Pergamon

Oral Oncol, Eur J Cancer, Vol. 30B, No. 3, pp. 179–185, 1994
Elsevier Science Ltd
Printed in Great Britain

Involvement of Cytochrome P450 2E1-like Isoform in the Activation of *N*-nitrosobis(2-oxopropyl)amine in the Rat Nasal Mucosa

Katherine Kazakoff, Patrick Iversen, Terence Lawson, Jeffery Baron, F. Peter Guengerich and Parviz M. Pour

Induction of tumours in the nasal olfactory region of MRC rats by *N*-nitrosobis(2-oxopropyl)amine (BOP) is inhibited by orchiectomy and restored by testosterone. These results suggest the involvement of a sex-specific enzyme in BOP bioactivation in rat nasal mucosa. The present study was undertaken to identify this enzyme. Enzyme-linked immunosorbent assay (ELISA) and the metabolism of known substrates (*p*-nitrophenol) pointed to a microsomal cytochrome P450 (P450) 2E1-like isoform as a candidate enzyme. A correlation was found between the enzyme activity in nasal mucosal microsomes and serum testosterone levels. Four times more activity was detected in the nasal mucosa than in the liver of male rats. Vanillin inhibited the activity of the nasal mucosal enzyme to a greater extent than that of the liver enzyme. The overall results suggest that a nasal mucosal P450 2E1-like isoform is involved in BOP metabolism.

Oral Oncol, Eur J Cancer, Vol. 30B, No. 3, pp. 179–185, 1994.

INTRODUCTION

THE NASAL cavity is a major port of entry into the body, and is exposed to a variety of xenobiotics, including inhaled odorants, steroids and carcinogens [1]. The nasal mucosa of many species, including humans, contains microsomal cytochrome P450 (P450) enzymes that can metabolise xenobiotics [2–8]. The activities of some of these enzymes are higher in the nasal mucosa than in the liver [4, 5]. The nasal mucosa of rats and hamsters are particularly sensitive to tumour induction by carcinogens, especially nitrosamines [3, 9]. The ability of the nasal mucosa of rats to metabolise nitrosamines to reactive products has been shown [3, 10, 11]. In the respiratory and olfactory epithelium of rats, P450 1A1 (β-NFB), P450 2B1 (PB-B) and P450 3A2 (PCN-E) have been demonstrated immunohistochemically [2, 12, 13]. Some P450 enzymes, including P450s 2C11 (UT-A), 2C12 (UT-I), 2G1, 3A2 and 2E1 are either sex specific [14] or tissue specific. P450 2G1, for example, has been reported to occur only in olfactory tissue [1, 15].

Correspondence to P.M. Pour.
P.M. Pour, K. Kazakoff and T. Lawson are at the Eppley Institute for Research in Cancer and Allied Diseases; P. Iversen is at the Department of Pharmacology, University of Nebraska Medical Center, 600 S 42nd Street, Omaha, Nebraska 68198-6805; J. Baron is at the Department of Pharmacology, The University of Iowa, College of Medicine Iowa City, Iowa 52242; and F.P. Guengerich is at the Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146, U.S.A.
Received 19 Apr. 1993; accepted 11 June 1993.

P450 2E1 is important for the activation of *N*-nitrosodimethylamine, *N*-nitrosodiethylamine and tobacco-specific nitrosamines [3]. A high incidence of nasal mucosal tumours in male rats induced by *N*-nitrosobis(2-oxopropyl)amine (BOP) can be prevented by orchiectomy [16] and restored by systemic testosterone treatment [17]. BOP is a dialkyl-*N*-nitrosamine that requires metabolic activation for carcinogenic action. Our observations suggest that the activation of BOP is catalysed by P450s whose activities may be modulated by testosterone.

To identify the enzyme in the rat nasal mucosa, the present studies were undertaken.

MATERIALS AND METHODS

Animals

Wistar-derived MRC rats from the Eppley colony, females with an average weight of 210 g and males with an average weight of 320 g, were housed under standard laboratory conditions (temperature, 21 ± 3°C; light/dark, 12 h/12 h; relative humidity, 40 ± 5%). They had free access to a pelleted diet (Wayne Lab Blox, Allied Mills, Chicago, Illinois, U.S.A.) and tap water.

Chemicals

Testosterone propionate (TP) (Sigma Chemical Co., St Louis, Missouri, U.S.A.) was suspended in olive oil (40 mg/ml) and was given subcutaneously (s.c.) (0.25 ml/100 g). BOP was obtained from Ash Stevens,

(Detroit, Michigan, U.S.A.). Vanillin, imadazole, capsaicin, pyrazole, *p*-nitrophenol, β-nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), 6-thioguanine, bovine serum albumin (BSA) and Trizma-base were obtained from Sigma Chemical Co., 2,2′-azino-di(3-ethyl-benzthiazoline-sufonate) horseradish peroxidase (ABTS HRP) from Kirkegaard and Perry Labs (Gaitherburg, Maryland, U.S.A.); and goat anti-rabbit or rabbit anti-goat horseradish peroxidase labelled conjugates from Bio-Rad (Richmond, California, U.S.A.); and Williams medium E (WE), fetal bovine serum (FBS) (non-dialysed) (10% v/v), penicillin G and streptomycin and antimycotic solution from BRL-Gibco (Grand Island, New York, U.S.A.).

