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Hormonal Regulation of Microsomal Flavin-Containing Monooxygenase: Tissue-Dependent Expression and Substrate Specificity

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SUMMARY

The substrate- and tissue-dependent hormonal regulation of flavin-containing monooxygenase (EC 1.14.13.8) was studied in male and female rats. Hypophysectomy of males reduced liver microsomal *N,N*-dimethylaniline *N*-oxidation, thiobenzamide *S*-oxidation, and imipramine *N*-oxidation, although the reduction was not as marked with the latter substrate. Castration also reduced flavin-containing monooxygenase-dependent activities, but not to the same extent as hypophysectomy. Administration of growth hormone or testosterone to hypophysectomized males only partially restored basal activities. In female rats, hypophysectomy had no effect on *N,N*-dimethylaniline *N*-oxidation or thiobenzamide *S*-oxidation and actually stimulated imipramine *N*-oxidation (98%). These effects were demonstrated to be tissue- and sex-dependent. For example, hypophysectomy markedly (300%) enhanced imipramine *N*-oxidation in male kidney and significantly decreased the same activity in male and female lung. Correlations between levels of the enzyme determined by immunoquantitation (with antibody to the rat liver enzyme) and activities toward these three substrates, in male and female liver, lung, and kidney, also provide evidence for the existence of multiple forms of flavin-containing monooxygenase, which appear to be under different hormonal regulation.

Most of the investigations devoted to the oxidative metabolism of xenobiotics by animals and humans implicate the microsomal heme-containing cytochrome P-450 system. However, an increasing number of reports have established the role of the FMO in the *N*- and *S*-oxygenation of numerous xenobiotics, including carcinogens, drugs, and pesticides (reviewed in Ref. 1). FMO, like cytochrome P-450, is widely distributed in mammalian tissues but is present at a high concentration in the endoplasmic reticulum of liver, kidney, and lung (reviewed in Ref. 2). One well studied characteristic of the cytochrome P-450 system is its inducibility by exogenous chemicals but, to date, studies have shown FMO to be refractory to such treatment. However, previous studies have shown that FMO activities could be modulated by endogenous factors, but the picture is rather confusing. Sex differences in the concentration or enzymatic activity of FMO have been observed in rats, mice, and rabbits (3). Sex-related differences in mouse liver microsomal activity appear to be due primarily to testosterone repression of the liver enzyme (4, 5). Rat liver FMO also appears to be positively regulated by testosterone, whereas estradiol exhibits repression of FMO levels (6). Estradiol also seems to play a role in determining the relative contribution of FMO in the *S*-oxygenation of *para*-methoxyphenyl 1,3-dithiolane observed in rat liver microsomes (7). During late gestation, changes in the hormonal milieu appear to be responsible for induction of FMO in rabbit lung, but not in liver (8, 9). Cortisol, through its diurnal secretion, also controls hepatic FMO activities in female mice (10). Diabetes can induce IMP *N*-oxidation in mice, an activity mediated exclusively by FMO (11).

Modulation of FMO activities is not restricted to the liver. Duffel *et al.* (5) and Cashman *et al.* (7) have found a different regulation pattern in lung and kidney from mice and rats, compared with liver. Gestation increases FMO activity in rabbit lung (8, 9) and in mouse placenta microsomes (12) but not in rabbit liver or in mouse liver or lung. Another example of hormonal regulation of extrahepatic FMO may be found in the >20-fold variation, during the estrous cycle, of the microsomal DMA *N*-oxidation catalyzed by the corpora lutea of the pig (13).

The studies discussed above indicate that FMO activity is under hormonal control, but the effects observed appear to be species and tissue-dependent.

It is generally accepted that the secretion of sex hormones is

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ABBREVIATIONS: FMO, flavin-containing monooxygenase; IMP, imipramine; DMA, *N,N*-dimethylaniline; TB, thiobenzamide; HGH, human growth hormone.

under the control of the hypothalamic-pituitary axis (14). Thus, hypophysectomy of male and female animals abolishes most of the sex differences (15). The involvement of some pituitary factor in the regulation of FMO cannot be ruled out. In order to obtain more information about the action of pituitary hormones on the expression of FMO, we have investigated the effects of castration and hypophysectomy in male adult rats and of hypophysectomy in female rats, using assays employed to characterize FMO, including IMP and DMA *N*-oxidation, TB *S*-oxidation, and immunochemical analysis by Western blotting with polyclonal antibodies raised to purified rat liver or rabbit lung FMO.

