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EVALUATION OF LORATADINE AS AN INDUCER OF LIVER MICROSOMAL CYTOCHROME P450 IN RATS AND MICE

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Abstract—The non-sedating anti-histamine, loratadine [ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-ylidene-1-piperidinecarboxylate], was administered orally in the diet to mature male rats at dosages of 4, 10 and 25 mg/kg/day for 2 weeks. The effects of these treatments on liver microsomal cytochrome P450 were evaluated by immunochemical and biochemical techniques, and were compared with the effects of treating rats with three different inducers of cytochrome P450, namely phenobarbital, 3-methylcholanthrene and dexamethasone. Treatment of rats with loratadine caused a dose-dependent increase in the levels of P450 2B1 and 2B2, the major phenobarbital-inducible P450 enzymes, as determined by Western immunoblotting. At the highest dosage tested, loratadine was less effective than phenobarbital as an inducer of 2B1 and 2B2, although the induction of these proteins could be detected immunochemically even at the lowest dosage of loratadine tested. Consistent with these observations, treatment of rats with loratadine caused a dose-dependent increase in the rate of two reactions that are catalyzed predominantly by 2B1/2, namely testosterone 16β-hydroxylation and 7-pentoxyresorufin *O*-dealkylation. At the highest dosage tested, loratadine caused a 7.3- and 8.5-fold increase in the rate of testosterone 16β-hydroxylation and 7-pentoxyresorufin *O*-dealkylation, respectively, compared with a 22- and 45-fold increase caused by phenobarbital treatment. Treatment of rats with loratadine caused a 1.4- to 2.0-fold increase in the 2β-, 6β- and 15β-hydroxylation of testosterone, which was associated with a similar increase in the levels of immunoreactive P450 3A1 and/or 3A2. As an inducer of P450 3A1/2, loratadine was slightly less effective than phenobarbital, and was considerably less effective than dexamethasone, which caused a 10- to 33-fold increase in testosterone 2β-, 6β- and 15β-hydroxylase activity. At the dosages tested, loratadine did not increase the levels of P450 1A1, the major 3-methylcholanthrene-inducible P450 enzyme, as determined by Western immunoblotting. The rate of 7-ethoxyresorufin *O*-dealkylation, which is catalyzed predominantly by P450 1A1, increased 1.9-fold after loratidine treatment, but this increase was less than that caused by phenobarbital treatment (2.2-fold), and was considerably less than that caused by 3-methylcholanthrene treatment (33-fold). The effects of treating mature male mice with loratadine on liver microsomal cytochrome P450 resembled the effects observed in rats. These results indicate that loratadine is a phenobarbital-type inducer of liver microsomal cytochrome P450 in rats and mice.

Preclinical testing of loratadine§ [ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-ylidene-1-piperidinecarboxylate] established that this non-sedating anti-histamine can increase the concentration of liver microsomal cytochrome P450 (up to 35%) and the rate of benzphetamine *N*-demethylation (up to 180%) and 7-ethoxycoumarin *O*-dealkylation (up to 70%) in rats and mice (Clement RP, Casciano CN and Cayen MN, unpublished results). The purpose of the present study was to determine by immunochemical and biochemical techniques which P450 enzymes are increased in rats and mice treated with loratadine (the structure of which is shown in Fig. 1).

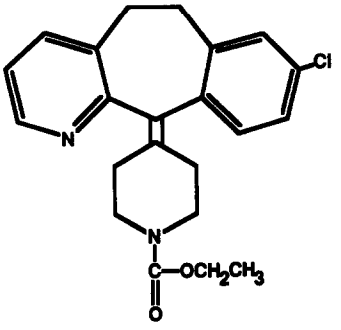

Fig. 1. Structure of the non-sedating anti-histamine, loratadine.

Phenobarbital, 3-methylcholanthrene and dexamethasone represent three different classes of

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§ Abbreviations and trivial names used: androstenedione, 4-androstene-3,17-dione; 6-dehydrotestosterone, 4,6-androstadien-17β-ol-3-one; loratadine, ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-ylidene-1-piperidinecarboxylate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; and testosterone, 17β-hydroxy-4-androsten-3-one.

chemicals that increase the concentration of liver microsomal cytochrome P450 and the rate of benzphetamine *N*-demethylation and/or the *O*-dealkylation of 7-ethoxycoumarin [1–5]. Treatment of rats with phenobarbital causes a marked induction of cytochromes P450 2B1 and 2B2,* which are structurally related isozymes (97% identical) with very similar substrate specificities. However, 2B1 is generally much more catalytically active than 2B2. Liver microsomes from untreated rats contain very low levels of 2B2 and virtually undetectable levels of 2B1. Treatment of rats with 3-methylcholanthrene causes a marked induction of cytochromes P450 1A1 and 1A2, which are structurally related enzymes (69% identical), but which catalyze different reactions. Liver microsomes from untreated rats contain low levels of 1A2 and virtually undetectable levels of 1A1. Treatment of rats with dexamethasone causes a marked induction of cytochrome P450 3A1, which is one of two independently regulated P450 3A isozymes with very similar structures (87%) and substrate specificites. P450 3A2, but not 3A1, is present in liver microsomes from untreated rats, although the levels of this enzyme decline markedly after puberty in female rats [1–5].

The effects of phenobarbital, 3-methylcholanthrene and dexamethasone on rat liver cytochrome P450 are not entirely disparate. Phenobarbital is a weak inducer of 3A1/2 whereas dexamethasone is a weak inducer of 2B1/2. All three inducers cause a 2- to 4-fold increase in the levels of 2A1, as well as a 50–75% decrease in the levels of 2C11 (which is present only in adult male rats).

The effects of treating rats with phenobarbital, 3-methylcholanthrene and dexamethasone on the levels of liver P450 2A1, 2B1/2, 2C11 and 3A1/2 can be monitored by changes in specific pathways of testosterone (17β-hydroxy-4-androsten-3-one) oxidation [5–8]. For all practical purposes, the rate of testosterone 2α-, 7α- and 16β-hydroxylation accurately reflects the levels of 2C11, 2A1 and 2B1/2, respectively. P450 2A1 also catalyzes the 6α-hydroxylation of testosterone, whereas P450 2C11 and 2B1/2 also catalyze the 16α-hydroxylation of testosterone, as well as the 17-oxidation of testosterone to 4-androstene-3,17-dione (androstenedione). In mature male rats, the overall effect of phenobarbital on the rate of formation of 16α-hydroxytestosterone and androstenedione depends on the extent to which 2C11 is suppressed versus the extent to which 2B1/2 is induced. The 2β-, 6β- and 15β-hydroxylation of testosterone collectively reflect the levels of P450 3A1 and/or 3A2 (the relative contribution of these two isozymes to testosterone oxidation depends on the source of the liver microsomes). P450 3A1/2 also oxidizes testosterone to 4,6-androstadien-17β-ol-3-one (6-dehydrotestosterone) [9], although this metabolite, which absorbs maximally at 280 nm, can be difficult to quantify when testosterone metabolites are monitored at 254 nm.