Treatment

Bilateral orchiectomy or ovariectomy was performed on 8-week-old rats under Nembutal anaesthesia [75 mg/kg body weight (b.w.)] injected intraperitoneally (i.p.). The testes or ovaries were ligated and removed. Wound clips were used to close the scrotal incision, while 5–0 chromic sutures were used to close the abdominal incision in females. Three weeks after orchiectomy or ovariectomy, rats were entered into the experiment. Some orchiectomised and control rats were given TP (100 mg/kg b.w.) s.c. daily for 5 days, and blood testosterone level was measured 1 day after the last injection. Before killing, a blood sample was taken from all the animals for determination of testosterone levels.

Hormone radioimmunoassay

Testosterone levels were measured using a coat-a-count radioimmunoassay (RIA) kit (Diagnostic Products Corp., Los Angeles, California, U.S.A.).

Antibodies

Three polyclonal antibodies against xenobiotic metabolising enzymes P450 2C11 (UT-A) [18], P450 1A1 (β-NFB) [18] and P450 2E1 (Oxford Biomedical Res., Oxford, Michigan, U.S.A.) were used. Antibodies were diluted to 40 μg/ml for the enzyme-linked immunosorbent assay (ELISA). The nomenclature of the P450s is adapted from Nebert *et al.* [19].

Preparation of microsomes

All animals were killed (rats treated with TP were euthanised 5 days after the initial TP injection) with excess Nembutal (100 mg/kg b.w., i.p.). The liver (left lobe) and nasal mucosa were removed and placed in ice-cold 0.05 mol/l Tris–sucrose buffer (pH 7.4). Tissues from four rats were pooled to obtain an adequate amount of nasal mucosal microsomes (6 mg). Microsomes were prepared by differential centrifugation [20]. The protein content was measured by the method of Lowry *et al.* [21].

ELISA

Liver and nasal mucosal microsomes from each test group were diluted to a protein concentration of 2 μg/ml with a sodium bicarbonate buffer (pH 9.6). These were titred out onto Nunclon 96 well polystyrene microwell plates (A/S NUNC, Roskilde, Denmark). Four protein concentrations per group were used: 0.25, 0.5, 1 and 2 μg/well. Plates were incubated overnight (4°C) to allow the protein to attach. The plates were washed with phosphate buffered saline (PBS), then 2% BSA in PBS was added to each well to block unbound sites. After incubation for 1 h, the plates were washed with PBS, and anti-P450s 1A1, 2C11 or 2E1 were added to the coated wells and then incubated for 2 h at room temperature. Goat anti-rabbit (1A1 and 2C11) and rabbit anti-goat (2E1) each labelled with horseradish peroxidase were added after the plates were washed. After incubation for 1 h at room temperature and washing with PBS, 100 μl of ABTS was added and then incubated for 30 min at room temperature. Absorbance was read at 405 nm using a through-the-plate spectrophotometer (Titertek Multiscan PLUS, Flow Lab, Mclean, Virginia, U.S.A.).

Enzyme activity

P450 2E1-like isoform activity was measured by the conversion of *p*-nitrophenol to *p*-nitrocatechol [22], with slight modifications. Each assay was performed with 400 μg of microsomal protein obtained from the pooled livers (left lobe) and nasal mucosa of four rats. Activities were measured in control and treated male and female rats. Each test was repeated three times with fresh microsomes. Microsomes were incubated for 10 min at 37°C with 0.25 mmol/l *p*-nitrophenol, 60 mmol/l NADPH and 2 ml 0.1 mol/l phosphate buffer (pH 7.4). The reaction was stopped with the addition of 1 ml of 15% trichloroacetic acid (TCA) and the precipitated protein removed by centrifugation. Sodium hydroxide (250 μl of a 10 mol/l solution) was added to 2.5 ml of the supernatant. Absorbance was read at 515 nm and the amount of conversion was calculated using the formula: A 515/0.4 mg protein/10 min/0.0117 = mmol/mg protein/min.

In vitro inhibition of *p*-nitrophenol conversion to *p*-nitrocatechol was tested with capsaicin, imadazole, vanillin and pyrazole, each dissolved in 0.5% ethanol. Vanillin, pyrazole, capsaicin or imadazole (40–640 μmol/l), nasal mucosa or liver microsomes (400 μg each) from untreated male rats, 0.25 mmol/l *p*-nitrophenol and 60 mmol/l NADPH were placed in 2.0 ml of 0.1 mol/l potassium phosphate buffer (pH 7.4). Control reactions consisted of microsomes, *p*-nitrophenol, NADPH and buffer.

For *in vivo* experiments, three groups of rats (four per group) were injected i.p. (1 ml solution/kg body weight) with vanillin 25 or 50 mg/kg (LD_{50} = 1580 mg/kg [23]), 6 h before killing. After killing, microsomes were prepared and the enzyme activity measured as described above. We used vanillin in the inhibition assays, because capsaicin was too toxic to be used *in vivo*, and pyrazole and imadazole did not inhibit P450 2E1-like isoform to the extent that vanillin did, especially in the nasal mucosa.

