localized in rat lung, by light microscopy [11, 12]. The low levels of activity, constitutively expressed in this tissue, can be increased, more than 30-fold, by treatment with 3-methylcholanthrene and other polycyclic hydrocarbons [13]. This induction increases the metabolism of benzo(*a*)pyrene by isolated rat lung [14] but has a minimal effect on the activation of various pneumotoxins [6, 15].

In contrast, the predominant isoenzyme in rat lung, CYP2B1 [12, 16–18], like its murine orthologue, has been shown to be critical in the activation of pneumotoxins which cause alveolar injury [6]. This injury is characterized by damage to the type I cells even though CYP2B has only been localized in the Clara and type II cells of rats and mice [12, 19, 20]. Traces of CYP4B1, have been found in pulmonary microsomes from rats and mice [21] and this enzyme has been localized in the Clara cells of rabbits [22]. It has been shown to be involved, together with CYP2B, in the activation of 4-ipomeanol in the rabbit [23]. The administration of 4-ipomeanol results in the selective injury of Clara cells of several species but in the rat, unlike the rabbit, pulmonary bioactivation of this pneumotoxin is predominantly dependent on the activity of CYP4B1 [24].

The isoenzymes of cytochrome P450, particularly CYP2B and CYP4B, are clearly of critical importance in the development of cell-specific lung injury and we have, therefore, used ultrastructural immunocytochemistry to determine their precise cellular distribution in the lungs of rats and mice.

MATERIALS AND METHODS

Animals. Outbred (MF1-aa) female mice (N = 8), weighing 20–25 g, and Porton-derived Wistar (LAC:P) female rats (N = 8), weighing 170–200 g, were housed in an air-conditioned room at 20–23° (40–60% relative humidity) with a 12 hr light/dark cycle. They were given free access to tap water and laboratory animal diet. Rats were kept in wire-bottomed cages and given Oxoid 41B diet (Labsure, K&K Greef Ltd, Croydon, U.K.). Mice were kept on shredded, pesticide-free maize stems and given Oxoid 'Breeding Diet' (Labsure, K&K Greef Ltd, Croydon, U.K.). All animals were anaesthetized with an i.p. injection of 7% chloral hydrate (400 mg/kg), lungs excised and slices, 0.6 mm thick, were

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prepared immediately, using a McIlwain tissue chopper.

In a second series of experiments, slices of lung tissue were prepared from a female New Zealand white rabbit; two female LAC:P rats and two female MF1-aa mice, four days after an i.p. dose of Aroclor 1254 (200 mg/kg) in arachis oil, and also from two female MF1-aa mice, 24 hr after the last of three daily i.p. doses of 3-methylcholanthrene (20 mg/kg) in arachis oil.

Chemicals. Aroclor 1254, electrical grade, was supplied by Monsanto (St Louis, MO, U.S.A.) and 3-methylcholanthrene was purchased from Sigma (Poole, Dorset, U.K.). Materials for electron microscopy were purchased from Agar Scientific (Stansted, U.K.). All other chemicals were of reagent grade and purchased from Fisons (Loughborough, U.K.). Polyclonal antibodies were used for all primary incubations. A rabbit-derived antibody to rat CYP1A1 was purchased from Oxygene Inc. (Dallas, TX, U.S.A.). Rabbit-derived antibodies to rat CYP2B1 and rat cytochrome P450 reductase were donated by Dr C. R. Wolf (ICRF Molecular Pharmacology Unit, University of Dundee, Ninewells Hospital, Dundee, U.K.) and Dr J. Hardwick (NE Ohio College of Medicine, Rootstown, OH, U.S.A.), respectively. Goat-derived antibody to rabbit CYP4B1 was donated by Dr R. M. Philpot (Laboratory of Pharmacology, NIEHS, NC, U.S.A.).

Immunogold (1 and 10 nm) and kits for silver enhancement were purchased from British Biocell International (Cardiff, U.K.). All solutions used for immunocytochemistry contained 1% (w/v) BSA (Sigma Chemical Co., Poole, Dorset, U.K.) and 0.1% (w/v) fish gelatin (British Biocell International). Dulbecco 'A' PBS tablets were purchased from Oxoid (Unipath Ltd, Basingstoke, U.K.).

Pre-embedding immunocytochemistry. Freshly prepared lung slices were fixed, for 15 min at 20° in either acetone, 4% formaldehyde (freshly prepared from paraformaldehyde) in Sorensen's phosphate buffer (pH 7.3), or 0.25% glutaraldehyde in PBS (pH 7.3) and washed overnight in PBS. Non-specific binding was minimized by treating slices with PBS containing 0.1% Tween 20 and 10% normal serum (obtained from the species used to provide the secondary antibody), before incubation with primary antibody. Rabbit-derived and goat-derived primary antibodies were localized with 1 nm colloidal gold conjugated to anti-rabbit and anti-goat IgG, respectively. Slices were incubated with primary antibody diluted 1:250 in PBS, at room temperature for 6 hr, washed overnight and then incubated with the appropriate secondary antibody, diluted 1:50 in PBS, for 2 hr.

Control incubations were carried out in parallel and involved omission of the primary antibody or its replacement with normal serum.

