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Interactive Binding at Cytochrome P-450 of Cell Growth Regulatory Bioamines, Steroid Hormones, Antihormones, and Drugs

Frank S. LaBella,[1]* Gary M. Queen,[1] and Lorne J. Brandes[1,2,3]

[1]Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba R3E 0W3, Canada
[2]Department of Medicine, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba R3E 0W3, Canada
[3]Manitoba Institute of Cell Biology, Winnipeg, Manitoba R3E 0V9, Canada

Abstract The virtually universal family of P-450 isozymes contribute to the regulation of cell growth by modulating the levels of steroids and other lipid messengers for cytoplasmic and nuclear processes, including gene expression. In microsomes from rat liver cells, the concentration (~1 nmole/mg protein) of cytochromes P-450 approximates that of intracellular binding sites (K_d 1.0–50 μM) for histamine. The potencies of certain therapeutic drugs to inhibit catalytic activity of, and histamine binding to, cytochromes P-450 in vitro were previously shown by us to be predictive of relative propensities to modulate tumor growth in rodents. Also, we demonstrated that growth-regulating polyamines potently interact with histamine at P-450. We now show that several classes of steroid hormones, antiestrogens, and antiandrogens, as well as various arylalkylamine drugs, all potently inhibit ^{3}H-histamine binding to cytochrome P-450 (K_i values: testosterone 0.28 μM, progesterone 0.56 μM, flutamide 1.7 μM, tamoxifen 9.0 μM). Furthermore, all the various hormone and drug ligands are mutually inhibitory in their binding to cytochrome P-450; e.g., K_i values of androstenedione and progesterone, to inhibit imipramine binding to P-450 (determined by spectral analysis), are 11 nM and 26 nM, respectively. The K_i value of imiprimine to inhibit binding of androstenedione to P-450 is 3.5 μM. We estimate the total P-450 content in microsomes to be greater in male than in female rats and correlated with the number of binding sites for histamine, but not for steroids and drugs that appear to be more selective for P-450 isozymes. Thus, for at least some isozymes, the homeostatic role of the monooxygenases may be governed by histamine, modulated by endogenous ligands, and perturbed by many foreign molecules. J. Cell. Biochem. 76:686–694, 2000. © 2000 Wiley-Liss, Inc.

Key words: histamine; polyamines; cytochrome P-450; cell growth; cell proliferation

A cell regulatory role of the virtually universal cytochrome P-450 monooxygenases is manifested in the metabolic transformation of endogenous lipids, including steroid hormones, fatty acids, and the eicosanoids. As pointed out by Nebert [1991], P-450 enzymes control the levels of endogenous lipid mediators that, in turn, modulate gene function, including expression of P-450 isozymes themselves. Our previous findings linked the apparent growth-modulating effects of histamine (HA) with binding of that bioamine to intracellular sites in microsomes and nuclei of various tissues [Brandes and LaBella, 1993], sites subsequently shown to be identical, at least in large part, to cytochromes P-450 [LaBella et al., 1992]. Furthermore, we showed for certain arylalkylamine drugs, including antidepressants, antiestrogens, antiandrogens, and antihistamines, an excellent correlation among potencies to inhibit (1) binding of HA to cytochrome P-450, (2) cytochrome P-450-mediated aminopyrine metabolism, and (3) lymphocyte mitogenesis, with pro-

Abbreviations used: HA, histamine; AU, absorbance units; PA, polyamines; DPPE, N,N-diethyl-2-[4-(phenylmethyl) phenoxy]ethanamine-HCl; EDTA, ethylenediamine tetraacetic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

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*Correspondence to: Frank S. LaBella, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba R3E 0W3, Canada.

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pensities to enhance tumor growth in mice [Brandes et al., 1992, 1994; LaBella and Brandes, 1996]. More recently, we showed a potent interaction of the cell growth-regulating polyamines (spermine, spermidine, and putrescine) with HA at cytochromes P-450 [Brandes et al., 1998]. In this report, we demonstrate that various sex steroids and certain of their drug antagonists bind to the same P-450 isozyme(s) previously shown to have an affinity for bioamines and a variety of arylalkylamine medicinals. Thus, among the ubiquitous P-450 monooxygenases, particular isozymes may represent common intracellular targets for diverse arrays of growth-regulating compounds of both endogenous and exogenous origin.

MATERIALS AND METHODS

Materials

Buffer constituents were obtained from Sigma Chemical Co. (St. Louis, MO) and from Mallinckrodt (Anachemia Science, Montreal, Quebec). HA, spermine, imipramine, tamoxifen, hydroxyzine, testosterone, progesterone, β-estradiol, aldosterone, cortisone, cortisol, and prednisolone were purchased from Sigma. Androstenedione was purchased from Steroloids (Wilton, NH). DPPE was synthesized in this laboratory. ^{3}H-HA (28 Ci/mmol), ^{3}H-imipramine (46.5 Ci/mmol), and ^{3}H androstenedione (27.5 Ci/mmol) were obtained from Mandel (Dupont-New England Nuclear Research Products, Guelph, ON). Male Sprague-Dawley rats were obtained from the Central Animal Care Facility, University of Manitoba.

Preparation of Microsomes

Tris buffer (pH 8.5) was found to be optimal for the spectral measurement of HA binding to P-450. Thus, for comparison of binding and spectral data among HA and other competitors, we employed microsomes prepared in sucrose and washed in Tris. Fresh or frozen (−80°C) livers from (200–250 g) male or female Spague-Dawley rats were minced, suspended (10% wt/vol) in 0.25 M sucrose, homogenized with 6–8 passes in a motor-driven Teflon-pestle glass vessel and centrifuged (15 min, 13,000*g*, 4°C). The supernatant was decanted and centrifuged (95 min, 144,000*g*, 4°C). The resulting pellet was resuspended in 5 mM Tris (pH 8.5) at a final concentration of 4 mg protein per ml, centrifuged (144,000*g*, 35 min, 4°C), resuspended in Tris buffer (10–20 mg protein per ml), and stored at −80°C. EDTA (1 mM) was added to the suspension before radioligand binding assays. In studies comparing P-450 levels in male and female rats, animals of both sexes were decapitated and liver cell microsomes prepared simultaneously.