The induction of 1A1/2 by 3-methylcholanthrene cannot be monitored by changes in testosterone oxidation [6, 7]. Fortunately, a series of 7-alkoxyresorufins has proven very useful for monitoring the induction of 1A1/2 by 3-methylcholanthrene-type inducers, as well as the induction of P450 2B1/2 by phenobarbital-type inducers [10–12]. P450 1A1/2 preferentially catalyzes the *O*-dealkylation of 7-methoxy- and 7-ethoxyresorufin, whereas P450 2B1/2 preferentially catalyzes the *O*-dealkylation of 7-pentoxy- and 7-benzyloxyresorufin. 7-Alkoxyresorufins have proven useful in monitoring the induction of 1A and 2B enzymes in species other than the rat [13].

In addition to measuring certain enzyme activities, changes in the levels of specific P450 enzymes can also be monitored by immunochemical techniques, such as immunoblotting [3–5, 14, 15]. Polyclonal antibodies raised against one P450 enzyme invariably cross-react with other P450 enzymes, due to the structural relatedness among this family of hemoproteins [14, 15]. In general antibodies that recognize P450 enzymes belonging to other gene families can be removed by immunoabsorption chromatography (by definition, mammalian P450 enzymes in the same gene family are >40% identical in amino acid sequence, and enzymes in the same subfamily are >55% identical [1]). However, it is often impractical to remove antibodies that recognize P450 enzymes within the same subfamily, because these proteins may possess remarkably similar amino acid sequences. In the present study, antibodies against rat 1A1, 2B1 and 3A1 were used together with measurements of selected enzyme activities to determine that loratadine is a phenobarbital-type inducer of liver microsomal cytochrome P450 in rats and mice.

MATERIALS AND METHODS

Chemicals. Horseradish peroxidase-conjugated, goat-*anti*-rabbit IgG (heavy and light chain specific) and rabbit peroxidase-*anti*-peroxidase (PAP) complex were purchased from the Cappel-Organon Teknika Co., West Chester, PA. Resorufin (hydroxyphenoxazone) and various 7-alkoxyresorufins were purchased from Molecular Probes (Junction City, OR). The sources of the other chemicals used in this study have been described elsewhere [7, 12, 16–20].

Animal treatments and preparation of liver microsomes. Male Sprague–Dawley rats (7 weeks old, from Charles River Laboratories Inc., Kingston, NY) were housed individually in stainless steel cages in a temperature-, humidity- and light-controlled facility (70–72°F, 48–52%, 12 hr light/dark). Rats were allowed free access to Agway Pro-Lab Rodent Chow No. 3200 and water. After a 2-week acclimation period, one group of rats (N = 4) was continued on rodent chow, whereas three other groups were fed loratadine in the diet for 15 or 16 days at dosages of 4, 10 or 25 mg/kg/day. A fifth group of rats was

* In previous publications, we have used the nomenclature system of Ryan and Levin [5], in which cytochromes P450a, P450b, P450e, P450h, P450m and P450p correspond to 2A1, 2B1, 2B2, 2C11, 2A2 and 3A1. Cytochrome P450 3A2 is a constitutively expressed, developmentally regulated isozyme of 3A1. Cytochrome P450 gene families can also be designated with roman numerals, e.g. 3A1 may also be written as IIIA1.

fed phenobarbital (85 mg/kg/day) in the diet. Rats were fasted for 12 hr prior to the preparation of liver microsomes. Liver microsomes were also prepared from 9-week-old-male Sprague–Dawley rats (from Charles River Laboratories, Wilmington, MA) after four consecutive daily injections (i.p.) of corn oil (10 mL/kg), phenobarbital (100 mg/kg), 3-methylcholanthrene (27 mg/kg) or dexamethasone (100 mg/kg).

Male CD-1 mice (7 weeks old, from Charles River Laboratories Inc., Kingston, NY) were housed as described above for Sprague–Dawley rats. Mice were allowed free access to Purina Rodent Chow No. 5002 and water. After a 2-week acclimation period, one group of mice (N = 8) was continued on rodent chow, whereas three other groups were fed loratadine in the diet for 15 or 16 days at dosages of 4, 12 or 40 mg/kg/day. A fifth group of mice was fed phenobarbital (85 mg/kg/day) in the diet. Mice were fasted for 12 hr prior to the preparation of microsomes from pools of two livers. Liver microsomes were also prepared from 8-week-old-male CF-1 mice (from Sasco, Omaha, NE) after three consecutive daily injections (s.c.) of phenobarbital (75 mg/kg), 3-methylcholanthrene (20 mg/kg) or pregnenolone-16α-carbonitrile (75 mg/kg), which is a dexamethasone-type inducer. These latter samples of mouse liver microsomes were provided by Drs. J. Liu and C. D. Klaassen of the University of Kansas Medical Center.

Protein purification and antibody production. Cytochromes P450 1A1, 2B1 and 3A1 were purified from rat liver microsomes as described previously [5, 7, 12, 16–18]. Polyclonal antibodies against these enzymes were raised in rabbits, and were subjected to immunoabsorption chromatography to remove antibodies that cross-reacted with P450 enzymes in other gene families [12, 16, 18]. After immunoabsorption chromatography, *anti*-1A1 was monospecific. In contrast, the immunoabsorbed antibody against 2B1 recognized 2B1 and 2B2, which are 97% identical in amino acid sequence [5]. Similarly, the immunoabsorbed antibody against 3A1 recognized 3A1 and 3A2, which are 87% identical in amino acid sequence [2], as well as a 50 kDa protein that appears to be a third member of the rat 3A gene family [19, 20].

Electrophoresis. Liver microsomes (10 μg protein) were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) according to the method of Laemmli [21], with minor procedural modifications [16]. The separating gel was 0.75 mm thick, 12.5 cm long, and composed of 7.5% acrylamide. Proteins were stained with Coomassie brilliant blue R250.

