0026-895X/91/030275-06$03.00/0

MOLECULAR PHARMACOLOGY, 39:275-280

ACCELERATED COMMUNICATION

Induction of Cytochrome P450IIE1 in the Obese Overfed Rat

JUDY L. RAUCY, JEROME M. LASKER, JAMES C. KRANER, DANIEL E. SALAZAR, CHARLES S. LIEBER, and GEORGE B. CORCORAN

University of New Mexico, Toxicology Program, College of Pharmacy, Albuquerque, New Mexico 87131 (J.L.R., J.C.K., D.E.S., G.B.C.), and the Alcohol Research and Treatment Center, Bronx Veterans Administration Medical Center and Mt. Sinai School of Medicine, New York, New York 10468 (J.M.L., C.S.L.)

Received September 13, 1990; Accepted December 12, 1990

SUMMARY

Cytochrome P450IIE1 (IIE1) is a microsomal xenobiotic-activating enzyme that is inducible not only by various chemical agents but also by fasting and diabetes. Using a rat model that mimics human obesity, we have found that hepatic IIE1 levels are also increased by this common clinical disorder. Liver microsomes from rats made obese by feeding with an energy-dense diet displayed elevated aggregate P450 content (+28%) and enhanced catalytic activities associated with IIE1, including low-K_m *N*-nitrosodimethylamine demethylation (+66%), aniline hydroxylation (+52%), *p*-nitrophenol hydroxylation (+170%), and acetaminophen-cysteine conjugate formation (+28%). In contrast, obesity had no significant effect on cytochrome b_5 content, P450 reductase activity, benzphetamine demethylation, or erythromycin demethylation, with the latter two reactions being linked with rat IIC11 and IIIA1, respectively. The enhancement of IIE1-dependent drug-metabolizing activities noted in liver microsomes from obese rats was paralleled by a similar increase (111%) in hepatic IIE1 protein content in these animals, as assessed on immunoblots developed with anti-hamster IIE1 IgG. Anti-IIE1-inhibitable rates of microsomal *p*-nitrophenol metabolism, a reaction highly correlated with IIE1 content ($r = 0.88$, $p < 0.01$), were over 3-fold higher in obese rats than in nonobese controls, providing additional evidence for the obesity-related increase of hepatic IIE1. The induction of IIE1 by the pathophysiological condition of obesity may provide a biochemical basis for the increased incidence of occult liver disease and certain cancers noted in obese individuals.

Obesity has been implicated as a risk factor in a number of drug-induced target organ toxicities (1, 2) and, in certain instances, these toxicities appear to be due to an increased production of reactive metabolites by microsomal oxidation (2, 3). Indeed, enhanced bioactivation of therapeutic agents and/or environmental pollutants by P450s may contribute to the high incidence of liver disease and certain cancers noted in obese humans (4). Although obesity has been shown to affect the metabolism of various xenobiotics (1, 5), the relationship between obesity and bioactivation of such agents has not been well characterized. In the obese individual, biotransformation may be influenced by fatty infiltration and fibrosis of the liver, pathophysiological processes that have been associated with this disorder, and by changes in dietary composition (4–6). Whether obesity causes changes in microsomal P450 enzyme composition is not known.

Induction of IIE1[1] is characterized by alterations in both the metabolic activity and the substrate specificity of liver microsomes. This enzyme is the major catalyst in the activation of several hepatotoxins, such as acetaminophen (7), ethanol (8), enflurane (9), and halothane (10), as well as the hepatocarcinogen NDMA. In fact, IIE1 has been implicated as the low-K_m NDMA demethylase in several species including humans (11–13). IIE1 also promotes the oxidation of acetone to acetol (14), which can be subsequently incorporated into glucose, suggesting that the enzyme may be involved in a physiological pathway leading to the production of glucose from ketone bodies. It has previously been reported that the low-K_m form of NDMA demethylase (IIE1) in rat liver microsomes is induced by fasting (15) and chemically induced diabetes (16), two conditions in which plasma ketone bodies are elevated. In addition to fasting and diabetes, IIE1 is inducible by certain xenobiotics, including acetone, ethanol, isoniazid, and pyrazole (17). Preliminary evidence from our laboratory suggests that obesity may be another

This research was supported by Department of Health and Human Services Grants AA-08139, AA-05934, and GM-41564, the Veterans Administration, a Grant-in-Aid from the American Heart Association (88-925), and funds contributed in part by the American Heart Association New Mexico Affiliate. Portions of this work were presented in preliminary form at the Annual Meeting of the American Society for Pharmacology and Experimental Therapeutics, 1989 (45).