Spectral Analysis of Ligand/P-450 Complexes

The P-450 absorbance difference spectra of P-450-bound HA was measured [Jefcoate, 1978; Estabrook and Werringloer, 1978] with a Milton Roy Spectronic 3000 Array Spectrophotometer; a Rapidscan computer software program controlled and operated the unit, and collected and plotted the spectral data. To obtain spectra, microsomes were suspended in 5 mM Tris buffer (pH 8.5; 1.0 mg protein/ml) containing HA, androstenedione, or imipramine and incubated (40 min, 22°C) in the absence or presence of increasing concentrations (10^{-7}–10^{-3} M) of drugs or hormones added to both the reference and sample (all stock solutions adjusted to pH 8.5). Total P-450 content was determined by the method of Omura and Sato [1964]. Spectral data were analyzed with ENZYME, a curve-fitting program employing weighted nonlinear least-squares analysis, as described by Lutz et al. [1986]. One- and two-site EC_{50} values and one-site K_i values, based on the Cheng-Prusoff equation [Cheng and Prusoff, 1973] were calculated using the weighted nonlinear regression option of PRISM (Graphpad Software, San Diego, CA). Two-site K_i values were calculated from two-site spectral K_s values and PRISM-derived two-site EC_{50} values.

Extinction coefficients for the P-450-substrate complex have been reported for type I and II substrates determined in rat liver microsomes, purified P-450's (3A and 2B) and bacterial P-450's. An average extinction coefficient (70 mM^{-1} cm^{-1}) of seven values [Diehl et al., 1970; Estabrook and Werringloer, 1978; Jefcoate, 1978; Luu-The, 1980; Roos et al., 1993; Woerlingler, 1995] within a range of 45–110 mM^{-1} cm^{-1} was used to calculate B_{max} values from the maximal spectral changes observed (Table I). Except for the high-affinity HA site, where the spectral B_{max} is 17 times the radioligand B_{max}, all other values comprise radioligand binding at both P-450 and non-P-450 sites.

TABLE I. Binding Site Affinities and Densities for Histamine, Imipramine, and Androstenedione as Determined by Radioligand (^3H) and Spectral (spec) Methods*

	High			
	^3H	spec	^3H	spec
	K_{d1}	K_{s1}	$Bmax_1$	
	(μM)		(pmol/mg)	
Histamine	1.0 ± 0.50	5.8 ± 2.2	3.3 ± 2.3	57 ± 14
Imipramine	0.21 ± 0.02	0.082 ± 0.02	324 ± 35	43 ± 4.3
Androstenedione	0.46 ± 0.10	0.28 ± 0.16	163 ± 58	86 ± 43

	Low					
	^3H	spec	^3H	spec	^3H	spec
	K_{d2}	K_{s2}	$Bmax_2$		$Bmax_2/Bmax_1$	
	(μM)		(pmol/mg)			
Histamine	52 ± 17	181 ± 39	744 ± 298	586 ± 43	228 ± 251	10.3 ± 4.1
Imipramine	8.6 ± 5.8	13 ± 4.6	1,940 ± 1,400	86 ± 8.6	6.0 ± 4.9	2.0 ± 0.4
Androstenedione	4.1 ± 0.9	3.0 ± 0.8	985 ± 58	286 ± 43	6.0 ± 2.5	3.0 ± 1.7

*Values are means ± SE.

Radioligand Binding Analysis

Aliquots (0.8 ml) of microsomal suspension (1.25 mg protein/ml) in Tris buffer (pH 8.5) were added to 1.5-ml Eppendorf tubes containing either 5 nM ^3H-HA, ^3H-androstenedione or ^3H-imipramine in the presence or absence of increasing concentrations (10^{-9}–10^{-4} M) of unlabeled ligand or other competitors (1-ml total volume). After incubation (40 min, 22°C), the tubes were placed in an ice bath for 10 min; twenty μl of 0.5 M $MgCl_2$ were added to promote protein aggregation after which the tubes were centrifuged (10,000*g*, 15 min, 4°C). Each pellet was dissolved in NaOH and radioactivity determined in a Beckman liquid scintillation counter. Data were analyzed using the LIGAND program [Munson and Rodbard, 1980].

RESULTS

Inhibition of HA Binding by Steroids

Radioligand binding. Two histamine sites of high (K_{d1} 1.0 ± 0.5 μM) and intermediate (K_{d2} 52 ± 17 μM) affinity, representing HA binding to monooxygenases [Brandes et al., 1998], were identified in male rat liver microsomes (Table II). Concentration inhibition curves for steroids on ^3H-HA binding to microsomes are shown in Figure 1; K_i values for steroids, steroid antagonists, and arylalkylamine drugs are presented in Table II. K_i values for the sex steroids at the high-affinity histamine site ranged from 0.28 ± 0.15 μM for testosterone, to 0.96 ± 0.44 μM for β-estradiol.