Effect of testosterone on enzyme activity

From all the rats used, serum testosterone levels were measured and plotted against the apparent K_m determined in the *p*-nitrophenol assay.

Effect of anti-P450 2E1 on enzyme activity

Liver and nasal mucosa microsomes from control male and female rats (four per group) were pooled. Microsomes (400 μg per assay) were placed in phosphate buffer together with 40 μg/ml anti-P450 2E1 and incubated for 30 min. The

Table 1. ELISA of nasal mucosa and liver microsomes reported as slope (absorbance/protein)

Antibody	Male	C-male	C-male-T	C-female	C-female-T	Female
N						
1A1	1.14±0.1*	0.82±0.1*	0.94±0.1	1.42±0.3	1.13±0.1	1.23±0.1
2C11	1.40±0.1†	0.56±0.1†	0.67±0.2	1.20±0.2	1.01±0.02	1.05±0.1
2E1	0.53±0.2	0.32±0.1‡	0.60±0.01‡	0.22±0.2	0.06±0.0	0.18±0.1
L						
1A1	2.02±1.4	3.28±1.0	1.78±0.9	0.47±0.4	0.45±0.4	0.49±0.3
2C11	2.26±0.1	3.16±1.5	2.30±0.5	0.67±0.2	0.55±0.2	0.66±0.3
2E1	0.43±0.1§	0.16±0.03§	0.22±0.04	0.22±0.1	0.18±0.02	0.22±0.1

C = castrated; T = testosterone treated. Absorbance was read a 405 nm. Data given are the mean ± S.E.M. *$P<0.02$; †$P<0.0006$; ‡$P<0.05$; §$P<0.03$.

remaining test components were added, and the activity was determined as described above.

Mutagenicity

Mutagenicity was measured in V79 cells using resistance to 6-thioguanine as the marker of mutagenicity, with liver and nasal mucosa homogenates as the activating system as described previously [24, 25].

Statistical analysis

For data comparison, the unpaired *t*-test was performed.

RESULTS

ELISA

Liver and nasal mucosa microsomes from six treatment groups were subjected to ELISA analysis using three anti-P450s (1A1, 2C11 and 2E1). The results, which were obtained from reading the absorbance at 405 nm, are shown in Table 1. The data is reported as the slope of the antibody response calculated by using the absorbance at three protein concentrations (0.25, 0.5 and 1 μg/well). The results show that expression of P450 1A1 and P450 2C11 diminished in the castrated male rat nasal mucosa but started to recover after testosterone administration. A similar trend was seen with P450 2E1-like isoform. In the liver, orchiectomy enhanced P450 1A1 and P450 2C11 levels, which also returned to control levels with testosterone treatment. The expression of P450 2E1-like isoform decreased slightly in the liver of orchiectomised rats and slightly recovered after testosterone treatment. Neither the nasal mucosa nor the livers of female rats displayed significant differences between groups.

P450 2E1-like isoform activity

There was more *p*-nitrophenol hydroxylase activity in the nasal mucosa than in the liver of control female and male rats (Fig. 1a and b). There was five times more P450 2E1-like isoform activity in the nasal mucosa than in the liver of female rats, four times more in the nasal mucosa than in the liver of male rats, and slightly more activity in males than in female (1.3-fold for liver and 1.2-fold for nasal mucosa). At the highest *p*-nitrophenol concentration (1 mmol/l), activity in the liver and nasal mucosa of females and in the liver of males had reached a plateau, whereas it was still increasing in the male nasal mucosa. The greater activity in the nasal mucosa was associated with an apparent K_m that is significantly different from the liver in both female (Fig. 1c) and male rats (Fig. 1d).

Effect of castration on P450 2E1-like isoform activity

Castration shifted the apparent K_m in both liver and nasal mucosa of the male rat (Fig. 2). Following castration, the apparent K_m was the same in the nasal mucosa and liver. This shift may indicate the presence of a competitive inhibitor in the castrated male rat. Female rats were not examined.

Effect of testosterone on P450 2E1-like isoform activity

Testosterone levels determined in the serum of rats taken at the time of killing were plotted against the apparent K_m determined in the *p*-nitrophenol assay. The lower the testosterone concentration, the lower the P450 2E1-like isoform affinity for the substrate (Fig. 3).

Effect of anti-P450 2E1 on enzyme activity

Anti-P450 2E1 added to microsomal preparations of male and female liver and nasal mucosa inhibited *p*-nitrophenol hydroxylase activity, with a significantly greater extent of inhibition being produced in the nasal mucosa of both male and female rats than in the liver (Table 2).