Experimental Procedures

Animals and treatments. Sprague-Dawley rats were obtained from Iffa-Credo (L'Asbresle, France). Castrated or hypophysectomized adult rats (7 weeks old, 200 g) were provided by the same source and treated 1 week after the operation with either HGH from Kabi Vitrum (0.4 IU/injection in sterile water, twice daily at 12-hr intervals for 7 days) or testosterone propionate (10 mg/kg in saline, daily for 10 days). Rats were killed 24 hr after the last injection, and livers were excised, weighed, and homogenized. Male New Zealand White rabbits (1.5–2 kg) were purchased from Wiss (Ste Savine, France). Microsomes were prepared as previously described (15).

Enzymatic activities. FMO activity was determined with three different substrates at saturation and assayed at pH 8.4 without cytochrome P-450 inhibitors. The conversion of TB to TB *S*-oxide was monitored at 370 nm (16). Assays were conducted at 37° in a Kontron 860 double-beam spectrophotometer, in a final volume of 1 ml containing 1 mg of microsomal protein and 0.1 mM TB. DMA *N*-oxide formation was determined by the procedure of Ziegler and Pettit (17). The kinetics of IMP *N*-oxidation by rat liver microsomes show the same complications observed for the oxidation of other amine substrates by purified FMO, i.e., a downward curvature of double-reciprocal plots at high substrate concentrations (16). [^{3}H]IMP chlorhydrate was used at a concentration of 0.5 mM (0.5 μCi/assay). To eliminate IMP *N*-oxide, formed during storage at −80° in the dark, [^{3}H]IMP was purified by high performance liquid chromatography just before use, as described elsewhere (11). Incubations were carried out in 1-ml total volume, for 10 min (to ensure initial rates) at 37°, with 0.5–1 mg of microsomal protein in the presence of an NADPH-generating system. Control incubations were carried out under the same conditions without the NADPH-generating system. At the end of the incubation, 0.2 ml of 10 M sodium hydroxide was added, and IMP and its metabolites were extracted twice with diethyl ether/dichloromethane (60:40). Extracts were evaporated to dryness under nitrogen and subjected to thin layer chromatography on silica plates (Merck 60 F254), which were developed with *n*-propanol/chloroform/27% ammonia (50:50:1). Spots were visualized under UV and scraped off for radioactivity counting. Proteins were determined according to the method of Lowry *et al.* (18).

Immunochemical determinations. The purification of FMO from rat liver (19) and rabbit lung (9) has been detailed elsewhere. Polyclonal antibodies to rat liver FMO were raised in female rabbits, and antibodies to rabbit lung FMO were raised in guinea pigs. To test the immunological reactivity of microsomal preparations, samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as described by Laemmli (20), and Western blotting was performed as described by Towbin *et al.* (21). Proteins were probed with the polyclonal anti-liver FMO or anti-lung FMO. Peroxidase activity was developed with 4-chloronaphthol. The nitrocellulose membranes were scanned for densitometric quantification using a computer design program from IMSTAR, as previously described (3).

Chemicals. IMP and IMP *N*-oxide were kindly provided by Ciba Geigy (Bâle, Switzerland), and [^{3}H]IMP (1.8 TBq/mmol) was from Amersham (Lesulis, France). All other reagents were of the finest grade available from commercial sources.

Results

Enzymatic activities in rat and rabbit tissues. FMO-dependent activities in different tissues vary with species and sex. The highest activities are found in the liver of male rats (Table 1). In male rats, IMP *N*-oxidation values are similar to those of DMA *N*-oxidation. No IMP *N*-oxidation is observed with rabbit lung microsomes, as has been reported previously (22, 23).