Western immunoblotting. Liver microsomes (2 or 10 μg protein) and purified P450 enzymes (0.5, 1 or 2 pmol) were analyzed by Western immunoblotting as described by Towbin *et al.* [22], with modifications described by Arlotto *et al.* [16]. Proteins were transferred electrophoretically from polyacrylamide gels to immobilon, after which non-specific binding sites were blocked with 5% non-fat dried milk in 10 mM Tris–HCl, pH 7.4, containing 50 mM NaCl and 1 mM EDTA. The immobilon membrane was incubated overnight with primary antibody (25 μg/mL of *anti*-1A1, *anti*-2B1 or *anti*-3A1), followed by a 4-hr incubation with secondary antibody (1.25 μg/mL of horseradish peroxidase-conjugated, goat-*anti*-rabbit-IgG), followed by a 1-hr incubation with 2.5 μg/mL of PAP complex. All antibodies were diluted in phosphate-buffered saline (PBS) containing 5% goat serum, 2% bovine serum albumin and 0.05% Tween 20. Horseradish peroxidase was located with 4CN-peroxidase substrate (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD). Between all reagent changes, the immobilon was rinsed three times for 2 min in 50 mL of PBS containing 0.05% Tween 20. All immunostaining procedures were performed at room temperature.

Testosterone oxidation. The pathways of testosterone oxidation catalyzed by liver microsomes were determined by HPLC, as described by Sonderfan *et al.* [7, 12]. Liver microsomes (0.5 mg/mL) were incubated for 5 min at 37° in 1-mL incubation mixtures containing potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), EDTA (1 mM), NADP (1 mM), glucose-6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (1 U/mL), the steroid 5α-reductase inhibitor 17β-*N,N*-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (1 μM) and testosterone (250 μM), at the final concentrations indicated. Reactions were started by addition of the NADPH-generating system and were stopped by addition of 6 mL dichloromethane. Each sample was spiked with 3 nmol of 11β-hydroxytestosterone (in 100 μL dichloromethane) and vigorously mixed on a batch vortexer (3 × 1 min). After the two phases were separated by low-speed centrifugation (2000 *g* for 10 min), the aqueous (upper) phase was aspirated and discarded. An aliquot (4 mL) of the organic phase was transferred to a culture tube (12 × 75 mm) and evaporated in a Speed-Vac concentrator (Savant Instruments, Farmingdale, NY). The residue was redissolved in 200 μL solvent A (see below), and a 50 μL aliquot was analyzed by HPLC.

High-performance liquid chromatography. Testosterone, androstenedione, 6-dehydrotestosterone, and fourteen monohydroxylated testosterone isomers were resolved at 40° on a Supelcosil reverse-phase C_{18} column (Supelco, Bellefonte, PA) with a Shimadzu LC-6A binary gradient HPLC system (Chimadzu Scientific Instruments, Columbia, MD). A concave gradient (curve 6) from 90% solvent A (methanol:water:acetonitrile, 39:60:1) to 85% solvent B (methanol:water:acetonitrile, 80:18:2) was operated over 22 min at 1.5 mL/min to elute testosterone and its potential metabolites, which were monitored at 254 nm with a variable wavelength UV detector (Shimadzu SPD-6A). Metabolites were quantified by comparison of their peak areas (integrated by a Shimadzu C-R3A recording data processor) with those of authentic standards.

To analyze the metabolites formed by mouse liver microsomes, a second HPLC procedure was used to resolve 6β-hydroxy- and 15α-hydroxytestosterone, as described by Arlotto *et al.* [16]. This latter metabolite is formed by both rat and mouse liver microsomes, but its rate of formation by rat liver microsomes is negligible (<3%) compared with

the rate of formation of 6β-hydroxytestosterone [18, 24].

O-Dealkylation of 7-ethoxy- and 7-pentoxyresorufin. 7-Alkoxyresorufin *O*-dealkylase activity was measured by the fluorimetric method of Burke *et al.* [10], with minor modifications [12]. Liver microsomes (40–1000 μg/mL) were incubated at 37° in 1-mL incubation mixtures containing potassium phosphate buffer (100 mM, pH 7.4), $MgCl_2$ (3 mM), EDTA (1 mM), NADP (1 mM), glucose-6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (1 U/mL) and either 7-pentoxyresorufin or 7-ethoxyresorufin (10 μM), at the final concentrations indicated. Reactions were started by addition of the NADPH-generating system, and were stopped after 5 min by addition of 2 mL of ice-cold acetone. Precipitated protein was removed by centrifugation (2000 *g* for 5 min). The amount of resorufin in the clear supernatant fraction was determined fluorimetrically ($\lambda_{ex} \approx 535$ nm, $\lambda_{em} \approx 585$ nm) with a Shimadzu RF 540 spectrofluorimeter. Zero-time incubations served as blanks, and blanks spiked with 0.5 to 2.0 nmol resorufin served as internal standards.

Other assays. Protein concentration was measured by the method of Lowry *et al.* [25], as modified by Miller [26]. The concentration of cytochrome P450 was determined by the method of Omura and Sato [27], from the carbon monoxide difference spectrum of dithionite-reduced microsomes, based on an extinction coefficient of 91 $mM^{-1}\,cm^{-1}$. The concentration of cytochrome b_5 was determined from the difference spectrum between oxidized and reduced cytochrome b_5, based on an extinction coefficient of 185 $mM^{-1}\,cm^{-1}$ [27].

Statistical analysis. Data were analyzed for statistically significant differences between controls and the multiple treatment groups by Dunnett's two-way analysis of variance at the 5% level of significance ($\alpha = 0.05$) [28].

RESULTS

Immunoblotting of liver microsomes from loratadine-treated rats. Loratadine was administered orally in the diet to mature male rats at dosages of 4, 10 and 25 mg/kg/day for 2 weeks. The dosages of loratadine were those used in carcinogenicity bioassays in rats. In a subchronic (90-day) toxicity study, the highest dosage of loratadine (32 mg/kg/day) caused hepatic changes including centrilobular hypertrophy and increased liver weight. The effects of these treatments on liver microsomal cytochrome P450 were evaluated by SDS–PAGE and Western immunoblotting, as shown in Figs. 2 and 3. Treatment of rats with loratadine caused a dose-dependent increase in the levels of P450 2B1 and 2B2, the major phenobarbital-inducible P450 enzymes (Fig. 3). At the highest dosage tested (25 mg/kg/day), loratadine was less effective than phenobarbital (85 mg/kg/day) an inducer of 2B1 and 2B2 (note that the amount of protein analysed by immunoblotting varied depending on the source of the liver microsomes). An increase in the levels of P450 2B1 and 2B2 could be detected immunochemically even at the lowest dosage of loratadine tested. Treatment of rats with loratadine caused less than a 2-fold increase in the levels of P450 3A1, the major dexamethasone-inducible P450 enzyme, and caused no detectable induction of 1A1, the major dexamethasone-inducible P450 enzymes, and caused no detectable induction of 1A1, the major 3-methylcholanthrene-inducible P450 enzyme.