TABLE II. Inhibition by Various Compounds of the Binding of ^3H-HA* to Microsomes

Steroid	Ki_1 (μM)**	Ki_2 (μM)**
Testosterone	0.28 ± 0.15	25 ± 3.1
Androstenedione	0.49 ± 0.23	51 ± 4.8
Progesterone	0.56 ± 0.25	57 ± 5.2
β-Estradiol	0.96 ± 0.44	82 ± 9.1
α-Estradiol	1.43 ± 0.67	195 ± 30
Aldosterone	1.61 ± 0.52	343 ± 49
Cortisone	9.6 ± 4.8	292 ± 52
Cortisol	16 ± 8.2	1,150 ± 593
Prednisolone	62 ± 50	>7,000
Drug		
Bupropion	0.20 ± 0.10	81 ± 11
Diethylstilbestrol	0.28 ± 0.15	34 ± 2.8
Imipramine	0.35 ± 0.12	73 ± 8
Clorgyline	0.42 ± 0.19	52 ± 5
Chlorpromazine	0.46 ± 0.20	61 ± 5
Flutamide	1.7 ± 0.53	127 ± 15
Nitromifen	2.5 ± 1.1	117 ± 11
Tamoxifen	9.0 ± 4.4	155 ± 19

*Kd_1 = 1.0 ± 0.50 μM; Kd_2 = 52 ± 17 μM.
**Mean ± SE.

The synthetic estrogen, diethylstilbestrol (DES), was equipotent to testosterone. Aldosterone (K_{i1} 1.61 ± 0.52 μM) was relatively potent, whereas the glucocorticoids, cortisol, cortisone, and prednisolone, were least potent (K_i range 9.6–62 μM). The psychotropic drugs, which include antidepressants, antipsychotics, and monoamine oxidase inhibitors, were as potent (K_i

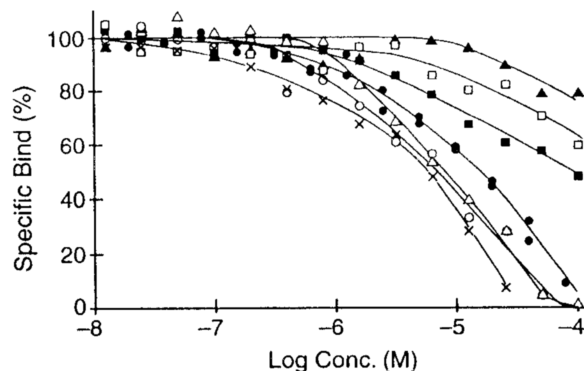

Fig. 1. Inhibition of [^{3}H]-histamine binding in male rat liver microsomes by histamine (●), prednisolone (▲), cortisol (□), aldosterone (■), β-estradiol (△), progesterone (○), and testosterone (x).

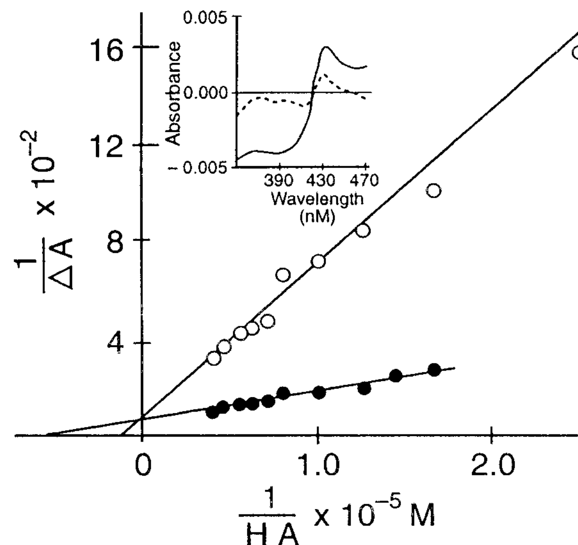

Fig.2. Inhibition by progesterone of the HA/P-450 complex determined from absorbance-difference spectra. Lineweaver-Burke plot: Concentration range of HA, (4–25 uM) (●), HA + progesterone (10 uM) (○). Inset: Absorbance-difference spectra HA (18 um) (—), HA (18 um) + progesterone (10 uM) (----).

range 0.2–0.46 µM) as the sex steroids, whereas the nonsteroidal antihormones, tamoxifen, flutamide, and nitromifen, (K_i range 1.7–9.0 µM) were less potent.

Spectral analysis. In reasonable agreement with the radioligand binding studies, spectral analysis showed two binding sites for HA in male rat liver microsomes (K_{s1} 5.8 ± 2.2 µM; K_{s2} 181 ± 40 µM) (Table I). Direct confirmation of the nature of the HA/steroid interaction resulted from the inhibitory effect of the steroids on the absorbance-difference spectrum generated by addition of HA to microsomes. Progesterone, a type I compound (binding to substrate site), competitively inhibited the binding of HA, a type II compound (binding to the heme iron), on at least one species of P-450 isozyme (Fig. 2, inset). At nonsaturating concentrations of HA, the higher affinity HA site sequestered proportionally more of the amine than did the lower affinity site; for example, at a concentration of 25 µM, 74 % of bound HA was associated with the high-affinity site. Under these conditions, progesterone (10 µM) inhibited HA binding by 75%, suggesting that this sex steroid competes selectively for the high-affinity HA site (K_i progesterone 2.5 ± 0.19 µM) (Fig. 2).

Binding of Steroids, Drugs, and HA to P-450: Mutual Inhibition

Radioligand binding and spectral assays revealed two binding sites for both the steroid, ^{3}H-androstenedione and the arylalkylamine, ^{3}H-imipramine; K_s values for both compounds, determined spectrally, were similar to the K_d values determined by radioligand studies (Table I). Affinity constants were determined from the spectral data: for androstendione, K_{s1} 0.28 ± 0.16 µM; K_{s2} 3.0 ± 0.80 µM, (Fig. 3A); for β-estradiol, K_{s1} 0.20 ± 0.07 µM; K_{s2} 5.05 ± 1.91 µM (Fig. 3B); for imipramine (spectra not shown), K_{s1} 0.082 ± 0.02 µM; K_{s2} 13 ± 4.6 µM.