The slices were washed thoroughly, post-fixed in PBS containing 2% glutaraldehyde and rinsed overnight in PBS. The colloidal gold staining was amplified by silver enhancement prior to fixation in 1% osmium tetroxide, and processing into Araldite resin [25].

Post-embedding immunocytochemistry. Slices, prepared as above, were fixed in 4% formaldehyde, freshly prepared from paraformaldehyde, in 0.1 M Sorensen's phosphate buffer (pH 7.3) for 1 hr and then washed in three changes of buffer. Slices were infiltrated with 'hard grade' LR White resin (London Resin Company, U.K.) and polymerization was carried out, using UV radiation, for 7 hr at 4° [25].

Ultrathin sections were mounted on parlodion-coated nickel grids. Non-specific binding was minimized by treatment with PBS containing 10% normal serum and 0.1% Tween 20 before incubation. All sections were incubated with primary antibody, at dilutions of 1:400 or 1:800 in PBS, for 20 hr, washed in PBS and then incubated with the secondary antibody conjugated to 10 nm colloidal gold (diluted 1:50 in PBS) for 2 hr. Control incubations were carried out in parallel and involved omission of the primary antibody or its replacement with normal serum. Sections were washed thoroughly in PBS, rinsed in distilled water, counterstained with 2% uranyl acetate (aqueous) for 30 min and examined using a Jeol 100CX electron microscope.

RESULTS

Fixation with glutaraldehyde provided excellent ultrastructural preservation of the lung tissue but abolished immunoreactivity with all antibodies tested. This reactivity was retained after fixation with acetone but morphological preservation was unsuitable for ultrastructural studies. Slices fixed with 4% formaldehyde were found to provide the best compromise between the retention of antigenicity and preservation of structural detail. Ultrastructural preservation was improved, in pre-embedding immunocytochemistry, by fixing incubated slices in glutaraldehyde and then in osmium tetroxide before they were embedded in resin. This treatment was particularly important for the retention of the characteristic appearance of lamellar bodies in type II cells.

Ultrafine (1 nm) gold particles were used to facilitate penetration into intact cells during pre-embedding immunocytochemistry. When used for post-embedding immunocytochemistry they did not result in labelling densities that were significantly greater than those achieved with 10 nm gold (data not shown) and so the larger, more readily discerned particles were used on resin sections.

Cytochrome P450 reductase

All preparations showed labelling, for cytochrome P450 reductase, in the cytoplasm of rat Clara cells with a sharp demarcation in the intensity of this labelling between these and the adjacent ciliated cells (Fig. 1a). Labelling was also found throughout the cytoplasm of the alveolar type II cells; the density of this labelling was intermediate between that over Clara and ciliated cells. The label was not, however, detected within the lamellar bodies of type II cells. This absence of label was observed after pre-embedding immunocytochemistry and also after post-embedding incubations of ultrathin sections, in which the lamellar bodies were poorly preserved (Fig. 1b). A similar distribution of antigenicity was evident in the lungs of mice (Fig. 1c) with low levels