Effect of hypophysectomy and castration on liver FMO activities. The metabolism of IMP, DMA, and TB depends on sexual hormone regulation in rat liver microsomes. Males demonstrate greater metabolism (220–455%) than females, especially for the *N*-oxidation of IMP (Fig. 1). In hypophysectomized male rats (Fig. 1), the oxidation of the three test substrates markedly decreases (approximately 87% for DMA and TB and 44% for IMP). Hypophysectomy has no effect in females, with respect to liver microsomal DMA *N*-oxidation or TB *S*-oxidation. In contrast, it has a pronounced stimulatory effect on IMP *N*-oxidation, to the degree that the sex difference disappears after hypophysectomy (Fig. 1). Intermittent injections of HGH (0.4 IU/injection, twice daily) cannot fully restore basal activity. Androgens also increase FMO activities in hypophysectomized male rats but cannot fully restore basal levels (Fig. 1). After castration, FMO-dependent activities are moderately depressed in males and all substrates are affected similarly (33–58% reduction). Castration mimics the effect of hypophysectomy in male rats only for IMP *N*-oxidation.

Effect of hormones on liver, kidney, and lung IMP *N*-oxidation in rats. In an earlier report of Duffel *et al.* (5), DMA *N*-oxidase was found to be higher in kidney and lung of female mice than male, in contrast to liver. In this study with rats, we observe the same effect with IMP *N*-oxidation (Fig. 2). For IMP *N*-oxidation, hypophysectomy of male rats results in a significant reduction of activity in liver and lung, in contrast to the marked increase seen in kidney (Fig. 2). In females, the picture is clearly different, because no changes are observed in kidney after hypophysectomy, whereas an increase in liver and a decrease in lung are seen. Whatever the tissue, hypophysectomy leads to a more or less complete abolition of the sex difference in IMP *N*-oxidation. Intermittent injections of HGH completely restore the basal activity in male rat kidney, in the same manner that testosterone treatment does. In con-

TABLE 1

Tissue- and substrate-dependent FMO activity

Microsomal oxidation activities were determined as described in Experimental Procedures. The results depict the mean ± standard deviation for three to five determinations.

Tissue	Microsomal activities		
	IMP *N*-oxidation	TB *S*-oxidation	DMA *N*-oxidation
	nmol/min/mg		
Male rat			
Liver	2.05 ± 0.25	2.62 ± 0.86	2.75 ± 0.61
Lung	1.33 ± 0.11	0.49	1.37
Kidney	0.56 ± 0.09	0.99	0.76
Female rat			
Liver	0.45 ± 0.06	1.04 ± 0.11	1.24 ± 0.43
Lung	1.46 ± 0.38	ND	ND
Kidney	0.94 ± 0.44	ND	ND
Rabbit			
Liver	1.88 ± 0.13	ND	ND
Lung	0.09 ± 0.02	ND	ND

* ND, not determined.