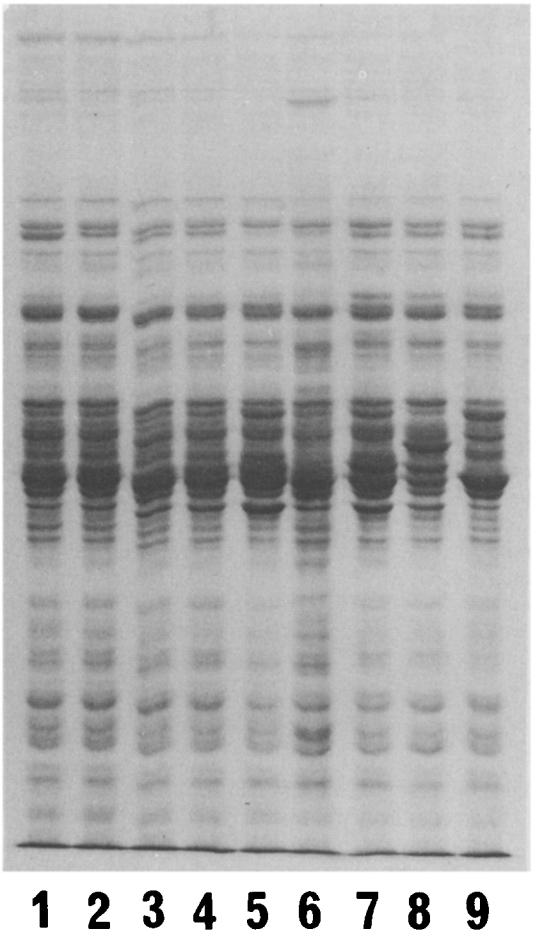

Fig. 2. SDS–polyacrylamide gel of liver microsomes from male rats treated with loratadine or known inducers of cytochrome P450. Liver microsomes (10 μg protein) from control and treated male Sprague–Dawley rats were subjected to SDS–PAGE as described in Materials and Methods. Lanes 1–5 contained liver microsomes from rats fed 0, 4, 10 or 25 mg/kg/day of loratadine or 85 mg/kg/day phenobarbital, respectively. Lanes 6–9 contained liver microsomes from rats injected i.p. with corn oil (control), phenobarbital, 3-methylcholanthrene or dexamethasone, respectively.

Testosterone oxidation by liver microsomes from loratadine-treated rats. The effects of treating rats with loratadine on the pathways of testosterone oxidation catalyzed by liver microsomes are shown in Table 1. The most pronounced effect of loratadine was a 7.3-fold increase in testosterone 16β-hydroxylation, which is catalyzed by 2B1/2. Induction of testosterone 16β-hydroxylase activity by loratadine was dose-dependent, but not statistically significant. At the highest dosage tested, loratadine was less

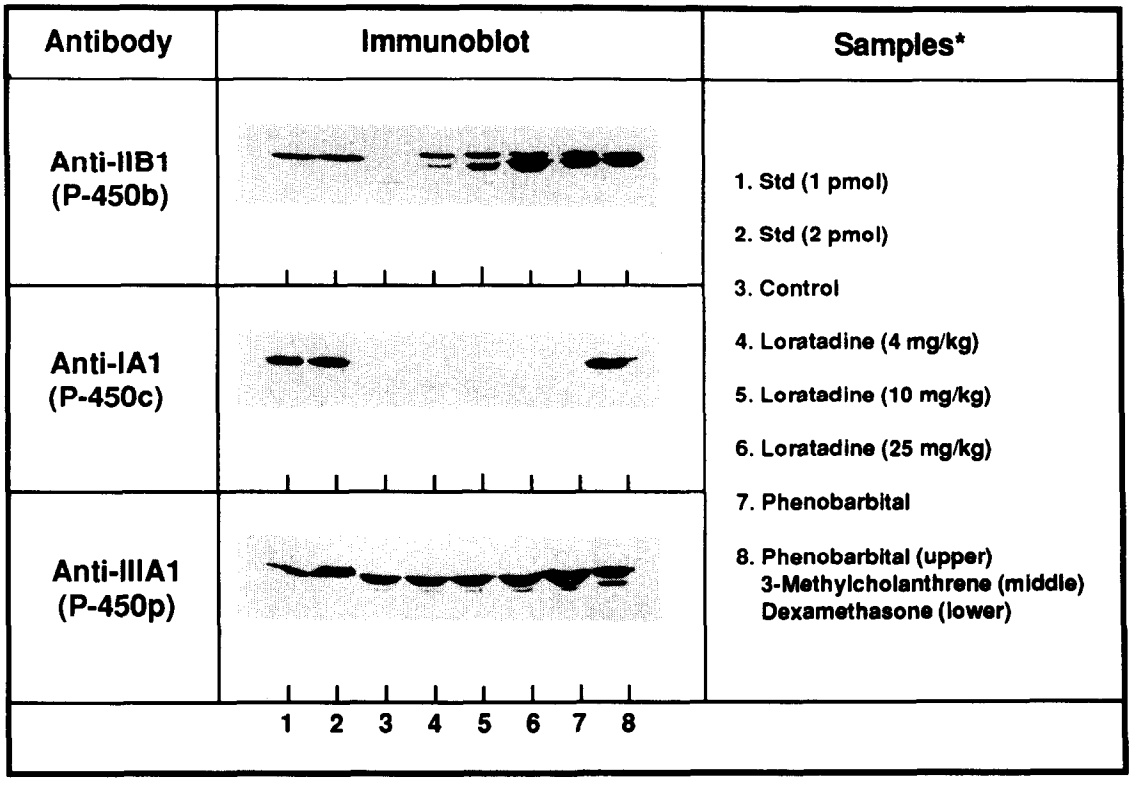

Fig. 3. Immunoblots of liver microsomes from male rats treated with loratadine or known inducers of cytochrome P450. Immunoblots of liver microsomes from control and treated male Sprague–Dawley rats were probed with antibody against rat 2B1, 1A1 or 3A1, as described in Materials and Methods. In all cases, lanes 3–6 contained 10 μg of liver microsomal protein from rats fed 0, 4, 10 or 25 mg/kg/day of loratadine. Lane 7 contained either 2 μg (upper panel) or 10 μg (middle and lower panels) of liver microsomal protein from rats fed 85 mg/kg/day phenobarbital. Lane 8 contained 2 μg of liver microsomal protein from rats treated i.p. with phenobarbital (upper), 3-methylcholanthrene (middle) or dexamethasone (lower). The standards (Std) were samples of the purified rat P450 enzymes against which the antibodies were raised.