[1] The P450 enzymes described in this work have been designated according to the recently defined P450 gene nomenclature (46).

ABBREVIATIONS: P450, cytochrome P450; IIE1, P450IIE1; IA2, P450IA2; IIC11, P450IIC11; IIIA1, P450IIIA1; NDMA, *N*-nitrosodimethylamine; IgG, immunoglobulin G; KPO_4, potassium phosphate.

pathophysiological stimulus that increases the hepatic concentration of IIE1, as shown by the significant enhancement of acetaminophen hepatotoxicity and microsomal ethanol oxidation in obese rats (1, 18). In obesity, consistently elevated ketone body concentrations are not routinely encountered, but the urine of overweight individuals tends to be more acidic than normal, suggestive of higher systemic exposure to ketone bodies (19). Consistent with this notion, energy-dense diets, of the type used to induce obesity in rodents, cause notable acidification of urine in normal human volunteers (20). Thus, ketosis could be the common feature for induction of IIE1 in the diabetic, fasted, and obese states. In the present study, we have utilized the overfed rat model of human obesity to further assess the effects of this pathophysiological condition on hepatic IIE1 and associated catalytic activities.

Materials and Methods

Animals. Male Sprague-Dawley weanling rats (100 g body weight; Blue Spruce Farms, Altamont NY) were fed either a standard pelleted diet (RMH-1000; Agway, Syracuse NY) or an obesity-producing energy-dense diet originally described by Wong *et al.* (21). Briefly, the obesity-producing diet contained (w/w) 60% vegetable shortening, 25% vitamin-free casein, 5% salt mixture, 3.55% sucrose, 2.2% vitamin fortification mixture, 2% cellulose fiber, 2% liver powder, and 0.25% DL-methionine. The pelleted control diet (Prolab RMH-1000; Agway) contained 6.5% fat, 14.4% protein, and 57.5% carbohydrate. Six rats were maintained on the energy-dense diet for 52 weeks, while five other animals were fed the pelleted control diet for the same amount of time; all animals had free access to food and tap water. For the purpose of this study, obesity was defined as body weight exceeding 125% that of pellet-fed control rats. Animals were killed by decapitation, livers were removed, and microsomes were prepared according to the method of Van der Hoeven and Coon (22). Microsomal P450 and cytochrome b_5 concentrations were determined as previously described (23). Protein concentrations were assessed according to the method of Bensadoun and Weinstein (24).

Enzyme assays. Aniline hydroxylation was determined as described by Schenkman *et al.* (25). Incubation mixtures contained 2.0 mM aniline, 0.2 mg of microsomal protein, and 1 mM NADPH in a final volume of 1.0 ml of 50 mM KPO_4 buffer, pH 7.4. After incubation for 10 min at 37°, the reaction was terminated by addition of 20% trichloroacetic acid. Benzphetamine and erythromycin demethylation were assayed by measurement of the formation of formaldehyde, according to the method of Nash (26). Either 1.0 mM benzphetamine or 1.0 mM erythromycin was added to 1.0 ml of 50 mM KPO_4 buffer, pH 7.4, 0.5 mg of microsomal protein, and 1.0 mM NADPH. The reaction was terminated, after 10 min at 37° with 20% trichloroacetic acid. *p*-Nitrophenol hydroxylation was determined as described by Reinke and Moyer (27). The reaction mixture consisted of 0.1 mM *p*-nitrophenol, 0.2 mg of microsomal protein, 1.0 mM ascorbate, and 1.0 mM NADPH, in a final volume of 1.0 ml of 100 mM KPO_4 buffer, pH 6.8. The formation of 4-nitrocatechol was determined spectrophotometrically.