Inhibition of P450 2E1-like isoform activity

P-nitrophenol hydroxylase activity was inhibited by capsaicin, pyrazole, imidazole and vanillin when both liver and nasal mucosa microsomes were used (Fig. 4). The IC_{50} were 80 μmol/l for capsaicin, 200 μmol/l for pyrazole and 110 μmol/l for vanillin. Capsaicin and vanillin were more effective then pyrazole or imidazole when nasal mucosal microsomes were used. The inhibitory effect of vanillin on P450 2E1-like isoform activity was also tested *in vivo* 6 h after treatment. The i.p. administration of either vanillin or ethanol inhibited enzyme activity in the nasal mucosa but not in the liver (Fig. 5). The inhibitory effect of vanillin on enzyme activity was dose-dependent.

Mutagenicity study

The mutagenicity of BOP (1 mmol/l) was measured in V79 cells after activation by homogenates of nasal mucosa and liver from intact male rats in the presence or absence of pyrazole and vanillin (Table 3). The nasal mucosal homogenate was more efficient at generating mutagens than the liver homogenate.

The concentrations of pyrazole (200 μmol/l) and vanillin (120 μmol/l) used were equal to the IC_{50}s based on the inhibitory action on cytochrome P450 2E1-like isoform. When BOP was activated by a liver homogenate, the inhibition of mutagenicity was 73% (pyrazole) and 68% (vanillin). The inhibition was 53% (pyrazole) and 82% (vanillin) when nasal mucosal homogenates were used. This inhibition is significant when compared to the mutagens generated by BOP only. Pyrazole and vanillin were not mutagenic.

DISCUSSION

The inhibitory effect of orchiectomy on the carcinogenicity of BOP in the nasal mucosa and the reversal of this inhibition by testosterone supplementation indicated that testosterone plays a role in nasal mucosal carcinogenesis in rats.

P450s are involved in the metabolism of xenobiotics, including nitrosocompounds. Some of the P450s are sex specific [14]. If a sex specific isoform regulates the metabolism of BOP in the nasal mucosa, it is expected that there is more of the isoform in males than females and that its activity decreases following orchiectomy and increases after subsequent testosterone treatment. In search for such a P450, we first screened nasal mucosa and liver microsomes with anti-P450s by an ELISA. The ELISA showed that the levels of isoforms, including P450 2E1-like isoform, decreased following castration and started to recover after testosterone treatment, but the results were not clear-cut. To clarify this matter, we examined *p*-nitrophenol hydroxylase activity, which (in nasal mucosal and liver microsomes) is a metabolic marker for the presence of P450 2E1 [26]. Castration shifted the apparent K_m for this reaction, there was a relationship between the serum level of testosterone and the apparent K_m of *p*-nitrophenol hydroxylase activity in the male's nasal mucosa. Inhibition of the enzyme activity by capsaicin, which we have shown to inhibit the activity of P450 2E1 [27], and by pyrazole and vanillin, which we have found to inhibit the activity of this isoform both *in vitro* and *in vivo*, further suggested that we are dealing with a P450 2E1-like isoform.

To further clarify the issue, we then examined the mutation levels of nasal mucosal and liver tissues BOP in the presence and absence of an inhibitor. The results of the mutagenicity experiment [24, 25] in the presence and absence of the inhibitors strengthen our assumption on the identity of the enzyme in the nasal mucosa. When liver tissue was used, pyrazole and vanillin inhibited BOP mutagenicity by 73 and 68%, respectively, while the comparable inhibitions of P450 2E1-like isoform activity were 52 and 54%. When nasal mucosal tissue was used, pyrazole and vanillin inhibited mutagenicity by 53 and 82%, respectively, and P450 2E1-like isoform activity by 25 and 59%. In all cases, the extent of inhibition of mutagenicity was greater than the inhibition of P450 2E1-like isoform activity.

The overall results, however, pointed to differences between the nasal mucosal and liver P450 2E1-like isoform. Activity was greater in the male's nasal mucosa than in the liver. A distinct difference was also found in response to vanillin, which inhibited activity in the nasal mucosa more than in the