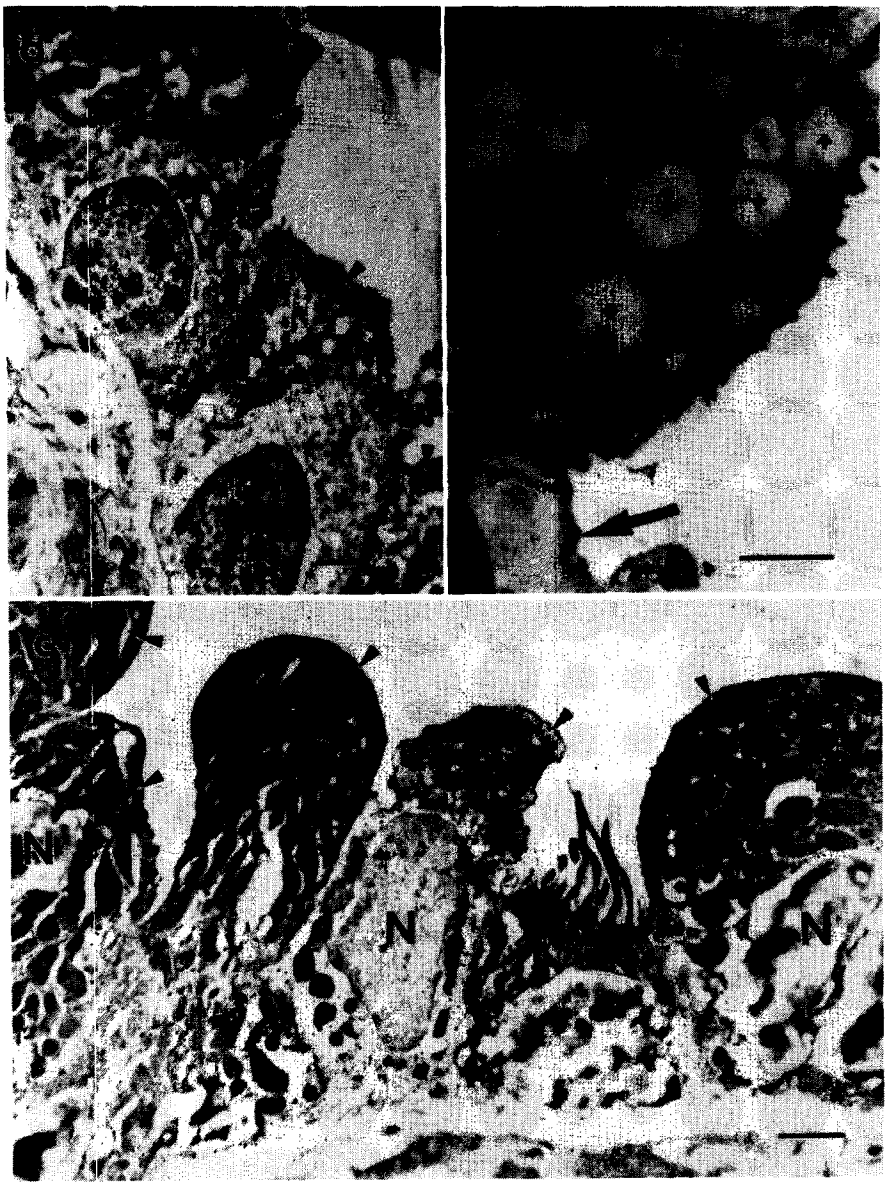

Fig. 1. (a) Incubation with an antibody to NADPH cytochrome P450 reductase has intensely labelled the apical cytoplasm of rat Clara cells (arrowheads), in contrast to the adjacent ciliated cells (*) which are only lightly labelled. (b) The cytoplasm of the rat type II cell is also labelled by this antibody but mitochondria and the lamellar bodies (+), which are electron-lucent in this non-osmicated section, are unlabelled. The adjacent type I cell (arrow) is not labelled. (c) The apical cytoplasm (arrowheads) of murine Clara cells is labelled by this antibody but the nuclei (N) of these cells together with the cytoplasm (*) and nucleus of a ciliated cell are only lightly labelled. Bars—1 μm.

of label in the ciliated cells, compared with the intensely labelled apical cytoplasm of Clara cells (Fig. 2a). The nuclei of both Clara and ciliated cells were virtually devoid of immunogold particles.

CYP1A1

This enzyme was not detected, by immunocytochemistry, in lung sections from any of the untreated animals. CYP1A1 was, however, localized in Clara cells (Fig. 2b) and, to a lesser extent, in endothelial cells (Fig. 2c) in the lungs of rats pretreated with Aroclor 1254. After post-embedding immunocytochemistry the endothelial cells were often difficult to distinguish from type I cells, especially at high magnifications, and so blood capillaries were always identified at low magnifications. Label was found over endothelial cells of all Aroclor-treated rats. In contrast, this treatment did not result in detectable levels of CYP1A1 in the Clara cells or endothelial cells of mouse lung.