Acetaminophen activation was determined as previously described by our laboratory (28). Radioactivity was used to quantitate acetaminophen-cysteine conjugate formation, and data were expressed as nmol of conjugate formed/30 min/mg of protein. *N*-Demethylation of NDMA was determined in a radiometric assay, according to the method of Levin *et al.* (12). Incubation mixtures contained 0.5 ml of 100 mM KPO_4, pH 6.8, 0.5 mM final concentration of a mixture of ^{14}C-labeled NDMA plus unlabeled NDMA, and 1.0 mM NADPH. After a 5-min incubation at 37°, the reaction was terminated by addition of 300 μl of 1.0 M sodium acetate, pH 4.5. P450 reductase activity was assayed by monitoring the rate of cytochrome *c* reduction at 550 nm in 0.3 M KPO_4, pH 7.7, at 30°, in 1.0-ml incubations containing 20 μg of microsomal protein and 5 mg/ml cytochrome *c*.

Antibody preparation. The preparation and characterization of monospecific polyclonal antibodies against hamster IA2 have been described elsewhere (28). Antibodies to hamster IIE1, which was purified from ethanol-treated animals[2] in a manner similar to the human IIE1 ortholog (29), were elicited in male New Zealand white rabbits (2.5-kg body weight, using an immunization protocol described by Raucy *et al.* (28). Immune-specific IgG fractions (and preimmune IgG obtained from rabbit sera before immunization) were purified using caprylic acid (30). Antibodies to hamster IIE1 were made monospecific by repeated passage of the IgG preparation over a Sepharose 4B column to which the heterologous cross-reacting hamster antigens had been coupled (28). After back-absorption, the antibody preparation recognized only a single M_r 53,000 protein in hamster liver microsomes, namely IIE1, the levels of which increased after treatment of animals with ethanol, pyrazole, or acetone. The antibody also cross-reacted with a similar inducible protein in rat (P450j; provided by A. Cederbaum) and in rabbit liver microsomes (LM3a; provided by D. Koop), but not with rat P450b, P450f, P450g, or P450h (provided by W. Levin). The monospecificity of anti-hamster IIE1 IgG on immunoblots of rat liver microsomes has been demonstrated previously (31).

Immunoblot analysis. Rat liver microsomes (3 μg of protein) were resolved using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (32). Separated proteins were then electrophoretically transferred to a nitrocellulose filter (33), and the filter was subsequently blocked for 1 hr with Blotto (34). The washed filter was then incubated overnight at 25° in Blotto containing either anti-hamster IIE1 IgG (1 μg/ml) or anti-hamster IA2 IgG (25 μg/ml). Following extensive washing with Blotto, the filter was further incubated for 1 hr with a 1:1000 dilution of biotinylated goat anti-rabbit IgG in Blotto. The final incubation consisted of 1:2000 dilution of streptavidin-horseradish peroxidase in Blotto, and it proceeded for 30 min at 25°. Peroxidase activity was subsequently detected with 4-chloro-1-napthol and hydrogen peroxide (33). Immunochemical staining was determined with a scanning densitometer (model GS-300; Hoefer Scientific Instruments) interfaced to a computer. Band areas were integrated using densitometry software (Hoefer Scientific Instruments).

Materials. Acetaminophen, cysteine, benzphetamine, erythromycin, aniline, *p*-aminophenol, *p*-nitrophenol, 4-nitrocatechol, 4-chloro-1-naphthol, cytochrome *c*, and hydrogen peroxide were obtained from Sigma Chemical Co. (St. Louis, MO). NADPH was from Boehringer Mannheim Biochemicals (Indianapolis, IN). High performance liquid chromatography grade solvents were obtained from VWR Scientific (San Francisco, CA) or Fisher Scientific (Plano, TX) and were filtered through a 0.22-μm Millipore GS filter (Bedford, MA) before use. Biotinylated goat anti-rabbit IgG and the streptavidin-peroxidase conjugate were from Calbiochem (La Jolla, CA). Nitrocellulose filters were from Schleicher and Schuell (Keene, NH). L-[^{14}C]Cysteine·HCl with a specific activity of 44.1 mCi/mmol, was obtained from Amersham (Arlington Heights, IL), and *N*,*N*-di-[^{14}C]methylnitrosamine (8 mCi/mmol), also from Amersham, was purified by reverse phase liquid chromatography, according to the method of Levin *et al.* (12), before use. All other chemicals were reagent grade or better.