Both androstenedione and imipramine were displaced by other type I compounds: the drug array inhibited both the binding of ^{3}H-imipramine (not shown) and of ^{3}H-androstenedione (Fig. 4, Table III) and of the P-450 /androstenedione complex determined spectrally (Fig. 5). Spectral analysis of the inhibition of imipramine (2 µM) binding to P-450 by drugs and steroids (Table IV and Fig. 6A,B) indicated that a one-site K_i value best fit the interaction with hydroxyzine, tamoxifen, DPPE or HA, whereas a two-site K_i value best fit the interaction with the steroids ($P < 0.0001$). Spectral analysis of the inhibition of androstenedione (1 µM) binding to P-450 by drugs and steroids (Table IV and Fig. 5) indicated competition at only the high-affinity (K_{s1} 0.28 ± 0.16 µM) site. The high-affinity imipramine/P-450 complex was most potently inhibited by androstenedione (11 nM), DPPE (26 nM) and progesterone (26 nM) (Table III), whereas the potencies of imipramine and other competing ligands at the high-affinity androstenedione site all were in the micromolar range (Table IV), suggesting that androstenedione and imipramine may not bind to the same P-450 isozyme or to identical sites

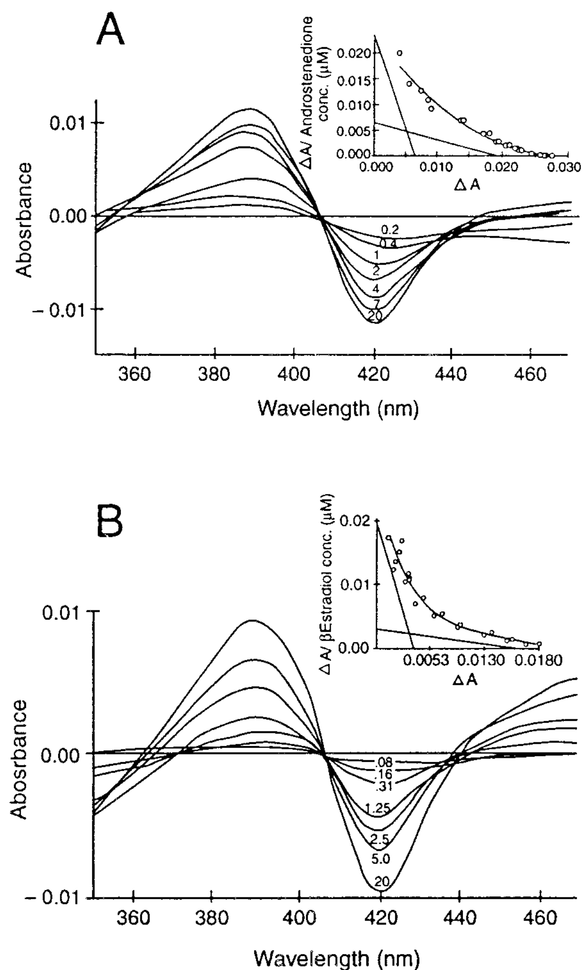

Fig. 3. Absorbance-difference spectrum and Scatchard plot (inset) for binding of (**A**) androstenedione, and (**B**) β-estradiol to P-450 in male rat liver microsomes. Values shown represent concentrations (μM) of ligand.

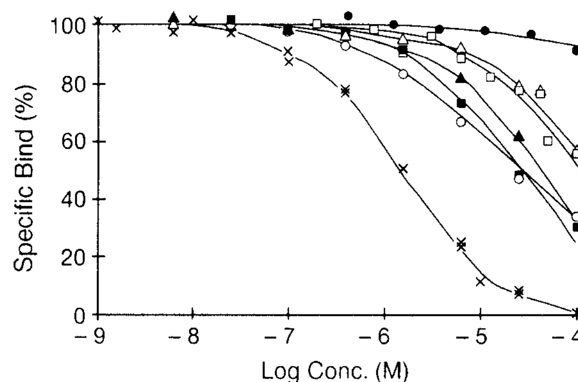

Fig. 4. Inhibition of [^{3}H]-androstenedione binding in male rat liver microsomes by androstenedione (x), histamine (●), DPPE (△), spermine (□), tamoxifen (▲), imipramine (■), and hydroxyzine (○).

TABLE III. Inhibition by Various Ligands of Binding of Androstenedione

	Radioligand assay*		Spectral analysis**
Ligand	Ki_1 (μM)	Ki_2 (μM)	Ki (μM)
Imipramine	5.4 ± 0.74†	134 ± 19	3.5 (2.5–5.0)‡
β-Estradiol	ND	ND	3.7 (2.8–4.9)
Hydroxyzine	3.6 ± 0.40	230 ± 33	8.2 (6.7–9.5)
Tamoxifen	15 ± 2.4	87 ± 12	12 (7.9–17)
DPPE	40 ± 5.2	591 ± 210	39 (31–51)
Cortisol	ND	ND	86 (56–131)
Spermine	13 ± 5.5	235 ± 56	214 (156–295)

ND, not determined.
*Androstenedione (5 nm): Kd_1 = 0.46 ± 0.10 μM; Kd_2 = 4.1 ± 0.9 μM.
**Androstenedione (1 μM): Ks_1 = 0.28 ± 0.16 μM; Ks_2 = 3.0 ± 0.8 μM.
†Mean ± SE.
‡95% confidence limits.

on the same P-450 . Except for spermine, there was a good correlation between K_i values to inhibit androstenedione binding in the spectral and radioligand assays (Table IV).