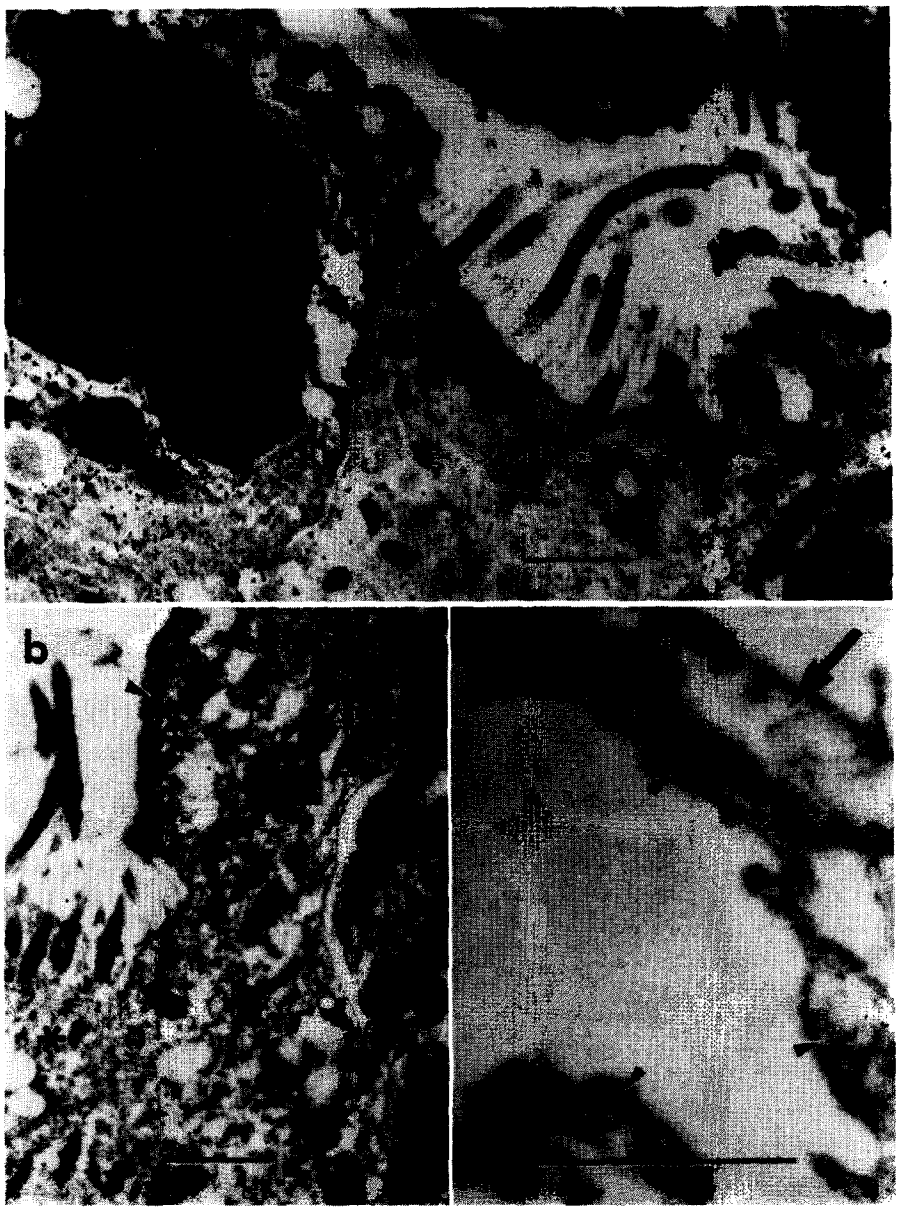

Fig. 2. (a) The cytoplasm of Clara cells in murine lung is labelled with antibody to NADPH cytochrome P450 reductase but the intervening ciliated cell (*) is only lightly labelled by this antibody. The nuclei of these cells (N) are virtually unlabelled. (b) The apical cytoplasm, but not the nucleus (N), of a Clara cell in an Aroclor 1254-treated rat is labelled (arrowhead) by an antibody raised against CYP1A1. The adjacent ciliated cell (*) is not labelled. (c) The cytoplasm of endothelial cells, in the alveolar septum of an Aroclor 1254-treated rat, is also labelled by this antibody (arrowheads) but the adjacent type I pneumocyte (arrow) is not labelled. Bars—1 μm.

Treatment with 3-methylcholanthrene did induce CYP1A in murine lung but immunogold was restricted to the cytoplasm of endothelial cells. There was no immunolabelling in murine Clara cells following either treatment.

CYP2B1

Pre-embedding immunocytochemistry resulted in labelling in the lungs of both rats and mice. The highest density of immunogold was located in the cytoplasm of Clara cells but the adjacent ciliated cells were almost devoid of label (Fig. 3a). Labelling was also found in the cytoplasm of type I (Fig. 3b) and type II cells of both species but all nuclei were virtually devoid of gold particles. A lower density of label was evident after post-embedding immunocytochemistry but colloidal gold was found over type I cells (Fig. 3c) and rather more intense labelling was present over type II cells and Clara cells (Fig. 4a).