effective than phenobarbital as an inducer of testosterone 16β-hydroxylase activity. Loratadine caused a small (<2.1-fold) but dose-dependent increase in those pathways of testosterone oxidation catalyzed by 3A1/2, which include 1β-, 2β-, 6β-, 15β- and 18-hydroxylation, and oxidation to 6-dehydrotestosterone. Testosterone 7α-hydroxylase activity, which reflects P450 2A1 levels, and testosterone 2α-hydroxylase activity, which reflects 2C11 levels, were not affected by loratadine treatment in a dose-dependent or statistically significant manner. In contrast to loratadine, the other inducers tested caused a suppression of testosterone 2α-hydroxylase activity. Phenobarbital, 3-methylcholanthrene and dexamethasone have been shown previously to suppress the expression of 2C11 in adult male rats [29, 30].

7-Alkoxyresorufin O-dealkylation by liver microsomes from loratadine-treated rats. The effects of treating rats with loratadine on the *O*-dealkylation of 7-pentoxy- and 7-ethoxyresorufin by liver microsomes are shown in Table 2. Loratadine treatment caused a dose-dependent but statistically insignificant increase in the *O*-dealkylation of 7-pentoxyresorufin, which is catalyzed by 2B1/2. At the highest dosage tested, loratadine was less effective than phenobarbital as an inducer of 7-pentoxyresorufin *O*-dealkylation (8.5-fold vs a 45-fold increase). At the highest dosage tested, loratadine caused a 1.9-fold increase in 7-ethoxyresorufin *O*-dealkylase activity, an effect similar to that elicited by phenobarbital. In contrast, treatment of rats with 3-methylcholanthrene caused a large increase (33-fold) in 7-ethoxyresorufin *O*-dealkylase activity.

Immunoblotting of liver microsomes from loratadine-treated mice. Loratadine was administered orally in the diet to male CD-1 mice at dosages of 4, 12 and 40 mg/kg/day for 2 weeks. The dosages of loratadine were those used in carcinogenicity bioassays in mice. The effects of these treatments on liver microsomal cytochrome P450 were evaluated by SDS–PAGE (Fig. 4) and Western immunoblotting (Fig. 5). Treatment of mice with loratadine caused a dose-dependent increase in the levels of a microsomal protein(s) recognized by antibody against rat 2B1. This same protein was intensified to a greater extent by treatment of mice with phenobarbital

Table 1. Testosterone oxidation by liver microsomes from male rats treated with loratadine

Treatment*	Testosterone oxidation† (pmol metabolite formed/mg protein/min)											
	1α/1β	2α	2β	6α	6β	7α	15β	16α	16β	A	18	DHT
Control	93 ± 35	2,680 ± 300	216 ± 58	13 ± 1	2,460 ± 780	263 ± 12	98 ± 22	3,950 ± 460	153 ± 43	1,600 ± 210	65 ± 25	623 ± 90
Loratadine (4 mg/kg/day)	106 ± 17	2,470 ± 540	249 ± 49	15 ± 2	2,960 ± 620	385 ± 30	103 ± 19	3,710 ± 790	226 ± 38	1,790 ± 270	75 ± 13	701 ± 76
Loratadine (10 mg/kg/day)	130 ± 20	2,780 ± 550	290 ± 48	17 ± 4	3,582 ± 530	430 ± 65	104 ± 12	4,290 ± 930	370 ± 120	1,880 ± 210	91 ± 13	741 ± 110
Loratadine (25 mg/kg/day)	178 ± 14‡	2,460 ± 240	395 ± 47‡	20 ± 1	5,030 ± 630‡	347 ± 29	135 ± 18	4,630 ± 320	1,110 ± 320	2,080 ± 300	136 ± 4‡	1,060 ± 160‡
Phenobarbital (85 mg/kg/day)	313 ± 35‡	615 ± 200‡	873 ± 140‡	54 ± 9‡	7,630 ± 1,000‡	1,160 ± 214‡	383 ± 73‡	4,160 ± 1,010	3,340 ± 1,250‡	2,470 ± 480	280 ± 39‡	1,650 ± 260‡
Control	41	1,880	130	<10	1,450	210	43	2,550	55	1,210	29	366
Phenobarbital (100 mg/kg/day)	330	1,000	840	34	7,940	920	390	4,700	3,700	2,770	256	1,630
3-Methylcholanthrene (27 mg/kg/day)	48	750	150	19	2,040	580	57	1,170	37	690	36	570
Dexamethasone (100 mg/kg/day)	600	990	2,110	43	14,000	570	1,400	1,940	420	1,070	506	2,880

* Male Sprague–Dawley rats (N = 4/group) were treated for 2 weeks with loratadine or phenobarbital in the diet (top), or they were injected i.p. for 4 days with corn oil (control), phenobarbital, 3-methylcholanthrene or dexamethasone (bottom), as described in Materials and Methods.

† Testosterone metabolites were resolved and quantified by HPLC. With the exception of A (androstenedione) and DHT (6-dehydrotestosterone), the abbreviations denote the hydroxytestosterone metabolite formed, e.g. 7α denotes 7α-hydroxytestosterone. Values are means ± SD.

‡ Statistically significant ($\alpha < 0.05$) by Dunnett's test.

Table 2. *O*-Dealkylation of 7-pentoxy- and 7-ethoxyresorufin by liver microsomes from male rats treated with loratadine

Treatment*	7-Pentoxyresorufin *O*-dealkylation† (pmol/mg protein/min)	7-Ethoxyresorufin *O*-dealkylation† (pmol/mg protein/min)
Control	13 ± 5	93 ± 16
Loratadine (4 mg/kg/day)	22 ± 2	144 ± 12
Loratadine (10 mg/kg/day)	36 ± 10	140 ± 25
Loratadine (25 mg/kg/day)	111 ± 27	177 ± 42‡
Phenobarbital (85 mg/kg/day)	584 ± 155‡	203 ± 49‡
Control	26	103
Phenobarbital (100 mg/kg/day)	879	387
3-Methylcholanthrene (27 mg/kg/day)	34	3410
Dexamethasone (100 mg/kg/day)	43	89

* Male Sprague–Dawley rats (N = 4/group) were treated for 2 weeks with loratadine or phenobarbital in the diet (top), or they were injected i.p. for 4 days with corn oil (control), phenobarbital, 3-methylcholanthrene or dexamethasone (bottom), as described in Materials and Methods.