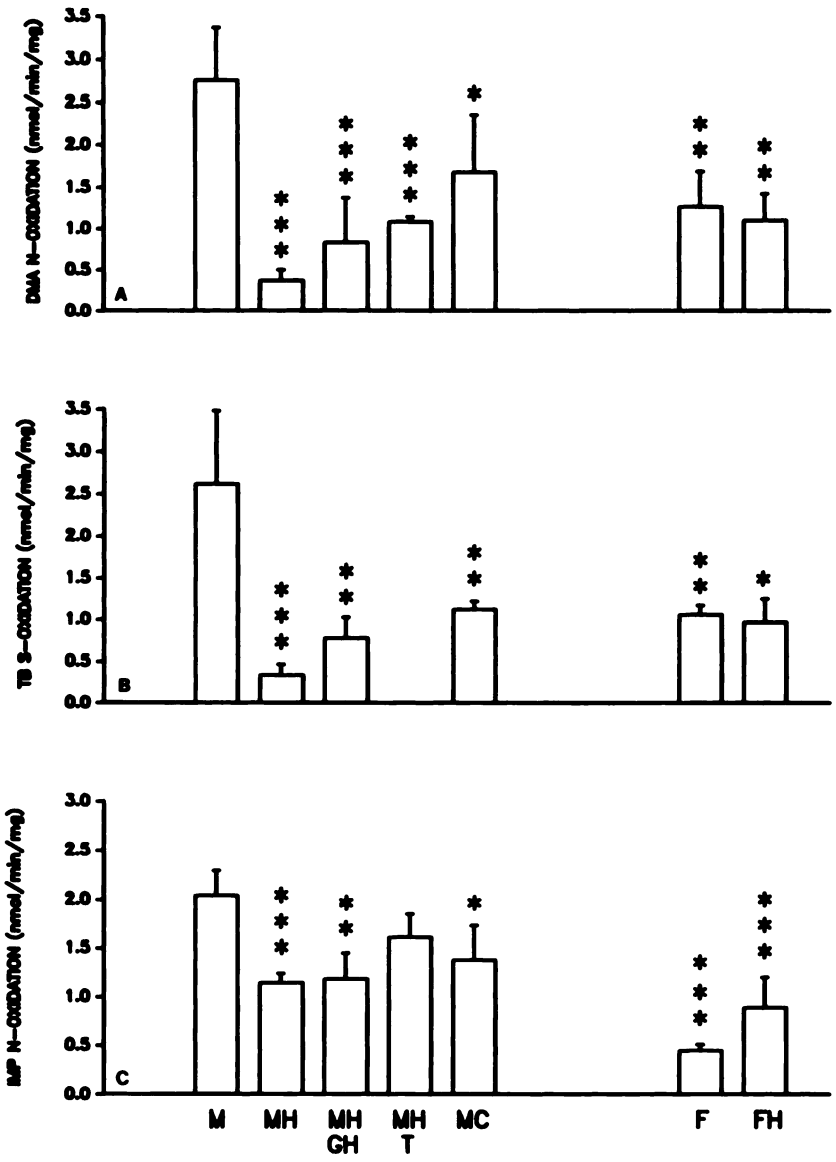

Fig. 1. Hormonal control of FMO activity toward three substrates in liver microsomes from male and female rats. FMO activity in liver microsomes of control males (*M*), hypophysectomized males (*MH*), hypophysectomized males treated with HGH (*MHGH*), hypophysectomized males treated with testosterone (*MHT*), castrated males (*MC*), control females (*F*), and hypophysectomized females (*FH*). The hormonal treatments are described in Experimental Procedures. A, B, and C, activity toward DMA, TB, and IMP, respectively. Enzymatic determinations were performed in triplicate, and the results represent the mean ± standard deviation for either four or five animals/treatment. Values for TB *S*-oxidation in the hypophysectomized testosterone-treated males were not determined. Results significantly different from the control male group are indicated; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. The results for IMP *N*-oxidation in the two female groups were statistically different from each other at the $p < 0.05$ level.

trast, in lung neither HGH nor testosterone has an effect on IMP *N*-oxidation. Castration has the same effect as hypophysectomy in liver and lung, i.e., lowering the basal activity, whereas no changes occur in male kidney after castration (Fig. 2). IMP *N*-oxidation values observed in rat tissues after hypophysectomy or castration are not as low as those obtained with rabbit lung microsomes (0.09 nmol/min/mg).

Effect of hypophysectomy and castration on FMO activity and liver mass. In both males and females, hypophysectomy results in a marked reduction of the liver mass, which can be partly reversed by treatment with either testosterone or HGH (Table 2). In castrated males, the liver mass is slightly higher than in control rats. The change in total hepatic IMP *N*-oxidase activity does not exactly parallel changes in specific activity, due to alterations of the liver mass after hormonal manipulation. For example, hypophysectomy decreases the specific activity in males by 44% and also reduces the liver weight by 58%. In contrast, hypophysectomy increases the specific activity in females by 98%, and the liver mass decreases by 27% (Table 2). Therefore, the variation in IMP *N*-oxidation can be attenuated by a modification of the liver mass but cannot be restricted to an alteration of the tissue involved.

Immunochemical reactivity. Microsomes from individual rats and rabbits were probed with anti-rat liver FMO (Fig. 3). A single band is observed with all tissues, but with a variation in the relative intensity of the stained bands. Densitometry of the stained bands has been calculated for each experimental group. When the FMO concentration is plotted versus activity (Fig. 4), a positive correlation is observed between the hepatic and pulmonary protein content and IMP *N*-oxidation ($r = 0.895$, $n = 12$), but not with renal FMO ($r = -0.075$, $n = 5$) (Table 3). Consequently, when all samples are pooled (liver, lung, and kidney), the correlation coefficient is lowered to 0.653 (Table 3). Similarly, DMA *N*-oxidation does not correlate with FMO protein in kidney ($r = -0.491$), whereas the correlation is positive in liver and lung ($r = 0.818$) (Table 3). A positive correlation between TB *S*-oxidation and FMO protein levels ($r = 0.570$) is observed in kidney and in liver plus lung ($r = 0.690$). When the same samples are probed with the polyclonal antibody to rabbit lung FMO, a single band is observed only in rabbit lung microsomes and not with microsomes from rat tissues (Fig. 5). No correlation with enzymatic activity is possible, and the high intensity of immunoreaction with rabbit