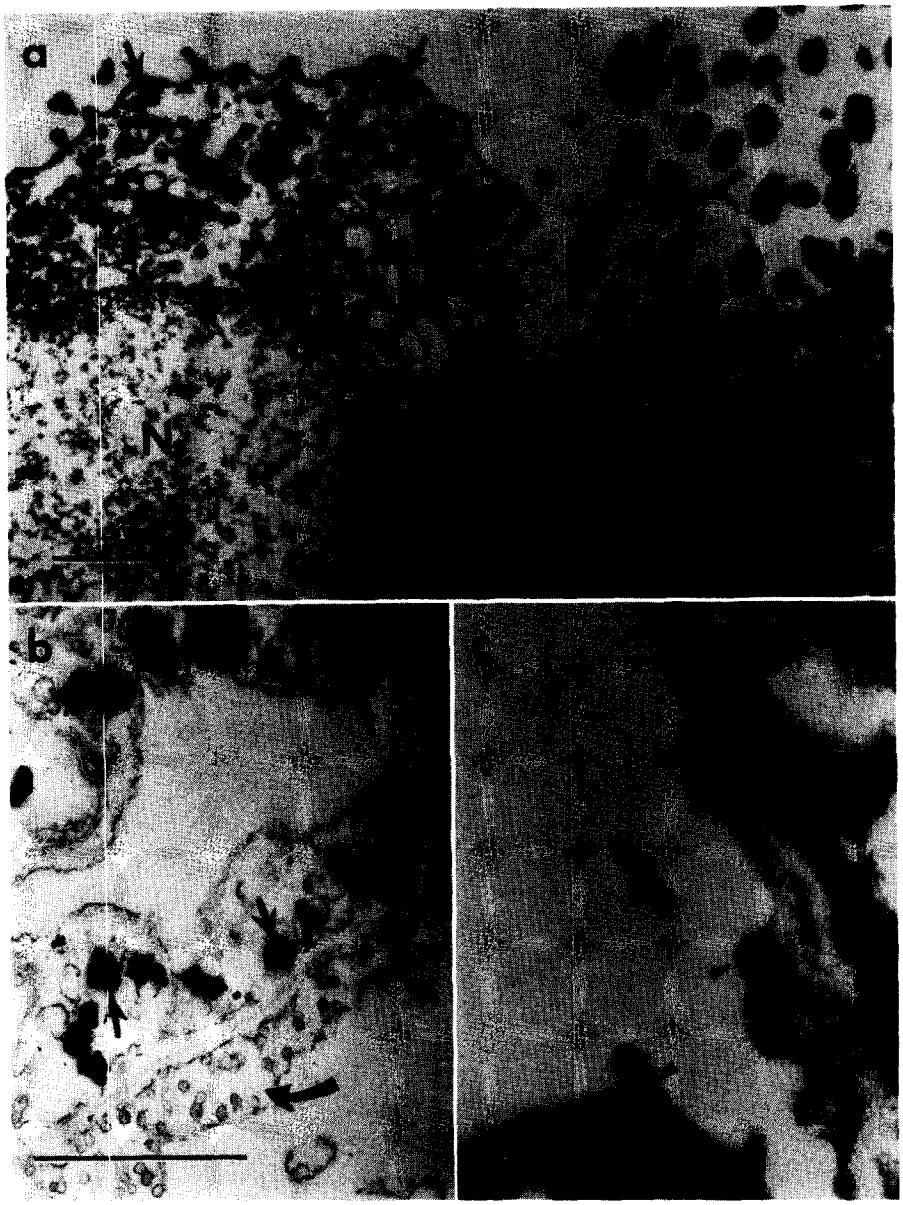

Fig. 3. (a) The apical cytoplasm, but not the nucleus (N) of the rat Clara cell, has been labelled, before embedding, by an antibody raised against CYP2B1. The ultrafine (1 nm) immunogold, used in this procedure, has been enhanced with silver (short arrows). (b) Pre-embedding immunocytochemistry also labelled the cytoplasm of type I cells (short arrows), but not the adjacent endothelial cells (curved arrow). (c) Post-embedding immunocytochemistry, using antibody to CYP2B1, labelled the cytoplasm of type I pneumocytes (arrowheads). Bars—1 μm.

CYP4B1

Pre-embedding immunocytochemistry, using antibody to CYP4B1, showed cytoplasmic labelling of both Clara and type II cells (Figs 4b and c) but no other cells were labelled. Similar incubations of murine lung slices did not result in any labelling. This antibody did not result in any post-embedding labelling of sections from rats or mice but sections of rabbit lung, incubated in parallel, did show labelling of both Clara and type II cells. This labelling, like that observed in rats, was restricted to the cytoplasm.

DISCUSSION

Antibodies to NADPH-cytochrome P450 reductase may be used to detect cells where one or more of the various isoenzymes of the cytochrome P450 system are present [26]. This flavoprotein has been demonstrated, by light microscopy, in isolated murine Clara cells [10] and also in the airway epithelium and alveolar lining cells of rat lung [8, 9]. Ultrastructural cytochemical studies have been largely restricted to rabbit lung, where reductase is only 'barely discernible in type II cells by immunogold labelling and TEM' [27] despite the high levels of