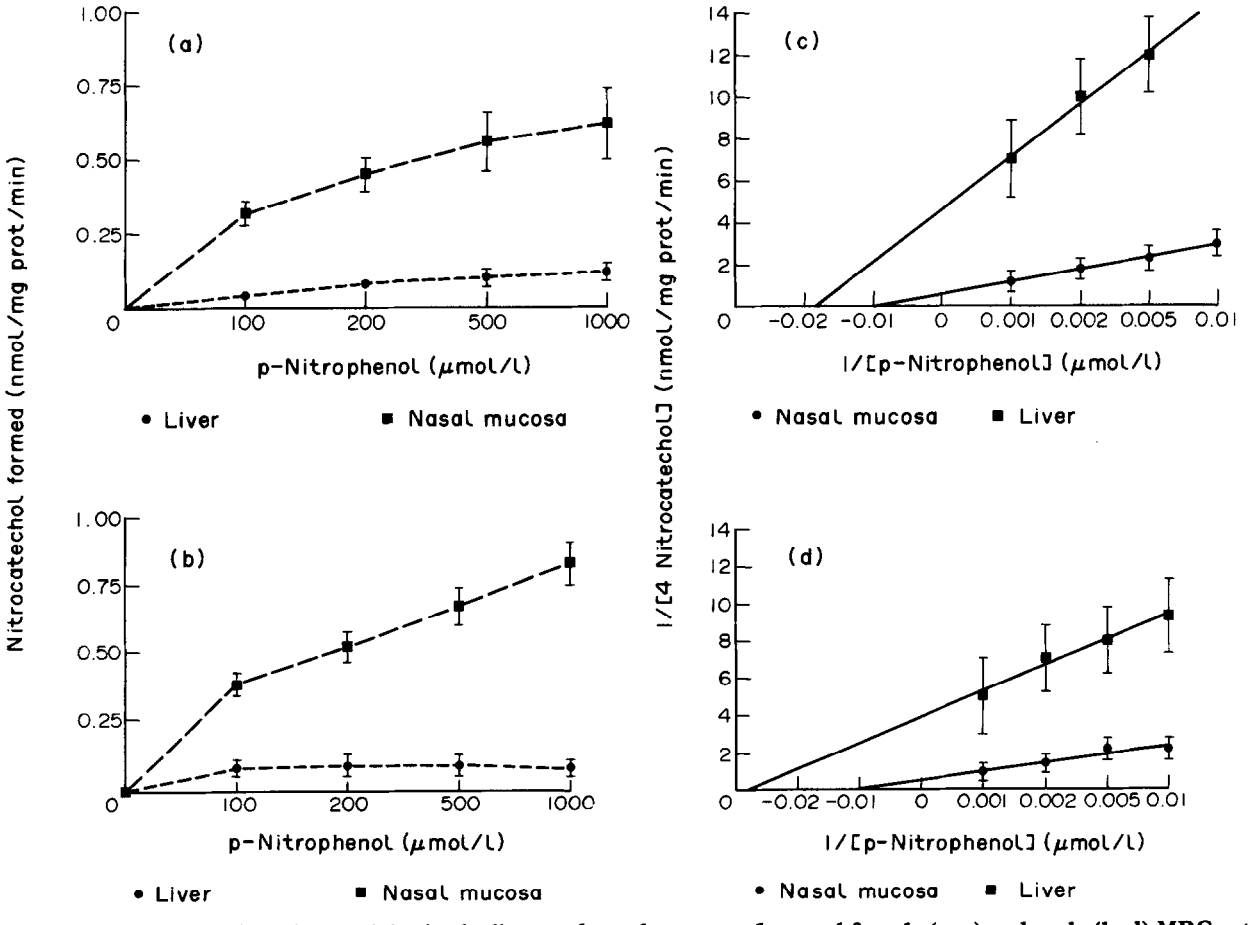

Fig. 1. *P*-nitrophenol hydroxylase activity in the liver and nasal mucosa of control female (a, c) and male (b, d) MRC rats.

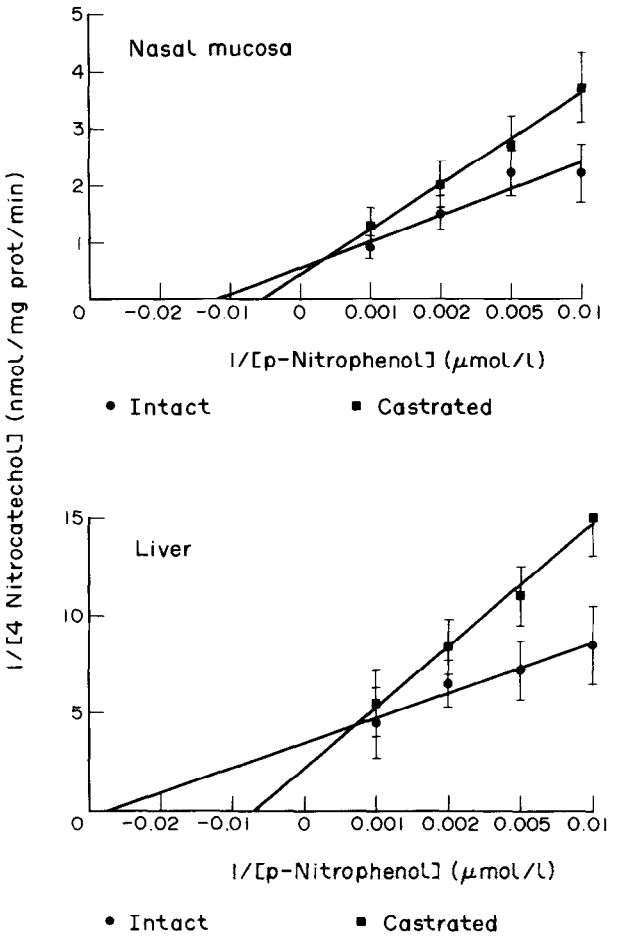

Fig. 2. *P*-nitrophenol hydroxylase activity in the nasal mucosa and liver of intact and castrated MRC rats.