Comparison of Ligand Binding Site Affinities and Densities in Liver Microsomes From Male and Female Rats: Evidence for CYP 3A2 as the Major Interactive Site

Total P-450 Content. The interaction of a wide variety of arylalkylamine drugs, bioamines and steroids at P-450 suggested to us that CYP 3A2 may be the specific target isozyme [Schuetz et al., 1996]. Moreover, livers from male rats contain significantly more 3A2 than those of female rats [Levin and Thomas, 1996]. We estimated that the total P-450 content of microsomes was 1.22 ± 0.05 greater (0.69 ± 0.04 vs 0.57 ± 0.02 nmoles/mg protein) in males than in females, as determined by carbon monoxide difference spectra, a ratio much less than that reported for the 3A2 isozyme alone.

Binding Sites for HA. Radioligand studies, at concentrations of HA ranging from 5 nM to 78 μM, demonstrated that the combined number of high- and low-affinity HA binding sites in male rat liver microsomes was 0.74 nmol/mg protein (Table I), approximating the total P-450 content. Absorbance difference spectra showed two binding sites for HA in both male and female rat livers; no significant sex differences in densities or affinities of HA sites were observed although binding affinities at both HA sites tended to be greater in males (data not

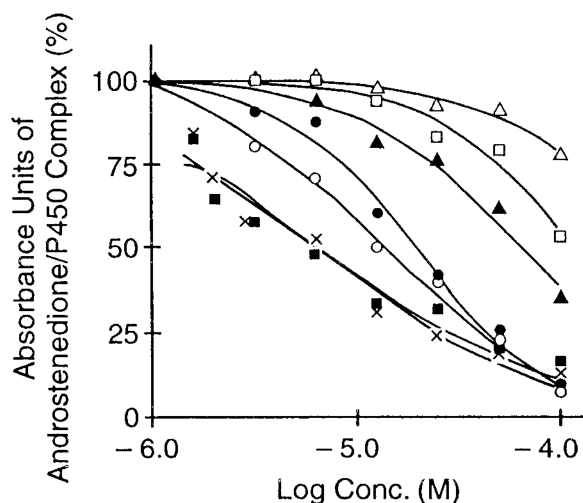

Fig. 5. Inhibition of androstenedione binding to cytochrome P-450 in male rat liver microsomes, determined by difference spectra, by spermine (△), cortisol (□), DPPE (▲), tamoxifen (●), hydroxyzine (○), imipramine (■), and β-estradiol (x).

TABLE IV. Inhibition by Various Ligands of Binding of Imipramine* to Cytochrome P-450 as Determined by Absorbance-Difference Spectrometry

Ligand	Imipramine Ki_1 (μM)	Imipramine Ki_2 (μM)
Hydroxyzine	2.4 (2.1–2.8)**	—
Androstenedione	0.011 (0.005–0.025)	221 (163–299)
Tamoxifen	5.6 (3.3–9.4)	—
β-Estradiol	0.27 (0.14–0.52)	1,648 (834–3260)
Progesterone	0.026 (0.011–0.060)	358 (226–568)
DPPE	0.026 ± 0.006†	—
Histamine	28 (24–32)	—

*Imipramine (2 μM): Ks_1 = 0.16 ± 0.02 μM; Ks_2 = 12 ± 2.6 μM.
**95% Confidence limits.
†Determined by inhibition of 0.2–3.0 μM imipramine by 30 nM DPPE, Lineweaver-Burke analysis, mean ± SE.

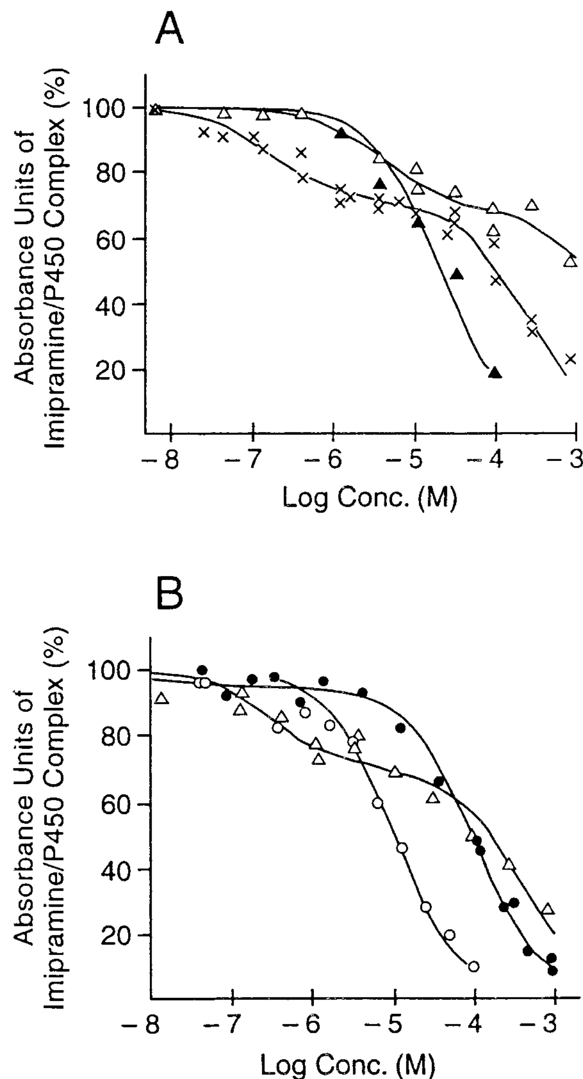

Fig. 6. Inhibition of imipramine binding to cytochrome P-450 (difference spectra) in male rat liver microsomes by (**A**) androstenedione (▼), β-estradiol (△) and tamoxifen (x); and (**B**), progesterone (△), hydroxyzine (○), and histamine (●).

shown). In addition, the absorbance differences generated by equivalent doses of HA from microsomes of male rats were 1.9 ± 0.3 times that of female rats (data not shown), reflecting higher-affinity interactions due to the different P-450 makeup in the male and the higher total P-450 content in males compared to females.