Results

Obesity produced by feeding rats an energy-dense semisynthetic diet for 52 weeks resulted in a marked increase in both body and liver weight. Rats fed the energy-dense diet exhibited a 44% increase in total body mass (735 versus 509 g, $p < 0.05$) and a similar (37%) increase in liver weight (22.2 versus 16.2 g, $p < 0.05$), compared with pellet-fed controls. The aggregate content of P450 in liver microsomes isolated from obese and

[2] J. M. Lasker, M. Tsutsmi, J. L. Raucy, B. Bloswick, and C. S. Lieber. Characterization of ethanol-inducible P450IIE1 and BNF-inducible P450IA2 from hamster liver microsomes: two structurally distinct enzymes with common metabolic properties. Submitted for publication.

nonobese animals (0.77 ± 0.22 versus 0.60 ± 0.02 nmol/mg of protein, respectively; $p < 0.05$) was significantly different; however, P450 reductase activity (163 nmol/min/mg in nonobese versus 165 nmol/min/mg in obese rats) was not. The hepatic microsomal content of cytochrome b_5 in rats fed the energy-dense diet (0.30 ± 0.04 nmol/mg) or the pellet diet (0.34 ± 0.10 nmol/mg) also exhibited no difference.

To assess changes in individual liver P450 enzymes, we first examined a profile of microsomal catalytic activities (Table 1). Of these activities, aniline, NDMA, *p*-nitrophenol, and acetaminophen oxidation were all significantly elevated in microsomes from obese animals. No changes in the rates of benzphetamine and erythromycin *N*-demethylation [catalyzed mainly by IIC11 (35) and IIIA1, respectively] were observed in microsomes from the two groups of rats (Table 1), suggesting that these two enzymes were not affected by obesity.

Because aniline hydroxylation and acetaminophen activation are reactions catalyzed by at least two P450 enzymes, IIE1 and IA2 (8, 28, 36, 37), the higher activity observed in microsomes from obese rats may be due to an increase in either or both enzymes. Therefore, we utilized immunoblot analysis, with polyclonal antibodies directed against hamster IIE1 and IA2, to evaluate enzyme contents in obese and nonobese rats. As shown in Fig. 1, an increase in staining intensity was observed with microsomes from obese animals when probed with the antibody to IIE1. Densitometric scanning of the immunoblot revealed an average increase in staining intensity of 111% (3286 ± 505 versus 1559 ± 480, $p < 0.05$) for microsomes from obese rats. However, no difference in staining intensity was observed between liver microsomes from control and obese animals on immunoblots developed with the antibody prepared to IA2 (not shown).

p-Nitrophenol hydroxylation was subsequently determined, using the same liver microsomes as those subjected to immunoblot analysis. Linear regression analysis comparing the rates of 4-nitrocatechol formation with IIE1 immunochemical staining intensity demonstrated a significant correlation ($r = 0.88$, $p < 0.01$) (Fig. 2). When separate analyses were performed on the two groups of rats, no correlation ($r = 0.59$, $p > 0.05$) was observed between staining intensity of microsomes from nonobese rats and *p*-nitrophenol hydroxylation, whereas a strong correlation existed for microsomes from obese rats ($r = 0.90$, $p < 0.01$). In addition, kinetic analysis performed on these microsomes revealed an increase in V_{max} (from 1.0 to 2.5 nmol/min/mg of protein in microsomes from control and obese animals, respectively), without a concomitant change in K_m (80 μM) for the *p*-nitrophenol hydroxylation reaction.