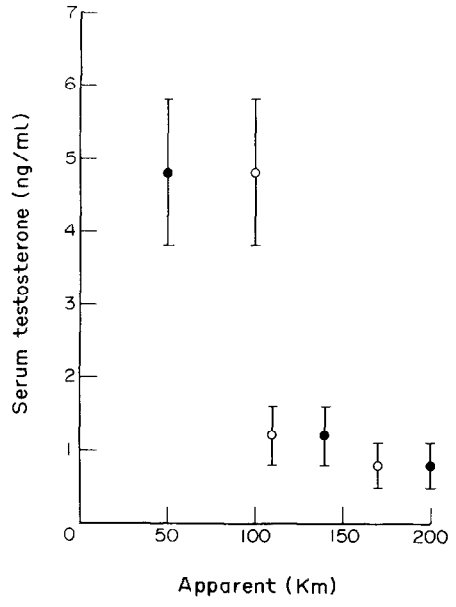

Fig. 3. The relationship between serum testosterone levels and the apparent K_m for *p*-nitrophenol hydroxylase in the liver (●) and nasal mucosa (○).

liver. We used vanillin as an enzyme inhibitor because our previous studies with capsaicin [27] indicated that the methoxy portion of capsaicin provides the reactive centre for

Table 2. Inhibition of P450 2E1 enzyme activity in the liver and nasal mucosa of male and female rats

	Anti-2E1		Control	
Tissue	Female	Male	Female	Male
Nasal	0.319±0.04*	0.180±0.03†	0.648±0.08*	0.533±0.10†
Liver	0.114±0.02	0.179±0.04	0.139±0.10	0.252±0.06

Data given are the means and S.E.M.s of nitrocatechol formed (nmol/mg protein/min). *$P<0.03$; †$P<0.025$.

enzyme inhibition. The aromatic ring appears to be the planar space filling portion of the molecule required for cytochrome P450 2E1 selectivity, and the side chain is believed to confer the major toxicity. Vanillin fulfills these requirements but lacks the toxicity of capsaicin.

The reactivity of nasal mucosal preparations with the polyclonal anti-P450 2E1 in an ELISA analysis also strongly supports the existence of differences between the liver and nasal mucosal isoform. It is known that there are considerable catalytic differences even among very closely related P450 proteins. A good example is P450 2A1, which has testosterone 7α-hydroxylation as a characteristic activity [28], whereas the very similar P450 2A6 (69% identity) is devoid of this activity [29]. This difference could be due to a single change in the sequence of the P450 2A1 molecule that could alter its catalytic specificity as well as reactivity with antibodies against a related family member. The failure of castration to inhibit hepato-

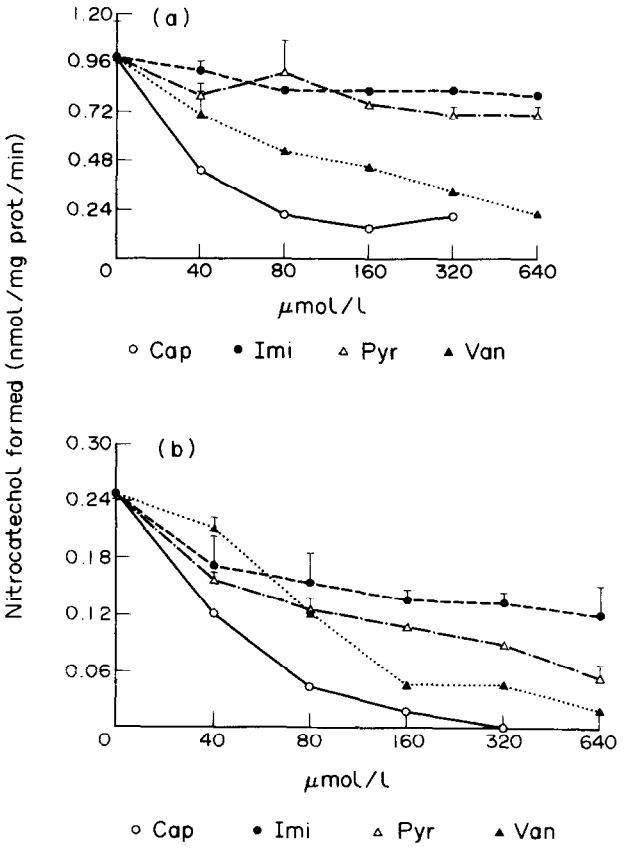

Fig. 4. Effects of capsaicin (Cap), imidazole (Imi), pyrazole (Pyr) and vanillin (Van) on *p*-nitrophenol hydroxylase activity in nasal mucosa (a) and liver (b) microsomes of MRC rats.