Binding Sites for Drugs and Steroids. Binding to P-450 of androstenedione, β-estradiol, imipramine, and hydroxyzine, as determined spectrally, showed that the aggregate male/female ratio (2.45 ± 1.1; range 1.3–3.5) of absorbance differences (which reflect both binding capacity and binding affinity) generated by these compounds is greater than the male/female ratio of total P-450 (1.22 ± .05), indicating a gender difference in the P-450 isozyme profile. The male/female ratio of absorbance differences, over a wide range of concentrations (Fig. 7), reflected greater binding of three of the four ligands in male rat microsomes.

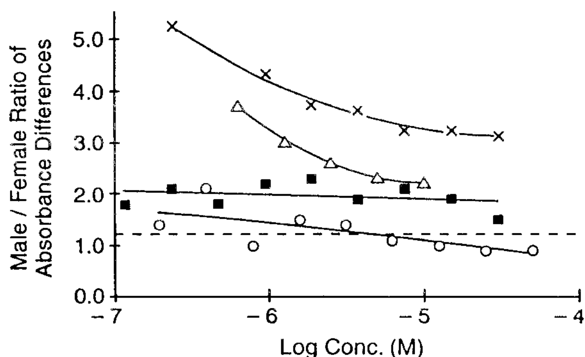

Fig. 7. Comparison of absorbance values (△A) in male and female rat liver microsomes for androstenedione (X), β-estradiol (△), imipramine (■) and hydroxyzine (○). Values were obtained from absorbance-difference spectra (see under Methods). Conc P-450 in males/conc P-450 in females (----). Binding capacity and affinity each are reflected in absorbance values. For androstenedione and β-estradiol, the male/female ratios (3.8 ± 2.7 and 3.0 ± 0.6, respectively) reflect a combination of greater binding affinity and capacity at P-450 sites in male rat liver microsomes (data not shown). For imipramine, the male/female ratio (2.0 ± 0.2) reflects a twofold higher capacity of the high-affinity P-450 sites in the male (data not shown), and for hydroxyzine, the male/female ratio (1.3 ± 0.4) reflects an eightfold greater affinity for the high-affinity P-450 sites in the male (data not shown).

DISCUSSION

The present results show that a diverse array of natural and synthetic hormones and arylalkylamine drugs, including antiestrogens, antiandrogens, antihistamines, phenothiazines, and antidepressants, compete with HA and with each other for binding to cytochromes P-450. On the basis of the known isozyme profile of P-450-mediated transformation of specific steroids and drugs, and of the observed male/female differences in total P-450 content, binding capacity, and/or affinity for the various ligands, CYP 3A2 in the rat appears to be one of the common isozyme targets for this multipartite interaction. CYP 3A2 in the rat, or its ortholog, CYP 3A4 in the human [Buetler et al., 1996], appears to make up a major portion of the P-450 complement in liver and gut endothelial cell microsomes [Kolars et al., 1994; Shimada et al., 1994] and, characteristically, metabolizes a large variety of hormones [Yamazaki and Shimada, 1997] and xenobiotics [Guengerich, 1994], including arylalkylamines, among them tamoxifen [Crewe et al., 1997], various antihistamines [Yumibe et al., 1996] and neuroleptic drugs [Shen, 1995]. CYP 3A4 also metabolizes many cytotoxic agents [Kivisto et al., 1995].

Another important function of the P-450 monooxygenases is to control the levels of small oxygenated species of molecules, including lipids and eicosanoids that, through modulating gene function, affect important cell processes such as proliferation [Nebert, 1991]. Previously, we suggested that, through its binding to the heme iron [LaBella et al., 1992], HA mediates growth by regulating the catalytic activity of P-450 enzymes [Brandes et al., 1994; LaBella and Brandes, 1996; Brandes et al., 1998]. Further buttressing this hypothesis is the demonstration, now, that total P-450 content approximates the total number of HA sites and that, in addition to the previously described interaction at P-450 between HA and polyamines [Brandes et al., 1998], both natural and synthetic growth-regulatory hormones, as well as their drug antagonists, potently inhibit HA binding to P-450 in rat liver microsomes. HA apparently is naturally present in hepatocytes; we observed that nuclei purified from rat liver cells contain a pool of HA that becomes depleted in parallel with a rise in DNA synthesis in the first 24 h after partial hepatectomy [Brandes et al., 1992]. In unpublished studies, we have obtained evidence in rat liver microsomes for the in situ presence of HA in the heme pocket of P-450 enzymes.

A common interaction at P-450 of HA, various arylalkylamine drugs and hormones may be biologically relevant in nonhepatic tissues. First, DPPE, an arylalkylamine tamoxifen analogue that does not bind to the estrogen receptor [Brandes and Hermonat, 1984], potently antagonizes ^{3}H-HA binding in microsomes [Brandes and LaBella, 1993], and inhibits both estradiol-induced uterine growth in immature oophorectomized female rats [Brandes and Hogg, 1990] and cortisol secretion in male rats subjected to cold-restraint stress [Glavin and Brandes, 1988]. Second, flutamide, an androgen receptor antagonist, also inhibits both ^{3}H-HA binding in microsomes (Table I) and P-450-mediated metabolism of testosterone in prostate tissue [Resnikov and Korpacheva, 1990].