† Values are means ± SD.

‡ Statistically significant ($\alpha < 0.05$) by Dunnett's test.

(note that the amount of protein analyzed by immunoblotting varied depending on the source of the liver microsomes). An increase in the levels of the mouse 2B protein could be detected immunochemically even at the lowest dosage of loratadine tested. Treatment of mice with loratadine did not intensify the microsomal protein recognized by antibody against rat P450 3A1 (Fig. 5). Treatment of mice with loratadine did not appear to induce 1A1, but this result requires some qualification (see Discussion). Antibody against rat 1A1 recognized a constitutively expressed protein in mouse liver microsomes, and the levels of this protein were decreased by phenobarbital treatment. This protein has a greater electrophoretic mobility than mouse 1A1 or 1A2, and its identity is unknown.

Testosterone oxidation by liver microsomes from loratadine-treated mice. The effects of treating mice with loratadine or phenobarbital on the pathways of testosterone oxidation catalyzed by liver microsomes are shown in Table 3. With two exceptions, the rate of all pathways of testosterone oxidation increased 1.5- to 5.5-fold in phenobarbital-treated mice. The two exceptions were the 7α- and 15α-hydroxylation of testosterone, which are catalyzed by different members of the 2A gene family [16, 31–32].* Treatment of mice with loratadine caused dose-dependent and/or statistically significant increases in several pathways of testosterone oxidation, but the magnitude of the increases was generally less than that elicited by phenobarbital treatment. It should be emphasized that testosterone 16β-hydroxylation is not a reliable indicator of the phenobarbital-inducible P450 enzymes in mice as it is in rats. The most pronounced effect of loratadine in mice was a 1.8-fold increase in testosterone 6α-hydroxylase activity. This same pathways was increased 5.5-fold in mice treated with phenobarbital, which confirms a previous report by Kelley *et al.* [33].

7-Alkoxyresorufin O-dealkylation by liver microsomes from loratadine-treated mice. The effects of treating mice with loratadine on the *O*-dealkylation of 7-pentoxy- and 7-ethoxyresorufin by liver microsomes are shown in Table 4. Loratadine treatment caused a dose-dependent increase in the *O*-dealkylation of 7-pentoxyresorufin. At the highest dosage tested, loratadine was less effective than phenobarbital as an inducer of 7-pentoxyresorufin *O*-dealkylation (3.3-fold vs a 13-fold increase). Loratadine did not cause a dose-dependent or statistically significant effect on 7-ethoxyresorufin *O*-dealkylase activity, which increased 1.6-fold in phenobarbital-treated mice. The lack of induction of 7-ethoxyresorufin *O*-dealkylase activity in mice treated with 3-methylcholanthrene is discussed below.

DISCUSSION

The results of the present study indicate that

* Lindberg RLP and Negishi M, Mouse testosterone 7α-hydroxylase (P450$_{7\alpha}$): Isolation, sequencing and expression of P450$_{7\alpha}$ cDNA and regulation by sex and dexamethasone in mouse Liver. Presented at the *VIIIth International Symposium on Microsomes and Drug Oxidations.* Abstract 278. Stockholm, Sweden, 1990.

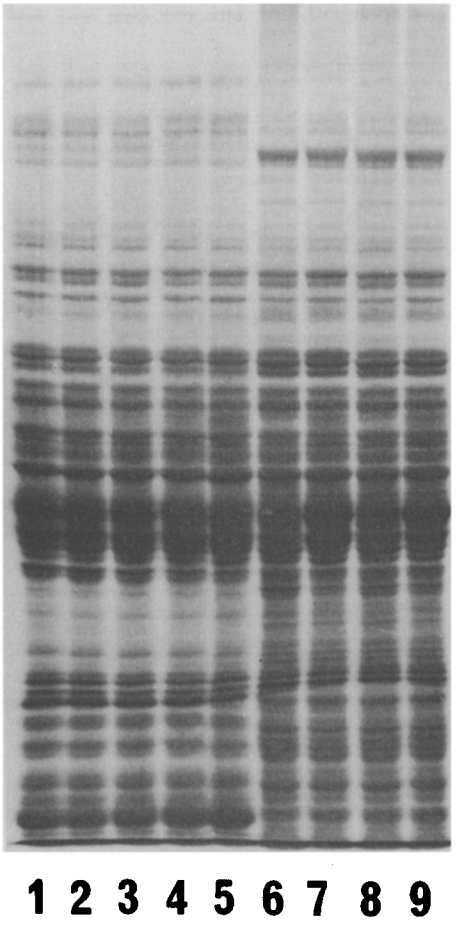

Fig. 4. SDS–polyacrylamide gel of liver microsomes from male mice treated with loratadine or known inducers of cytochrome P450. Liver microsomes (10 μg protein) from control and treated male mice were subjected to SDS–PAGE as described in Materials and Methods. Lanes 1–5 contained liver microsomes from CD-1 mice fed 0, 4, 12 or 40 mg/kg/day of loratadine or 85 mg/kg/day phenobarbital, respectively. Lanes 6–9 contained liver microsomes from CF-1 mice injected s.c. with corn oil (control), phenobarbital, 3-methylcholanthrene or pregnenolone-16α-carbonitrile, respectively.

loratadine is a phenobarbital-type inducer of cytochrome P450 in rats and mice. Treatment of male Sprague–Dawley rats with loratadine caused a dose-dependent increase in the levels of P450 2B1/2, as determined by immunoblotting, and an increase in the rate of testosterone 16β-hydroxylation and the *O*-dealkylation of 7-pentoxyresorufin. At the dosages tested, loratadine was less effective than phenobarbital as an inducer of 2B1/2. At the highest dosage tested (25 mg/kg/day), loratadine caused a 7.3- and 8.5-fold increase in the rate of testosterone 16β-hydroxylation and 7-pentoxyresorufin *O*-dealkylation, respectively, compared with a 22- and 45-fold increase caused by phenobarbital treatment (85 mg/kg/day). Like phenobarbital, loratadine caused a modest increase in testosterone 1β-, 2β-, 6β-, 15β- and 18-hydroxylase activity (as well as 6-dehydrotestosterone formation), which was associated with a similar increase in the levels of immunoreactive P450 3A1 and/or 3A2. At the dosages tested, treatment of rats with loratadine did not appear to increase 1A1 levels, although the highest dosage of loratadine caused a slight increase in the *O*-dealkylation of 7-ethoxyresorufin. The magnitude of this effect (<2-fold increase) suggests that the increase in 7-ethoxyresorufin *O*-dealkylation caused by loratadine was due to the induction of 2B1, which can catalyze the *O*-dealkylation of 7-ethoxyresorufin, albeit to a much lesser extent than 1A1 [12]. In contrast to phenobarbital, treatment of rats with loratadine did not decrease the rate of testosterone 2α-hydroxylation, which suggests that loratadine did not suppress the levels of P450 2C11. The induction of 2B1/2 by phenobarbital involves an increase in the levels of 2B1/2 mRNA due to increased transcription of the *2B1/2* genes [34]. Whether the induction of 2B1/2 by loratadine involves transcriptional activation of the *2B1/2* genes remains to be determined.