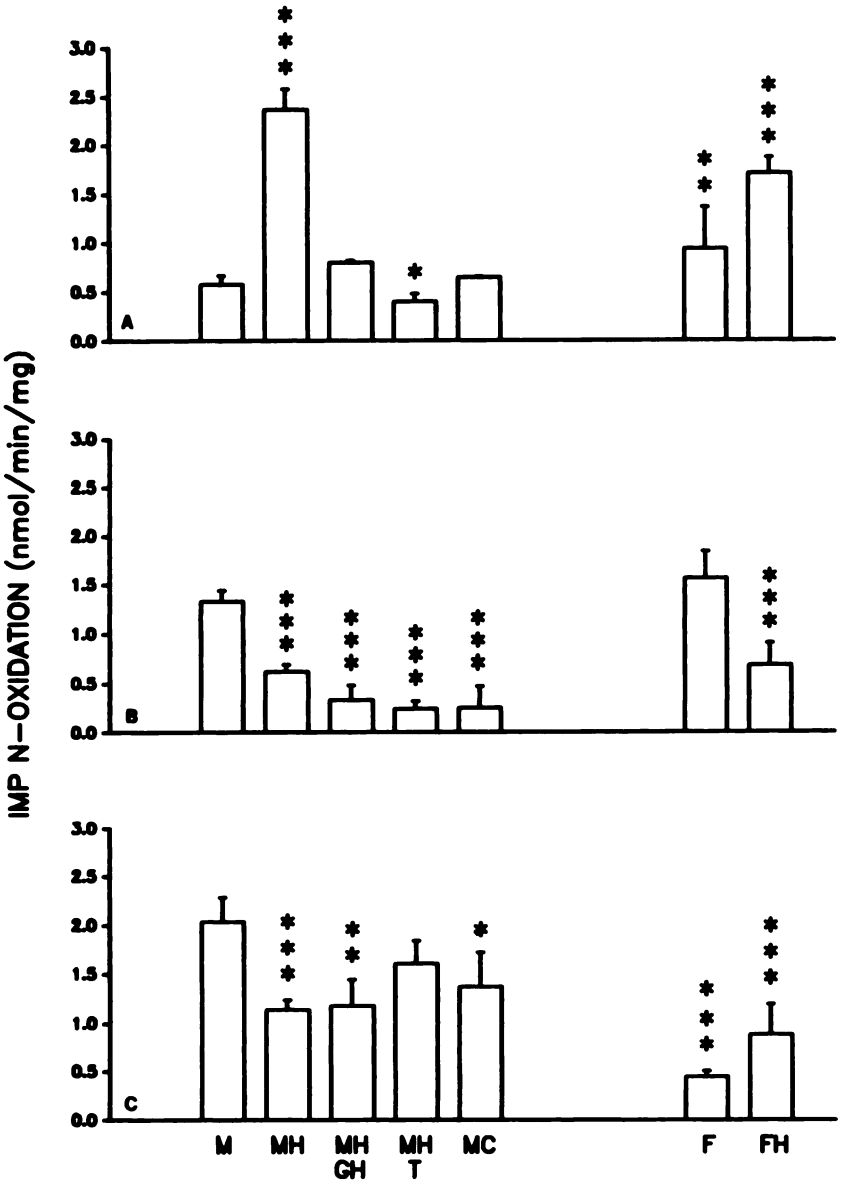

Fig. 2. Effect of hormonal treatments on IMP *N*-oxidation in kidney, lung, and liver of male and female rats. The abbreviations are as defined for Fig. 1. Hormonal manipulations were as described in Experimental Procedures. The values shown in A, B, and C, mean ± standard deviation of IMP *N*-oxidation for the kidney, lung, and liver, respectively. Enzymatic determinations were performed in triplicate with microsomal preparations from four or five animals/group. The level of statistical significance is indicated as described for Fig. 1.

lung microsomes contrasts with the lack of IMP *N*-oxidation with these microsomes (0.09 ± 0.02 nmol/min/mg).