Although concentrated mainly in liver, cytochromes P-450 are virtually universal; the isozyme profile is characteristic of cell type, functional state and the influence of chemical modulators, both endogenous and exogenous. Induction of the monooxygenases is generally recognized as an indicator of potential carcino-

genesis [Guengerich, 1988] or tumor promotion [Lucier, 1992]. For example, an association between induction of CYP 2B and promotion of experimental cancer growth has been shown for barbiturates, hydantoins, oxazolidinediones, and hexachlorocyclohexanes [Rice et al., 1994; Diwan et al., 1993]. CYP 1A is implicated in cell mitosis and proliferation in general and is responsible for activating 90% of those chemicals metabolically transformed to carcinogens [Bartsch et al., 1991]. A general feature of hepatocarcinogenesis in mice is overexpression of CYP 2A5 [Jounaidi et al., 1994]. Apart from their metabolism of chemicals, P-450 enzymes also metabolize steroid hormones and arachidonic acid (AA) to intermediates that modulate gene function, both directly and indirectly. A wide variety of AA metabolites generated by lipoxygenases and cyclooxygenases also undergoes further transformation by P-450 monooxygenases [Capdevila et al., 1992]. Like hormonal products derived from monooxygenase activity, P-450 metabolites of AA, similarly, are implicated in growth responses, including the stimulation of mitogenesis, for example, in mesangial [Harris et al., 1990; Sellmayer et al., 1991] and smooth muscle cells [Graber et al., 1997; Uddin et al., 1998].

In summary, we have demonstrated that a host of endogenous and exogenous growth-regulatory substances, including bioamines, steroid hormones, anti-hormones, and drugs, bind interactively to P-450 enzymes. One isozyme with which at least certain of these agents may interact is tentatively identified as CYP 3A2, established in the rat as a major monooxygenase with respect to both its abundance and wide ranging affinity for endogenous and exogenous compounds. Common to all the ligands is antagonism of the binding of HA to the heme moiety of the enzyme, an action which may perturb steady-state levels of P-450-generated lipids and eicosanoids involved in cell-growth regulation [LaBella and Brandes, 1996].

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REFERENCES

Bartsch H, Petruzzelli S, De Flora S, Hietanen E, Camus A-M, Castegnaro M, Geneste O, Camoirano A, Saracci R, Guintini C. 1991. Carcinogen metabolism and DNA adducts in human lung tissues as affected by tobacco smoking or metabolic phenotype: a case-control study on lung cancer patients. Mutat Res 250:103–114.

Brandes LJ, Hermonat MW. 1984. A diphenylmethane derivative specific for the antiestrogen binding site in rat liver microsomes. Biochem Biophys Res Commun 123: 724–728.

Brandes LJ, Hogg GR. 1990. Study of the in vivo antioestrogenic action of N,N-diethyl-2-[4-(phenylmethyl)phenoxy] ethamine HCl (DPPE), a novel histamine antagonist and antioestrogen binding site ligand. J Reprod Fertil 89: 59–67.

Brandes LJ, LaBella FS. 1993. Identification of intracellular histamine receptors (H_{IC}) that regulate cell proliferation. Adv Biosci 89:31–41.

Brandes LJ, Bogdanovic RP, Tong J, Davie JR, LaBella FS. 1992. Intracellular histamine and liver regeneration: high-affinity binding of histamine to chromatin, low affinity binding to matrix, and depletion of a nuclear storage pool after partial hepatectomy. Biochem Biophys Res Commun 184:840–847.

Brandes LJ, Arron RJ, Bogdanovic RP, Tong J, Zaborniak CLF, Hogg GR, Warrington RC, Fang W, LaBella FS. 1992. Stimulation of malignant growth in rodents by antidepressant drugs at clinically relevant doses. Cancer Res 52:3796–3800.

Brandes LJ, Warrington RC, Arron RJ, Bogdanovic RP, Fang W, Queen G, Stein D, Tong J, Zaborniak CLF, LaBella FS. 1994. Enhanced cancer growth in mice administered daily human-equivalent doses of some H^1-antihistamines: predictive in vitro correlates. J Natl Cancer Inst 86:770–775.

Brandes LJ, Queen GM, LaBella FS. 1998. Potent interaction of histamine and polyamines at microsomal cytochrome P450, nuclei, and chromatin from rat hepatocytes. J Cell Biochem 68:1–11.

Buetler TH, Bammler TK, Hayes JD, Eaton DL. 1996. Oltiproz-mediated changes in aflatoxin B(1) biotransformation in rat liver: implications for human chemointervention. Cancer Res 56:2306–2313.

Capdevila JH, Falck JR, Estabrook W. 1992. Cytochrome P450 and the arachidonate cascade. FASEB J 6:731–736.

Cheng Y, Prusoff WH. 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. Biochem Pharmacol 23:3099–3108.

Crewe HK, Ellis SW, Leonard MS, Tucker GT. 1997. Variable contribution of cytochromes 2D6, 2C9 and 3A4 to the 4-hydroxylation of tamoxifen by human liver microsomes. Biochem Pharmacol 53:171–178.

Diehl H, Schadekin J, Ullrich V. 1970. Studies on kinetics of cytochrome P-450 reduction in rat liver microsomes. Hoppe-Seylers Z Physiol Chem 351:1359–1371.

Diwan BA, Henneman JR, Nims RW, Rice JM. 1993. Tumor promotion by an anticonvulsant agent, phenytoin, in mouse liver: correlation with CYP2B induction. Carcinogenesis 14:2227–2231.

Estabrook RW, Werringloer J. 1978. The measurement of difference spectra: application to the cytochromes of microsomes. Methods Enzymol 52:212–220.

Glavin GB, Brandes LJ. 1988. Antiulcerogenic and antisecretory effects of a novel diphenylmethane derivative and antiestrogen binding site ligand. Can J Physiol Pharmacol 66:1139–1143.

Graber MN, Alfonso A, Gill DL. 1997. Recovery of Ca^{2+} pools and growth in Ca^{2+} pool-depleted cells is mediated by specific epoxyeicosatrienoic acids derived from arachidonic acid. J Biol Chem 272:29546–29553.

Guengerich FP. 1988. Roles of cytochrome P450 enzymes in chemical carcinogenesis and cancer chemotherapy. Cancer Res 48:2946–2954.