Treatment of male CD-1 mice with loratadine caused a dose-dependent increase in the levels of a microsomal protein(s) recognized by antibody against rat 2B1. This same protein was intensified to a greater extent by treatment of mice with phenobarbital. At the dosages tested, treatment of mice with loratadine caused no detectable induction of proteins immunochemically related to rat 3A1. Treatment of mice with loratadine did not appear to induce 1A1, but this result requires some qualification. CD-1 mice are derived from Swiss mice [35], which are *Ah*-non-responsive, i.e. they do not respond to treatment with polycyclic aromatic hydrocarbons by increasing the synthesis of P450 1A1 [36, 37]. Although not derived from Swiss mice [35], CF-1 mice also appear to be *Ah*-non-responsive, as indicated by the lack of induction of 1A1 in CF-1 mice treated with 3-methylcholanthrene (Fig. 5).

As in the rat, the induction of 2B enzymes in the mouse was associated with a large increase in 7-pentoxyresorufin *O*-dealkylase activity. However, in contrast to the rat, the induction of 2B enzymes in the mouse resulted in only a modest increase in testosterone 16β-hydroxylase activity. This species difference can be attributed mainly to the high rate of testosterone 16β-hydroxylation catalyzed by liver microsomes from untreated mice. The constitutive enzyme responsible for catalyzing this reaction in mouse liver microsomes does not appear to be a 2B enzyme because such enzymes could not be detected when immunoblots of liver microsomes from control mice were probed with *anti*-rat 2B1 (Fig. 5). Mouse liver microsomes also possess high testosterone 15α-hydroxylase activity, which is catalyzed by 2A4/5 [1, 38]. Rat liver microsomes contain a similar 15α-hydroxylating enzyme, namely 2A2. Although purified 2A2 as an effective catalyst of testosterone 15α-hydroxylation, the microsomal enzyme is not; hence, the 15α-hydroxylation of testosterone represents only a minor pathway of testosterone oxidation in rat liver microsomes [18, 24]. Another difference between mice and rats is that only rats express 2C11; hence, only rat liver microsomes

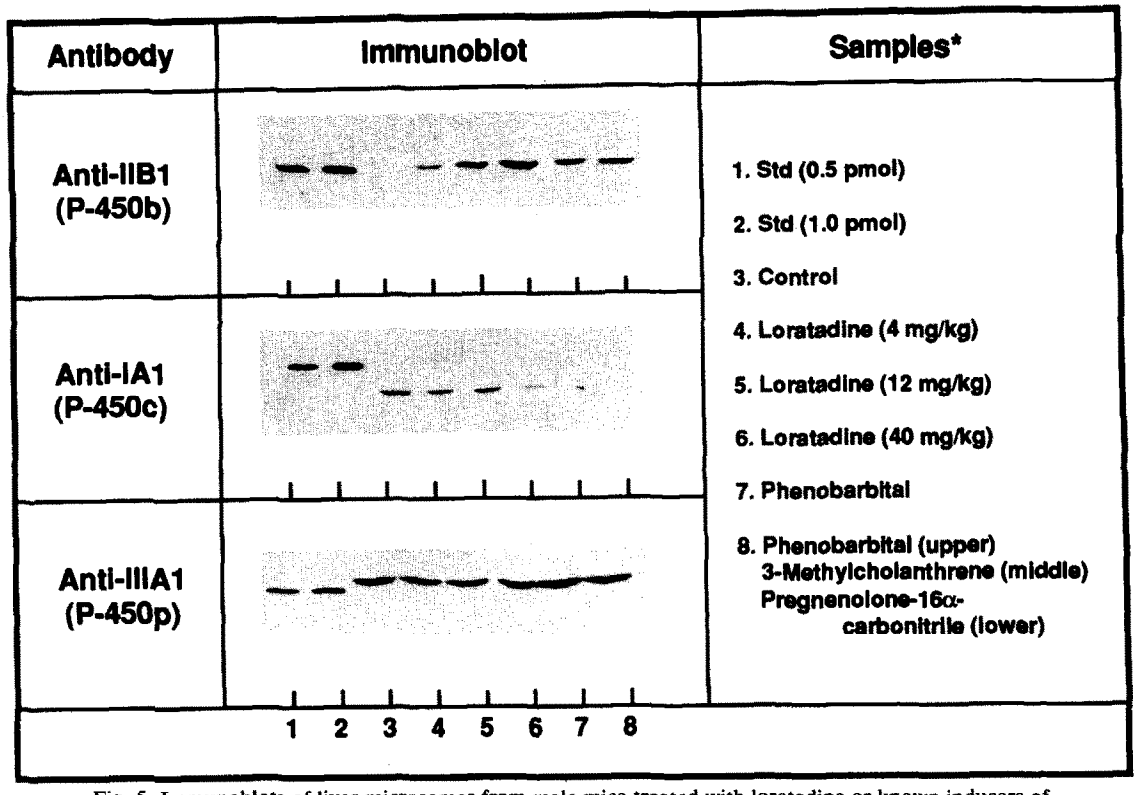

Fig. 5. Immunoblots of liver microsomes from male mice treated with loratadine or known inducers of cytochrome P450. Immunoblots of liver microsomes from control and treated male mice were probed with antibody against rat 2B1, 1A1 or 3A1, as described in Materials and Methods. In all cases, lanes 3–6 contained 10 μg of liver microsomal protein from CD-1 mice fed 0, 4, 12 or 40 mg/kg/day of loratadine. Lane 7 contained either 2 μg (upper panel) or 10 μg (middle and lower panels) of liver microsomal protein from mice fed 85 mg/kg/day phenobarbital. Lane 8 contained 2 μg of liver microsomal protein from CF-1 mice treated s.c. with phenobarbital (upper), 3-methylcholanthrene (middle) or pregnenolone-16α-carbonitrile (lower). The standards (Std) were samples of the purified rat P450 enzymes against which the antibodies were raised.

catalyze a high rate of conversion of testosterone to 2α-hydroxytestosterone, 16α-hydroxytestosterone and androstenedione.