Discussion

The results presented here confirm the sex differences in FMO activities in rats and indicate the dominant role of hormonal regulation in the control of drug metabolism by this enzyme. Male rats showed more hepatic metabolism than females, as has been described for many phase I and II enzymes (24). The observed sex difference is reversed in extrahepatic tissues. Lung and kidney microsomes from female rats exhibited higher IMP *N*-oxidase activity than did males. One major mechanism of the regulation of xenobiotic biotransformation is hormonal control. This control is directed mainly at the liver but is not always a direct effect of the hormone on this organ. In the case of hepatic constitutive cytochromes P-450, male- and female-specific isoenzymes have been demonstrated to be under the control of sex steroids. A dual regulation has been described; androgens imprint the brain of the neonatal rat and maintain some sex differences in the adult period by an action via the pituitary gland (25). Little work has been published on the effects of the hormonal regulation of FMO. According to these results, the pituitary gland seems to play a major role in the control of this enzyme, particularly in males. However, when HGH was injected subcutaneously twice daily, a partial restoration of basal activity was observed, suggesting that HGH was not the sole factor involved in the regulation.

Castrated males exhibited decreased FMO-dependent monooxygenase activities (33 and 58%), in the same manner as hypophysectomized males. Testosterone treatment had an effect similar to that of HGH treatment. These results indicate the existence of at least two types of control of hepatic FMO-dependent drug metabolism in the rat. Gonadal control, acting either directly or via the pituitary gland on the liver, would be one possibility. FMO is only partially dependent on androgenic stimulation for expression in male rats. Nongonadal factors probably play a role in the regulation of this FMO, because Dannan *et al.* (6) have observed that sex differences were only

TABLE 2

Liver mass and IMP *N*-oxidase activity in rat liver microsomes

The results are the mean ± standard deviation for each group, and the statistical significance is given with reference to the untreated value in the same sex. IMP *N*-oxidase was assayed as described previously (11). Numbers in parentheses are the numbers of rats/group.

Rats	Liver mass	IMP *N*-oxidase
	g	*nmol/min/mg of protein*
Males		
Untreated (5)	10.0 ± 1.0	2.05 ± 0.25
Hypophysectomized (4)	4.2 ± 0.4[a]	1.15 ± 0.10[a]
Hypophysectomized/HGH (5)	7.3 ± 0.8[b]	1.18 ± 0.27[c]
Hypophysectomized/testosterone (5)	5.9 ± 0.9[a]	1.61 ± 0.24
Castrated (4)	12.8 ± 1.5[b]	1.38 ± 0.35[b]
Females		
Untreated (4)	8.0 ± 0.4	0.45 ± 0.26
Hypophysectomized (4)	5.8 ± 0.8[a]	0.89 ± 0.34

[a] $p < 0.001$.
[b] $p < 0.05$.
[c] $p < 0.01$.

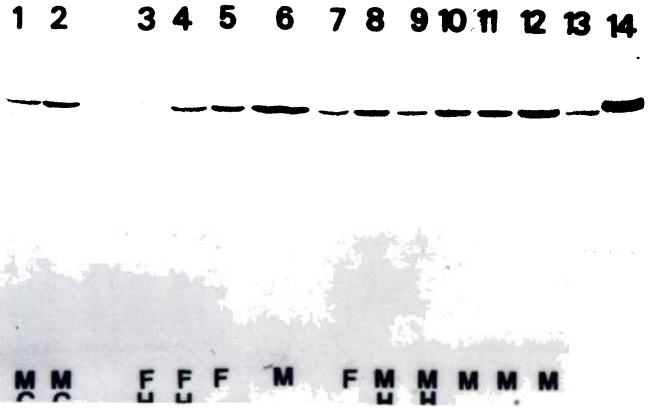

Fig. 3. Immunochemical cross-reactivity between rat and rabbit microsomes from various tissues and antibody to rat liver FMO. Microsomes (50 μg) from male and female rat liver, kidney, and lung and from female rabbit lung and liver were probed with rabbit antiserum to rat liver FMO after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting, as described in Experimental Procedures. The numbered samples are identified as follows: *1*, lung from castrated male rats; *2*, liver from castrated male rats; *3*, kidney from hypophysectomized female rats; *4*, liver from hypophysectomized female rats; *5*, lung from female rats; *6*, liver from male rats (100 μg); *7*, liver from female rats; *8*, kidney from hypophysectomized male rats; *9*, liver from hypophysectomized male rats; *10*, lung from male rats; *11*, kidney from male rats; *12*, liver from male rats; *13*, rabbit lung; *14*, rabbit liver. Experiments were performed in triplicate with all the samples from the different groups.