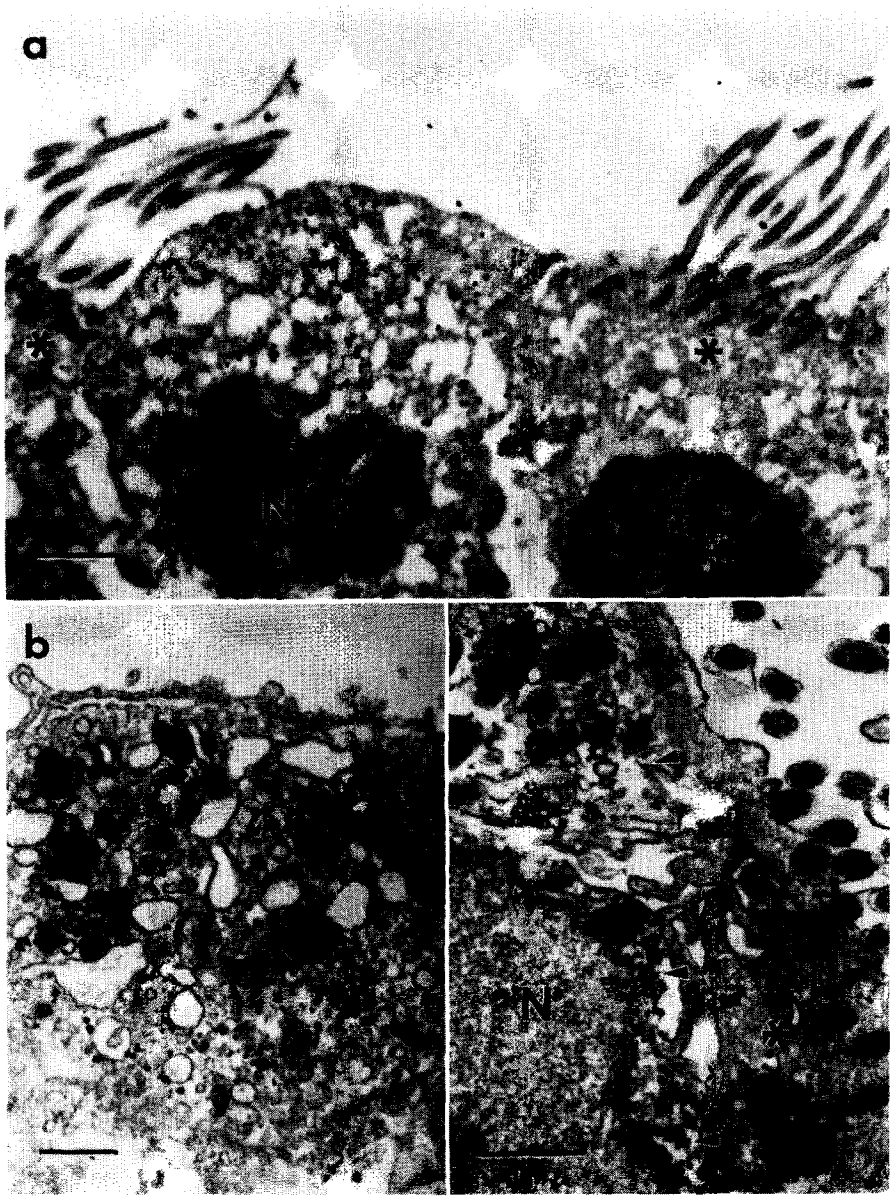

Fig. 4. (a) The apical cytoplasm of a rat Clara cell has been labelled on a section incubated with an antibody raised against CYP2B1. The nuclei (N) of these cells, together with the nuclei and cytoplasm (*) of adjacent ciliated cells are only lightly labelled. (b) The cytoplasm of a rat type II pneumocyte has been labelled, by pre-embedding immunocytochemistry, using an antibody raised against CYP4B1 but the lamellar bodies (+) are unlabelled. (c) The lateral and apical cytoplasm, but not the nucleus (N), of the Clara cell has also been labelled by this antibody (arrowheads) but the adjacent ciliated cell (*) is not labelled. Bars—1 μm.

activity detected in isolated cells [28]. Labelling of organelles, including the residues of lamellar bodies in type II cells, has been reported in rabbit lung [22] but our results show exclusion of label from the nucleus and cytoplasmic granules of labelled Clara and type II cells of both species, indicating the restriction of this enzyme to the cytoplasmic matrix. Antibodies to NADPH-cytochrome P450 reductase proved to be a poor predictor for the localization of CYP isoenzymes and similar results have been reported in rabbit lung [22]. The absence of labelling in the type I pneumocytes and endothelial cells may reflect fixation-induced changes in the antigenicity of cytochrome P450 reductase but it is more likely to result from the low ratio of this enzyme to the CYP isoenzymes [29].

CYP1A1 has been reported, at low levels, in the Clara cells of untreated rats but treatment with β-naphthoflavone [11] or 3-methylcholanthrene [12] greatly increased the intensity of labelling. The low

levels of EROD*, an indicator of CYP1A1 activity present in rat lung microsomes, are increased by treatment with β-naphthoflavone, 3-methylcholanthrene or, even more strongly, with Aroclor 1254 [30]. Aroclor 1254 treatment clearly increased the pulmonary concentration of CYP1A1 protein, in our sections of rat lung, to detectable levels. This induction in Clara cells was similar to that observed after the treatment of rats with β-naphthoflavone [11]. The increase in labelling in vascular endothelial cells observed after Aroclor 1254 is similar to that reported after the treatment of rats with 3-methylcholanthrene [12].

CYP1A1 was also induced, by 3-methylcholanthrene, in the endothelial cells of mice but, as reported by Forkert *et al.* [19], this induction did not extend to the Clara cells. In isolated lungs, induction of this enzyme has been shown to accelerate clearance of benzo(*a*)pyrene from the bloodstream [14], and thus may reflect a primary role in the modification of circulating compounds. This role would be consistent with the predominant localization of this enzyme in the endothelial lining of the pulmonary vasculature. In contrast, CYP1A1 does not seem to have a significant role in the bioactivation of several pneumotoxins which have been shown to be activated by at least one CYP isoenzyme [6]. These toxins include butylated hydroxytoluene, *O*,*O*,*S*-trimethylphosphorothioate and methylcyclopentadienyl manganese tricarbonyl, which result in injury to the type I alveolar cells, and 4-ipomeanol, which injures the Clara cells of the bronchioles [24]. The antibody used to localize CYP1A1 does not modify the activation of these pneumotoxins by rat lung microsomes [6, 24] and treatment of rats with Aroclor 1254 or β-naphthoflavone, which induce a >30-fold increase in the level of EROD activity, is similarly ineffective [6, 24].

The antibody to CYP2B1, used in this study, may cross react with other members of the CYP2B subfamily but CYP2B1 is reported to be the major isoenzyme of cytochrome P450 in rat lung [12, 16–18, 28]. This isoenzyme has been shown to be responsible for the bioactivation, in rats, of the pneumotoxins, *O*,*O*,*S*-trimethylphosphorothioate and methylcyclopentadienyl manganese tricarbonyl [6]. In mice, the antibody to CYP2B1 probably labels the murine orthologue of CYP2B1, which has been shown to activate butylated hydroxytoluene [6, 31].

Pre-embedding incubations, which are particularly valuable for the detection of extracellular proteins [25], demonstrated the presence of CYP2B in the cytoplasm of Clara, ciliated, type I and type II cells. These results were confirmed by post-embedding incubations, which ensure uniform access of reagents to all subcellular compartments. The intense labelling observed in the apical cytoplasm of Clara cells, from rat, mouse and rabbit, is consistent with an association with the abundant smooth endoplasmic reticulum present in this region. A similar localization has been reported in rabbit lung but in this species CYP2B has also been reported on microvilli, in the Golgi region of ciliated cells and in endothelial cells [22, 27]. The absence of such overall labelling, in our samples, may reflect interspecies differences but the presence of extracellular enzyme, reported by these authors, probably results from apocrine secretion by Clara cells. This process would account for the presence of CYP activity in bronchoalveolar lavages from rats [32]. Apocrine secretion by Clara cells has, however, been associated with lung irritation and injury [33]. The normal, merocrine secretory activity of these cells probably releases enzymes concentrated in secretory granules, e.g. glutathione *S*-transferases rather than those concentrated in the endoplasmic reticulum [34]. The thin cytoplasmic processes of type I pneumocytes cannot be resolved by light microscopy but ultrastructural immunocytochemistry has enabled us to demonstrate the presence of CYP2B in these cells. The localization of this enzyme throughout the alveolar epithelium indicates that it is probably synthesized within mature type I cells, and is not merely a remnant from the progenitor type II cells.