Guengerich FP. 1994. Catalytic selectivity of human cytochrome P450 enzymes: relevance to drug metabolism and toxicity. Toxicol Lett 70:133–138.

Harris RC, Homma T, Jacobson HR, Capdevilla J. 1990. Epoxyeicosatrienoic acids activate Na^+/H^+ exchange and are mitogenic in cultured rat glomerular mesangial cells. J Cell Physiol 144:429–437.

Jefcoate CR. 1978. Measurement of substrate and inhibitor binding to microsomal cytochrome P450 by optical-difference spectroscopy. Methods Enzymol 52:258–279.

Jounaidi Y, Bonfils C, Perin F, Negishi M, Lange R. 1994. Overexpression of a cytochrome P-450 of the 2A family (CYP2A-5) in chemically induced hepatomas from female mice. Eur J Biochem 219:791–798.

Kivisto KT, Kroemer HK, Eichelbaum M. 1995. The role of human cytochrome P450 enzymes in the metabolism of anticancer agents: implications for drug interactions. Br J Clin Pharmacol 40:523–530.

Kolars JC, Lown KS, Schmiedlin-Ren P, Ghosh M, Fang C, Wrighton SA, Merion RM, Watkins PB. 1994. CYP 3A gene expression in human gut epithelium. Pharmacogenetics 4:247–259.

LaBella FS, Queen G, Glavin G, Durant G, Stein D, Brandes LJ. 1992. ^{3}H receptor antagonist, thioperamide, inhibits adrenal steroidogenesis and histamine binding to adrenocortical microsomes and binds to cytochrome P450. Br J Pharmacol 107:161–164.

LaBella FS, Brandes LJ. 1996. Enhancement of tumor growth by drugs with some common molecular actions. Mol Carcinogen 16:68–76.

Levin W, Thomas PE. 1996. Induction of the male-specific cytochrome P450 3A2 in female rats by phenytoin. Arch Biochem Biophys 332:153–162.

Lucier GW. 1992. Receptor mediated carcinogenesis. In: Vainio H. Magee PN, McGregor DB, McMichael AJ, editors. Mechanisms of carcinogenesis in risk identification. Lyon: International Agency for Research in Cancer. p 87–112.

Lutz RA, Bull C, Rodbard D. 1986. Computer analyses of enzyme-substrate-inhibitor kinetic data with automatic model selection using IBM-PC compatible microcomputers. Enzyme 36:197–211.

Luu-The V, Cumps J, Dumont P. 1980. Metyrapone-reduced cytochrome P450 complex: A specific method for the determination of the phenobarbital inducible form of rat hepatic microsomal cytochrome P450. BBRC 93:776–781.

Munson RJ, Rodbard D. 1980. LIGAND: a versatile computerized approach for characterization of ligand binding systems. Anal Biochem 107:220–239.

Nebert DW. 1991. Proposed role of drug-metabolizing enzymes: regulation of steady state levels of the ligands that affect growth, homeostasis, differentiation and neuroendocrine functions. Mol Endocrinol 5:1203–1214.

Omura T, Sato R. 1964. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 239:2370–2378.

Resnikov A, Korpacheva T. 1990. Nonsteroid antiandrogen inhibiting effect on testosterone metabolism in rat prostate and liver. Endocrinol Exp 24:437–447.

Rice JM, Diwan BA, Hu H, Ward JM, Nims RW, Lubet RA. 1994. Enhancement of hepatocarcinogenesis and induction of specific cytochrome P450-dependent monooxygenase activities by the barbiturates allobarbital, aprobarbital, pentobarbital, secobarbital and 5-phenyl- and 5-ethylbarbituric acids. Carcinogenesis 15:395–402.

Roos PH, Golub-Ciosk B, Kallweit P, Kauczinski D, Hanstein WG. 1993. Formation of ligand and metabolite complexes as a means for selective quantitation of cytochrome P450 isozymes. Biochem Pharmacol 45:2239–2250.

Schuetz EG, Beck WT, Schuetz JD. 1996. Modulators and substrates of p-glycoprotein and cytochrome P450 3A coordinately up-regulate these proteins in human colon carcinoma cells. Mol Pharmacol 49:311–318.

Sellmayer A, Uedelhoven WM, Weber PC, Bonventre JV. 1991. Endogenous non-cyclooxygenase metabolites of arachidonic acid modulate growth and mRNA levels of immediate-early response genes in rat mesangial cells. J Biol Chem 266:3800–3807.

Shen WW. 1995. Cytochrome P450 monooxygenases and interactions of psychotropic drugs: a five year update. Int J Psychiatry Med 25:277–290.

Shimada T, Yamazaki H, Himura M, Invi Y, Guengerich FP. 1994. Inter-individual variations in human liver cytochromes P450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 270:414–423.

Uddin MR, Muthalif MM, Karzoun NA, Benter IF, Malik KU. 1998. Cytochrome P450 metabolites mediate norepinephrine-induced mitogenic signaling. Hypertension (1 pt 2):242–247.

Werringloer, Estabrook RW. 1975. Heterogeneity of liver microsomal cytochrome P450: The spectral characterization of reactants with reduced cytochrome P450. Arch Biochem Biophys 167:270–286.

Yamazaki H, Shimada T. 1997. Progesterone and testosterone hydroxylation by cytochrome P450 2C19, 2C9 and 3A4 in human liver microsomes. Arch Biochem Biophys 346:161–169.

Yumibe N, Huie K, Chen KJ, Snow M, Clement RP, Coyen MN. 1996. Identification of human liver cytochrome P450 enzymes that metabolize the nonsedating antihistamine loratadine. Formation of descarboethoxyloratadine by CYP 3A4 and CYP 2D6. Biochem Pharmacol 51:165–172.