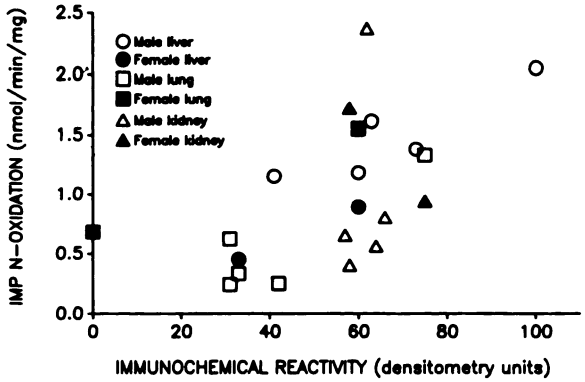

Fig. 4. Correlation between FMO activity and immunochemical reactivity to antibody to rat liver FMO. IMP *N*-oxidation as performed as described in Experimental Procedures and plotted against the amount of protein that cross-reacted with antibody to rat liver FMO on Western blots (see Experimental Procedures), as determined by densitometry. The results represent the mean of three Western blots versus the mean of enzymatic activities.

TABLE 3

Correlation between FMO activities and immunochemical reactivity

FMO activities were taken from the data in Table 1. Immunochemical reactivity of microsomes from various tissues was determined by densitometry of the Western blot probed with antibody to rat liver FMO (Fig. 3), as described in Experimental Procedures.

Activity	Correlation		
	Liver + lung + kidney	Liver + lung	Kidney
IMP *N*-oxidation	+0.653	+0.895	−0.075
TB *S*-oxidation	+0.610	+0.690	+0.570
DMA *N*-oxidation	+0.771	+0.818	−0.491

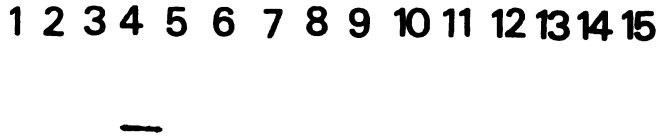

Fig. 5. Immunochemical cross-reactivity between rat and rabbit microsomes from various tissues and antibody to rabbit lung FMO. Microsomes (50 μg) from various rat and rabbit tissues were analyzed by Western blotting as described for Fig. 3, except that the blots were probed with guinea pig anti-rabbit lung FMO IgG. The only sample to cross-react with the antibody is in *lane 4*, which contained rabbit lung microsomes.

partially abolished upon neonatal gonadectomy. The effect of castration may be explained as the removal of a positive feedback of androgen on the release of a pituitary factor or an antagonism by androgens, at the level of the liver, of a pituitary hormone. Whatever the mechanism, the pituitary gland and testes are involved in the regulation of FMO in males. In females, Skett *et al.* (25) have observed that ovariectomy had no effect on the hepatic metabolism of IMP, whereas hypophysectomy increased IMP *N*-oxidation. These results suggest that the pituitary gland is the dominant controlling factor in female rats and the ovaries are not involved.

However, this regulation seems to be substrate and tissue dependent. Hepatic IMP *N*-oxidation does not exhibit the same pattern of regulation as TB *S*- or DMA *N*-oxidation, particularly in female rats. At the present time, there is no single procedure that can conveniently measure activity of FMO in all three tissues. *N*-oxidation of DMA and *S*-oxidation of TB are usually employed. DMA is a substrate for all FMO isoenzymes; however, the procedure is insensitive and its application to tissues with relatively low activity is limited. The *S*-oxidation of TB is a rapid and simple method, but this compound is also a substrate for cytochrome P-450-dependent *S*-oxidation (16). IMP *N*-oxidation is more sensitive than DMA *N*-oxidation and