The antibody to CYP4B1, used in this study, was raised against the rabbit-derived protein and has been shown to react with CYP4B1 on immunoblots of lung microsomes from both rats and mice [21]. The absence of labelling in our samples of mouse lung may reflect strain and sex differences but the distribution of labelling, in the Clara and type II cells of rats, was similar to that observed in rabbit lung. The selective injury of Clara cells by 4-ipomeanol was first described in rabbit lung and was shown to be characterized by covalent binding of radiolabelled toxin in the target cells [2]. In this species, bioactivation of 4-ipomeanol probably involves the activity of both CYP2B and CYP4B1 [24]. In rats, the pulmonary bioactivation of 4-ipomeanol is predominantly dependent on the activity of CYP4B1, indeed the antibody used in this study prevented the covalent binding of the radiolabelled compound to lung microsomes [24]. Thus, the presence of CYP4B1 in rat Clara cells is consistent with the particular susceptibility of these cells to 4-ipomeanol. The localization of CYP4B1 in murine Clara cells, also reported by Chichester *et al.* [10], and the susceptibility of these cells to 4-ipomeanol, indicates that a similar situation may exist in mice. The resistance of type II cells to 4-ipomeanol, in rabbits, rats and mice, despite the presence of CYP4B1, may reflect differences in detoxification mechanisms. The bioactivation of several pneumotoxins which cause injury to the type I cells of the alveoli, in rats and mice, has been shown to result from the activity of pulmonary CYP2B [30]. The particular vulnerability of type I cells has been attributed to their inability to deactivate the resulting metabolites [25]. The present findings indicate that type I cells are, indeed, capable of bioactivating these pneumotoxins.

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* Abbreviation: EROD, ethoxyresorufin *O*-dealkylation.