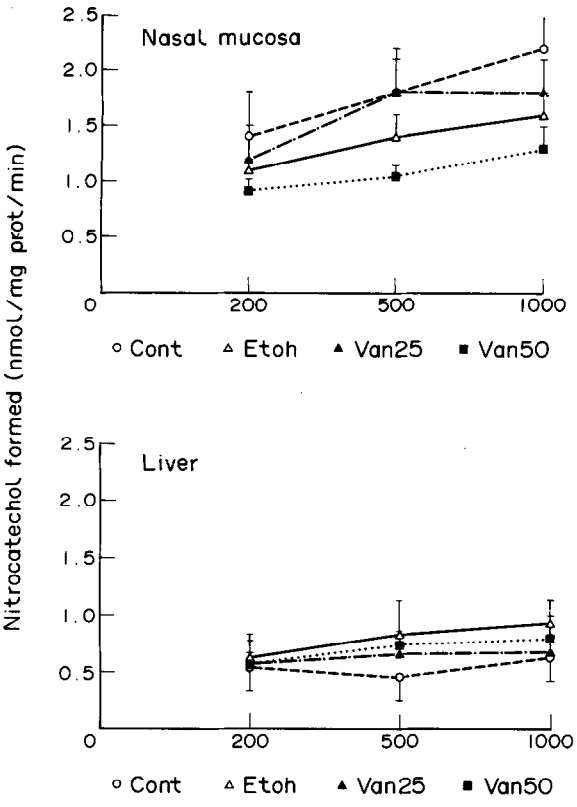

Fig. 5. *In vivo* effects of ethanol (Etoh) and vanillin (Van) on the nasal mucosa enzyme activity of male MRC rats. Cont = control. Two doses of vanillin were used: 25 mg/kg b.w. (Van25) and 50 mg/kg b.w. (Van50).

carcinogenicity of BOP is an additional hint of differing identity and function of the P450 in the nasal mucosa and liver of rats.

Overall findings suggest that the P450 in our study may correspond with the recently described olfactory-specific P450 2G1 (P450olf) [30]. The induction of nasal mucosal tumours by BOP restricted to the olfactory region [16, 17] supports this view. It has been shown that the P450-dependent metabolism of various substrates is higher in olfactory epithelium than in the liver and that the enzymes in the olfactory epithelium have different kinetic and substrate specificities [1]. These enzymes are also more sensitive to P450 inhibitors than those in the liver [31], as we also found in our study. Vanillin inhibited the activity of the nasal mucosal protein much more than that of the liver. Because P450 2G1 does not show an identity greater than 53% with other known P450 sequences [1], the lack of the reactivity of this isoform with the antibody to P450 2E1 is self-explanatory. Further studies are required to substantiate the identity of the enzyme in our studies.

Table 3. Mutagenicity of BOP (1 mmol/l) activated by liver and nasal mucosal homogenates from male MRC-WISTAR rats and incubated with pyrazole (200 μmol/l) and vanillin (120 μmol/l)*

Treatment	Mutants/10^6 cells†‡	
	Liver	Nasal mucosa
Control§	0±0 (59)	0±0 (54)
BOP	41±2 (54)§§	60±6 (35)‖§§
BOP + pyrazole¶	10±2 (61)††§§	25±6 (45)††‡‡
BOP + vanillin**	15±1 (69)††¶¶	15±2 (69)††‡‡§§
Pyrazole¶	0±0 (49)§§	0±0 (30)‖‖
Vanillin**	0±0 (48)§§	0±0 (40)‖‖

*10 mg protein/flask; †Mean ± S.E. ($n=16$); ‡Absolute survival plating efficiencies (%) in parentheses; §V79 cells, no BOP; ‖$n=8$; 200 μmol/l; **120 μmol/l; ††$P<0.0001$ compared with the BOP group; ‡‡$P<0.0001$; §§$P<0.0001$; ‖‖$P<0.0003$; ¶¶$P<0.04$.