In rats, loratadine caused statistically significant changes in liver microsomal testosterone oxidation at the highest dosage tested (25 mg/kg/day). At the lowest and intermediate dosages tested (i.e. 4 and 10 mg/kg/day), treatment of rats with loratadine had no statistically significant effects on the oxidation of testosterone or the *O*-dealkylation of 7-ethoxy- or 7-pentoxyresorufin by liver microsomes. In mice, loratadine caused statistically significant changes in liver microsomal testosterone oxidation and 7-pentoxyresorufin *O*-dealkylation at both the intermediate and highest dosage tested (12 and 40 mg/kg/day). At the lowest dosage tested (i.e. 4 mg/kg/day), treatment of mice with loratadine had no statistically significant effects on the oxidation of testosterone, but caused a statistically significant increase in the *O*-dealkylation of 7-pentoxyresorufin by liver microsomes. These results suggest that, as an inducer of liver microsomal cytochrome P450, the NOEL (no observable effect level) for loratadine is ~4 and 10 mg/kg/day in mice and rats, respectively.

The structural features that determine the ability of a chemical to induce P450 1A enzymes have been fairly well defined, whereas those that determine the ability of a chemical to induce P450 2B enzymes have not. Inducers of P450 1A enzymes tend to be planar compounds, such as polycyclic aromatic hydrocarbons and halogenated aromatic compounds. Among the polychlorinated biphenyls that induce P450 enzymes, those congeners that can adopt a co-planar conformation (i.e. those lacking *ortho*-substituents) are 3-ethylcholanthrene-type inducers of P450 1A enzymes, whereas those that are sterically hindered from adopting a co-planar conformation (i.e. those with two *ortho*-substituents) are phenobarbital-type inducers of P450 2B enzymes [4, 39]. Loratadine is a non-planar molecule because the two aromatic rings lie above the plane of the 7-membered rings whereas the piperidine ring lies below (these features are not readily apparent from the structure shown in Fig. 1). The propensity for P450 2B enzyme inducers to be non-planar compounds is evident from a partial list of such inducers: barbiturates, DDT, SFK 525A, *trans*-stilbene oxide and 1,4-bis[2-(3,5-dichloropyridyloxy)]-benzene (TCPOBOP) [40, 41].

Table 3. Testosterone oxidation by liver microsomes from male mice treated with loratadine

Treatment*	Testosterone oxidation† (pmol metabolite formed/mg protein/min)										
	1α/1β	2α	2β	6α	6β	7α	15α	15β	16α	16β	18
Control	198 ± 34	42 ± 4	192 ± 33	126 ± 20	3,020 ± 290	368 ± 137	356 ± 180	198 ± 35	305 ± 64	301 ± 35	104 ± 16
Loratadine (4 mg/kg/day)	186 ± 24	48 ± 4	186 ± 28	167 ± 26	3,040 ± 580	217 ± 73	333 ± 116	180 ± 16	355 ± 35	380 ± 40	103 ± 11
Loratadine (12 mg/kg/day)	196 ± 10	59 ± 5	189 ± 4	243 ± 35‡	2,940 ± 130	382 ± 119	395 ± 52	200 ± 20	382 ± 34	391 ± 25‡	103 ± 9
Loratadine (40 mg/kg/day)	204 ± 30	75 ± 7‡	197 ± 28	232 ± 67‡	3,260 ± 570	300 ± 151	286 ± 33	196 ± 34	481 ± 50‡	329 ± 78	101 ± 17
Phenobarbital (85 mg/kg/day)	434 ± 27‡	63 ± 6‡	393 ± 12‡	688 ± 55‡	5,880 ± 130‡	353 ± 98	587 ± 213	454 ± 63‡	510 ± 21‡	612 ± 19‡	177 ± 3‡
Control	182	41	173	141	2,580	438	332	195	368	455	81
Phenobarbital (75 mg/kg/day)	893	112	777	584	11,800	530	861	850	754	1,440	348
3-Methylcholanthrene (20 mg/kg/day)	172	36	161	141	2,470	470	304	172	326	414	80
Pregnenolone-16α-carbonitrile (75 mg/kg/day)	733	87	663	127	11,100	389	274	616	412	614	322

* Male CD-1 mice were treated for 2 weeks with loratadine (N = 4/group) or phenobarbital (N = 3) in the diet (top), or male CF-1 mice were injected s.c. for 3 days with corn oil (control), phenobarbital, 3-methylcholanthrene or pregnenolone-16α-carbonitrile (bottom), as described in Materials and Methods.

† Testosterone metabolites were resolved and quantified by HPLC. The abbreviations denote the hydroxytestosterone metabolite formed, e.g. 7α denotes 7α-hydroxytestosterone. Rates of formation of androstenedione and 6-dehydrotestosterone are not shown because these two metabolites were not consistently resolved by HPLC. Values are means ± SD.

‡ Statistically significant ($\alpha < 0.05$) by Dunnett's test.

Table 4. *O*-Dealkylation of 7-pentoxy- and 7-ethoxyresorufin by liver microsomes from male mice treated with loratadine

Treatment*	7-Pentoxyresorufin *O*-dealkylation† (pmol/mg protein/min)	7-Ethoxyresorufin *O*-dealkylation† (pmol/mg protein/min)
Control	30 ± 3	109 ± 16
Loratadine (4 mg/kg/day)	59 ± 7‡	119 ± 19
Loratadine (12 mg/kg/day)	74 ± 13‡	137 ± 28
Loratadine (40 mg/kg/day)	98 ± 7‡	125 ± 19
Phenobarbital (85 mg/kg/day)	400 ± 30‡	169 ± 17‡
Control	35	214
Phenobarbital (75 mg/kg/day)	681	468
3-Methylcholanthrene (20 mg/kg/day)	38	220
Pregnenolone-16α-carbonitrile (75 mg/kg/day)	65	245

* Male CD-1 mice were treated for 2 weeks with loratadine (N = 4/group) or phenobarbital (N = 3) in the diet (top), or male CF-1 mice were injected s.c. for 3 days with corn oil (control), phenobarbital, 3-methylcholanthrene or pregnenolone-16α-carbonitrile (bottom), as described in Materials and Methods.

† Values are means ± SD.

‡ Statistically significant ($\alpha < 0.05$) by Dunnett's test.

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