REFERENCES

1. Serabjit-Singh CJ, Wolf CR and Philpot RM, Cytochrome P-450: Localization in rabbit lung. *Science* **207**: 1469–1470, 1980.
2. Boyd MR, Metabolic activation and lung toxicity: a basis for cell-selective pulmonary damage by foreign chemicals. *Environ Health Perspect* **55**: 47–55, 1984.
3. Adamson IYR, Bowden DH, Cote MG and Witschi H, Lung injury induced by butylated hydroxytoluene: Cytodynamic and biochemical studies in mice. *Lab Invest* **36**: 26–32, 1977.
4. Dinsdale D, Verschoyle RD and Cabral JRP, Cellular responses to trialkylphosphorothioate-induced injury in rat lung. *Arch Toxicol* **51**: 79–89, 1982.
5. Kehrer JP and Kacew S, Systematically applied chemicals that damage lung tissue. *Toxicology* **35**: 251–293, 1985.
6. Verschoyle RD, Wolf CR and Dinsdale D, CYP2B1 is responsible for the pulmonary bioactivation and toxicity of butylated hydroxytoluene, O,O,S-trimethylphosphorothioate and methylcyclopentadienyl manganese tricarbonyl. *J Pharmacol Exp Ther* **266**: 958–963, 1993.
7. Soucek P and Gut I, Cytochromes P450 in rats: structures, functions, properties and relevant human forms. *Xenobiotica* **22**: 83–103, 1992.
8. Dees JH, Coe LD, Yasukochi Y and Masters BS, Immunofluorescence of NADPH-cytochrome c (P-450) reductase in rat and minipig tissues injected with phenobarbital. *Science* **208**; 1473–1475, 1980.
9. Voigt JM, Kawabata TT, Burke JP, Martin MV, Guengerich FP and Baron J, In situ localization and distribution of xenobiotic-activating enzymes and aryl hydrocarbon hydroxylase activity in lungs of untreated rats. *Mol Pharmacol* **37**: 182–191, 1990.
10. Chichester CH, Philpot RM, Weir AJ, Buckpitt AR and Plopper CG, Characterization of the cytochrome P-450 monooxygenase system in nonciliated bronchiolar epithelial (clara) cells isolated from mouse lung. *Am J Resp Cell Mol Biol* **4**: 179–186, 1991.
11. Foster JR, Elcombe CR, Boobis AR, Davies DS, Sesardic D, Quade J, Robson RT, Hayward C and Lock EA, Immunocytochemical localization of cytochrome P-450 in hepatic and extra-hepatic tissues of the rat with a monoclonal antibody against cytochrome P-450c. *Biochem Pharmacol* **35**: 4543–4554, 1986.
12. Keith IM, Olson EB, Wilson NM and Jefcoate CR, Immunological identification and effects of 3-methylcholanthrene and phenobarbital on rat pulmonary cytochrome P-450. *Cancer Res* **47**: 1878–1882, 1987.
13. Boobis AR, Sesardic D, Murray BP, Edwards RJ, Singleton AM, Rich KJ, Murray S, De La Torre R, Segura J, Pelkonen O, Pasanen M, Kobayashi S, Zhi-Guang T and Davies DS, Species variation in the response of the cytochrome P-450-dependent monooxygenase system to inducers and inhibitors. *Xenobiotica* **20**: 1139–1161, 1990.
14. Roth RA and Vinegar A, Action by the lungs on circulating xenobiotic agents, with a case study of physiologically based pharmacokinetic modeling of benzo(a)pyrene disposition. *Pharmacol Ther* **48**: 143–155, 1990.
15. Verschoyle RD, Carthew P, Wolf CR and Dinsdale D, The toxicity of 1-nitronaphthalene: Effects of chemical modulation of cytochrome P450. *Toxicol Appl Pharmacol* **122**: 208–213, 1993.
16. Omiecinski CJ, Tissue-specific expression of rat mRNAs homologous to cytochromes P-450b and P-450e. *Nucleic Acids Res* **14**: 1525–1539, 1986.
17. Christou M, Wilson NM and Jefcoate CR, Expression and function of three cytochrome P450 isozymes in rat extrahepatic tissues. *Arch Biochem Biophys* **258**: 519–534, 1987.
18. Wheeler CW and Guenthner TM, Cytochrome P-450-dependent metabolism of xenobiotics in human lung. *J Biochem Toxicol* **6**: 163–169, 1991.
19. Forket PG, Vessey ML, Elce JS, Park SS, Gelboin HV and Cole SPC, Localization of phenobarbital- and 3-methylcholanthrene-inducible cytochromes P-450 in mouse lung with monoclonal antibodies. *Res Commun Chem Pathol Pharmacol* **53**: 147–157, 1986.
20. Rich KJ, Sesardic D, Foster JR, Davies DS and Boobis AR, Immunohistochemical localization of cytochrome P450b/e in hepatic and extrahepatic tissues of the rat. *Biochem Pharmacol* **38**: 3305–3322, 1989.
21. Vanderslice RR, Domin BA, Carver GT and Philpot RM, Species-dependent expression and induction of homologues of rabbit cytochrome P-450 isozyme 5 in liver and lung. *Mol Pharmacol* **31**: 320–325, 1987.
22. Serabjit-Singh, CJ, Nishio SJ, Philpot RM and Plopper CG, The distribution of cytochrome p-450 monooxygenase in cells of the rabbit lung; an ultrastructural immunocytochemical characterization. *Mol Pharmacol* **33**: 279–289, 1988.
23. Wolf CR, Statham CN, McMenamin MG, Bend JR, Boyd MR and Philpot RM, The relationship between the catalytic activities of rabbit pulmonary cytochrome P-450 isozymes and the lung-specific toxicity of the furan derivative, 4-ipomeanol. *Mol Pharmacol* **22** (3): 738–744, 1982.
24. Verschoyle RD, Philpot RM, Wolf CR and Dinsdale D, CYP4B1 activates 4-ipomeanol in rat lung. *Toxicol Appl Pharmacol* **123**: 193–198, 1993.
25. Dinsdale D, Green JA, Manson MM and Lee MJ, The ultrastructural localization of γ-glutamyltranspeptidase in rat lung: correlation with the histochemical demonstration of enzyme activity. *Histochem J* **24**: 144–152, 1992.
26. Hall PM, Stupans I, Burgess W, Birkett DJ and McManus ME, Immunohistochemical localization of NADPH-cytochrome P450 reductase in human tissues. *Carcinogenesis* **10**: 521–530, 1989.
27. Overby L, Nishio SJ, Lawton MP, Plopper CG and Philpot RM, Cellular localization of flavin-containing monooxygenase in rabbit lung. *Exp Lung Res* **18**: 131–144, 1992.
28. Domin BA, Devereux TR and Philpot RM, The cytochrome P-450 monooxygenase system of rabbit lung enzyme components, activities, and induction in the nonciliated bronchiolar epithelial (Clara) cell, alveolar type II cell, and alveolar macrophage. *Mol Pharmacol* **30**: 296–303, 1986.
29. Guengerich FP, Wang P and Mason PS, Immunological comparison of rat, rabbit, and human liver NADPH-cytochrome P-450 reductases. *Biochemistry* **20**: 2379–2385, 1981.
30. Verschoyle RD, Dinsdale D and Wolf CR, Inhibition and induction of cytochrome P450 isoenzymes in rat lung. *J Pharmacol Exp Ther* **265**: 386–391, 1993.
31. Bolton JL and Thompson JA, Oxidation of butylated hydroxytoluene to toxic metabolites: Factors influencing hydroxylation and quinone methide formation by hepatic and pulmonary microsomes. *Drug Metab Dispos* **19**: 467–472, 1991.
32. Clouter A, Lung Clara cells and their reaction to bronchiolar toxins. Thesis, University of Wales, Cardiff, U.K., 1993.
33. Widdicombe JG and Pack RJ, The Clara cell. *Eur J Resp Dis* **63**: 202–220, 1982.
34. Lee MJ and Dinsdale D, Immunolocalization of glutathione S-transferase isoenzymes in the bronchiolar epithelium of rats and mice. *Am J Physiol* **267**: L766–L774, 1994.