1. *Nef P, Heldman J, Lazard D, et al.* Olfactory-specific cytochrome P450: cDNA cloning of a novel neuroepithelial enzyme possibly involved in chemoreception. *J Biol Chem* 1989, **264**, 6780–6785.
2. Baron J. Burke JP, Guengerich FP, Jakoby W, Voigt JM. Sites for xenobiotic activation and detoxication within the respiratory tract: implications for chemically induced toxicity. *Toxicol Appl Pharmacol* 1988, **93**, 493–505.
3. Brittebo EB, Castonguay A, Furuya K, Hecht SS. Metabolism of tobacco-specific nitrosamines by cultured rat nasal mucosa. *Cancer Res* 1983, **43**, 4343–4348.
4. Dahl AR. Activation of carcinogens and other xenobiotics by nasal cytochrome P450. In Boobis Ar, Caldwell J, de Matteis F, Elcombe CR, eds. *Microsomes and Drug Oxidations*. London, Taylor and Francis, 1985, 299–309.
5. Dahl AR. The effect of cytochrome P450-dependent metabolism and other enzyme activities on olfaction. In Margolis FL, Getchell TV, eds. *Molecular Neurobiology of the Olfactory System*. New York, Plenum Press, 1988, 51–70.
6. Gervasi PG, Longo V, Naldi F, Panattoni G, Ursino F. Xenobiotic-metabolizing enzymes in human respiratory nasal mucosa. *Biochem Pharmacol* 1991, **41**, 177–184.
7. Gervasi PG, Longo V, Ursino F, Panattoni G. Drug metabolizing enzymes in respiratory mucosa of humans. Comparison with rats. In Scuster I, ed, *Proceedings of the 6th International Conference on Biochemistry and Biophysics of Cytochrome P450*. London, Taylor and Francis, 1989, 97–100.
8. Longo V, Citti L, Gervasi PG. Biotransformation enzymes in nasal mucosa and liver of Sprague-Dawley rats. *Toxicol Lett* 1988, **44**, 289–297.
9. Pour PM, Grandjean CJ, Knepper S. Selective induction of nasal cavity tumors in rats by diallylnitrosamine. *J Cancer Res Clin Oncol* 1985, **109**, 5–8.
10. Lai DY, Aros JC. Minireview: dialkylnitrosamine bioactivation and carcinogenesis. *Life Sci* 1980, **27**, 2149–2265.
11. Yoo J-SH, Ishizaki H, Yang CS. Roles of cytochrome 450 2E1 in the dealkylation and denitrosation of *N*-nitrosomethylamine in rat liver microsomes. *Carcinogenesis* 1990, **11**, 2239–2243.
12. Voigt JM, Guengerich FP, Baron J. Localization of a cytochrome P450 isoform (cytochrome P450 PB-B) and NADPH-cytochrome P450 reductase in rat nasal mucosa. *Cancer Lett* 1985, **27**, 241–247.
13. Baron J. *In situ* sites for xenobiotic activation and detoxification: Implications for the differential susceptibility of cells to the toxic action of environmental chemicals. In Graumann W, Druckker J, eds. *Histo-and Cytochemistry as a Tool in Environmental Toxicology*. Stuttgart, Fisher Verlag, 1991, 32–44.
14. Waxman DJ, Dannan GA, Guengerich FP. Regulation of rat hepatic cytochrome P450: age-dependent expression, hormonal imprinting and xenobiotic inducibility of sex-specific isoforms. *Biochemistry* 1985, **23**, 4409–4417.
15. Ding X, Coon MJ. Purification and characterization of two unique forms of cytochrome P450 from rabbit nasal microsomes. *Biochemistry* 1988, **27**, 8330–8337.
16. Pour PM, Götz U. Prevention of *N*-nitroso(2-oxopropyl)amine-induced nasal cavity tumors in rats by orchiectomy. *J Natl Cancer Inst* 1983, **70**, 353–357.
17. Pour PM, Stepan K. The role of testosterone in the nasal cavity tumours induced by *N*-nitrosobis(2-oxopropyl)amine in rats. *Carcinogenesis* 1988, **9**, 1417–1420.
18. Guengerich FP, Dannan GA, Wright ST, Martin MV, Kaminsky LS. Purification and characterization of liver microsomal cytochromes P450: electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated

from rats treated with phenobarbital or β-naphtoflavone. *Biochemistry* 1982, **21**, 6019–6030.
19. Nebert DM, Nelson DR, Coon MJ, *et al.* The P450 superfamily: update on new sequences, gene mapping, and recommended nomenclature. *DNA Cell Biology* 1991, **10**, 1–14.
20. Hong J-Y, Yang CS. The nature of microsomal *N*-nitrosodimethylamine demethylase and its role in carcinogen activation. *Carcinogenesis* 1985, **6**, 1805–1809.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
22. Koop DR. Hydroxylation of *p*-nitrophenol by rabbit ethanol-inducible cytochrome P450 isoform 3a. *Molec Pharmacol* 1986, **29**, 399–404.
23. Jenner PM, Hagan EC, Taylor JM, Cook EL, Fitzhugh OH. Food flavourings and compounds of related structure I. Acute oral toxicity. *Food Cosmet Toxicol* 1964, **2**, 327–343.
24. Lawson TA. Activation of *N*-nitroso(2-oxopropyl)amine by liver and nasal mucosa tissue from intact and castrated male rats. *Cancer Lett* 1990, **53**, 39–43.
25. Lawson T. Kolar C, Nagel D. Mutagenicity of *N*-nitrosobis(2-oxopropyl)amine in V79 cells when activated by homogenates of liver and pancreas acinar and duct tissues from Syrian golden hamsters and MRC-Wistar rats. *Mut Res* 1990, **240**, 237–240.
26. Koop DR. Oxidative and reductive metabolism by cytochrome P450 2E1. *FASEB* 1992, **6**, 724–730.
27. Gannett PM, Iversen P, Lawson T. The mechanism of inhibition of cytochrome P450 IIE1 by dihydrocapsaicin. *Bioorg Chem* 1990, **18**, 185–198.
28. Levin W, Thomas PE, Ryan DE, Wood AW. Isoform specificity of testosterone 7α-hydroxylation in rat hepatic microsomes: is cytochrome P450a the sole catalyst? *Arch Biochem Biophys* 1987, **258**, 630–635.
29. Yun C-H, Shimada T, Guengerich FP. Purification and characterization of human microsomal cytochrome P450 2A6. *Mol Pharmacol* 1991, **40**, 679–685. 1630.
30. Coon MJ, Ding X, Pernecky SJ, Vaz AS DN. Cytochrome P450: progress and predictions. *FASEB* 1992, **6**, 669–673.
31. Jenner J, Dodd G. The interaction of metyrapone and α-naphtholflavone with rat olfactory cytochrome P-450. *Biochem Pharmacol* 1988, **37**, 558–559.

Acknowledgements—This study was supported by Grant 1RO1CA35042 and Core Grants CA36727 and CA44353 from the National Cancer Institute/NIH and Grant SIG-16 from the American Cancer Society.