can be quantified in all tissues tested. No interference by cytochrome P-450-dependent *N*-oxidation is observed in rat tissues, inasmuch as a complete inhibition by thermal inactivation is observed within 1 min for liver and kidney (26) and for lung (data not shown). The *N*-oxidation of IMP by rat liver and kidney microsomes was severely inhibited (85–90%) by increasing amounts of anti-rat liver FMO antibodies (26). Inhibition of DMA and TB oxidation, under the same conditions, was much less severe (40 and 50% in rat liver and kidney microsomes, respectively). Of particular interest is the absence of IMP *N*-oxidation observed with rabbit lung microsomes, which is consistent with previous results (22, 23, 27). Rabbit lung FMO is characterized by a more restricted substrate binding site (compared with the liver enzyme), which excludes compounds, such as IMP, with a diameter of >8 Å, unless the oxygenated heteroatom extends at least 6–8 Å from the bulky ring system (27). Thus, IMP *N*-oxidation was a convenient analytical procedure to study FMO regulation in rat tissues. Results show that hypophysectomy has a minor effect on IMP *N*-oxidation in males and increases the activity in females, in contrast to the other activities examined. Castration and hypophysectomy have similar effects on the metabolism of IMP, i.e., abolishing sex differences. Thus, the hormonal differences observed in substrate metabolism could possibly be explained by a contribution of cytochrome P-450 in TB and DMA oxidation. However, in this study we observed a different hormonal pattern of regulation in kidney than in liver for the same substrate (IMP), which could be explained by the existence of distinct proteins that are differently regulated. We have previously described a similar phenomenon in human tissues (26).

The existence of two distinct proteins in a single species was demonstrated unambiguously by the isolation of FMO from lung microsomes of rabbit (22, 28). This protein exhibited differences in substrate specificity and immunochemical cross-reactivity, compared with the liver enzyme. IMP and chlorpromazine, which are good substrates for hepatic FMO, are not oxygenated by the lung enzyme (22, 23). Tynes and Philpot (29) provided evidence of distinct lung and liver forms of FMO in male rabbits, mice, rats, guinea pigs, and hamsters. In rats, the liver and kidney were seen to contain a single identical form. The liver and kidney form was also present in rat lung, along with a distinct FMO (29). Recently, Lawton *et al.* (30) have sequenced both the liver and lung FMO cDNA from rabbits and demonstrated them to be distinct gene products, with about 56% identity. Two distinct forms of FMO have been isolated from liver microsomes of rabbits (31) and guinea pigs (32), and neither form corresponds to the lung enzyme. The two guinea pig liver FMOs demonstrated distinct but overlapping substrate specificity. Therefore, there are now examples of at least three distinct FMO isoenzymes in a single species.

Immunoquantitation of rat tissues by Western blotting with the polyclonal antibody raised to rat liver FMO confirms the existence of two or more distinct proteins with overlapping substrate specificities. Our antibody recognized a single band on Western blots with rat and rabbit microsomes. Similar results were observed by Dannan and Guengerich (3) with antibody to pig liver FMO, suggesting that our antibody to rat liver FMO is directed to the liver FMO previously described. We have previously shown that immunoglobulins to rat liver FMO inhibit IMP *N*-oxidation and, to a lesser extent, TB and DMA oxidation in rat liver and kidney microsomes, suggesting that a common protein exists in these tissues (26). However, immunoquantification of FMO protein in rat kidney shows that this protein exhibits no variation after hormonal treatment and does not correlate with IMP *N*-oxidation. These results suggest the existence of another protein in kidney that is more specific in catalyzing DMA *N*-oxidation. This kidney FMO appears to be regulated only by pituitary factor(s). This could be by the appearance of a new protein or the induction of protein present in kidney but repressed by growth hormone. Inhibition by HGH of the synthesis of the male-specific form of cytochrome P-450 has been demonstrated previously by Vockentanz and Virgo (33). The lack of immunochemical reactivity of kidney microsomes with the polyclonal antibody to rabbit lung FMO on Western blots is consistent with previous results from another laboratory (29) and confirms that the protein present in rat kidney is not identical to the rabbit lung enzyme. Further studies with a direct approach are needed to confirm this hypothesis.

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