Immunoinhibition by anti-IA2 IgG and anti-IIE1 IgG of *p*-nitrophenol hydroxylation was also examined in microsomes from obese and normal rats (Table 2). Optimal amounts of each antibody needed to produce maximal inhibition of the reaction had been previously determined.[2] Anti-IA2 IgG (5 mg of IgG/nmol of microsomal P450) inhibited 42% and 26% of the *p*-nitrophenol hydroxylation by microsomes from nonobese and obese animals, respectively. When anti-IIE1 IgG (2 mg of IgG/nmol of P450) was substituted, the converse situation was observed; a greater extent of inhibition occurred in microsomes from obese (77%) than nonobese (60%) animals, and the antibody-inhibitable rate (the difference between rates determined in the presence of preimmune IgG and anti-IIE1 IgG) increased more than 3-fold, from 0.58 nmol/min/mg in microsomes from nonobese to 2.00 nmol/min/mg in microsomes from obese rats. Interestingly, the sum of the inhibited rates produced by anti-IIE1 IgG and anti-IA2 IgG in microsomes from either nonobese or obese rats was approximately equal to the rate determined in the presence of preimmune IgG. In order to rule out the possibility that the inhibition of *p*-nitrophenol metabolism by anti-IA2 IgG was due to antibody cross-reaction with IIE1, the capacity of this antibody preparation to inhibit low-K_m NDMA demethylation in microsomes from control, ethanol-treated, and obese rats was assessed [in rat liver microsomes, low-K_m NDMA metabolism is catalyzed exclusively by IIE1 (12)]. As expected, the higher concentration of microsomal IIE1 in ethanol-treated and obese animals resulted in higher rates of microsomal demethylation, but anti-IA2 IgG (5 mg of IgG/nmol of microsomal P450) neither inhibited this enhanced activity nor had any effect on demethylation rates in microsomes from control animals (<12% decrease in activity in microsomes from all three groups of rats).

TABLE 1

Catalytic activities of liver microsomes from obese and control rats

Values are the mean ± standard deviation of two or more determinations in microsomes from five nonobese and six obese animals.

Catalytic activity (substrate concentration)	Rate of oxidation		
	Control	Obese	Change
	nmol of product formed/min/mg of protein		%
Aniline hydroxylation (2 mM)	0.48 ± 0.07	0.73 ± 0.10[a]	+52
p-Nitrophenol hydroxylation (0.1 mM)	0.96 ± 0.29	2.59 ± 0.66[a]	+170
N-Nitrosodimethylamine demethylation (0.5 mM)	0.24 ± 0.05	0.40 ± 0.07[a]	+67
Acetaminophen activation (10 mM)	1.62 ± 0.43	2.07 ± 0.52[a,b]	+28
Benzphetamine demethylation (1 mM)	4.40 ± 0.74	3.94 ± 0.74	−10
Erythromycin demethylation (1 mM)	1.89 ± 0.22	1.86 ± 0.32	−2

[a] Significantly different from control value ($p < 0.05$) by Student's *t* test.
[b] nmol of product formed/30 min/mg of microsome protein.

Discussion

In the present study, we have further characterized the changes in hepatic oxidative drug metabolism that occur as a result of obesity. We have shown for the first time in the obese overfed rat that the concentration of a P450 enzyme, IIE1, increases significantly in liver microsomes. This increase in hepatic IIE1 concentration was manifested by enhanced rates of microsomal metabolism of acetaminophen, aniline, and NDMA, all of which are relatively specific substrates for IIE1. Noted also in microsomes from obese rats was enhanced metabolism of the phenolic compound *p*-nitrophenol, as a result of its increased IIE1-catalyzed oxidation but not of that mediated by a second P450 enzyme, IA2. The finding that the effect of obesity on liver P450 enzymes appeared to be somewhat selective for IIE1 was shown by the failure of this condition to alter the microsomal concentrations of three other P450s, namely IA2, IIC11, and IIIA1, as assessed either immunochemically or catalytically. The obese state had no effect on liver microsomal content of cytochrome b_5 and P450 reductase, both of which are required for P450 function.

The increases in liver microsomal IIE1 content and associated catalytic activities observed in obese rats, as compared with pellet-fed controls, are less than those reported with

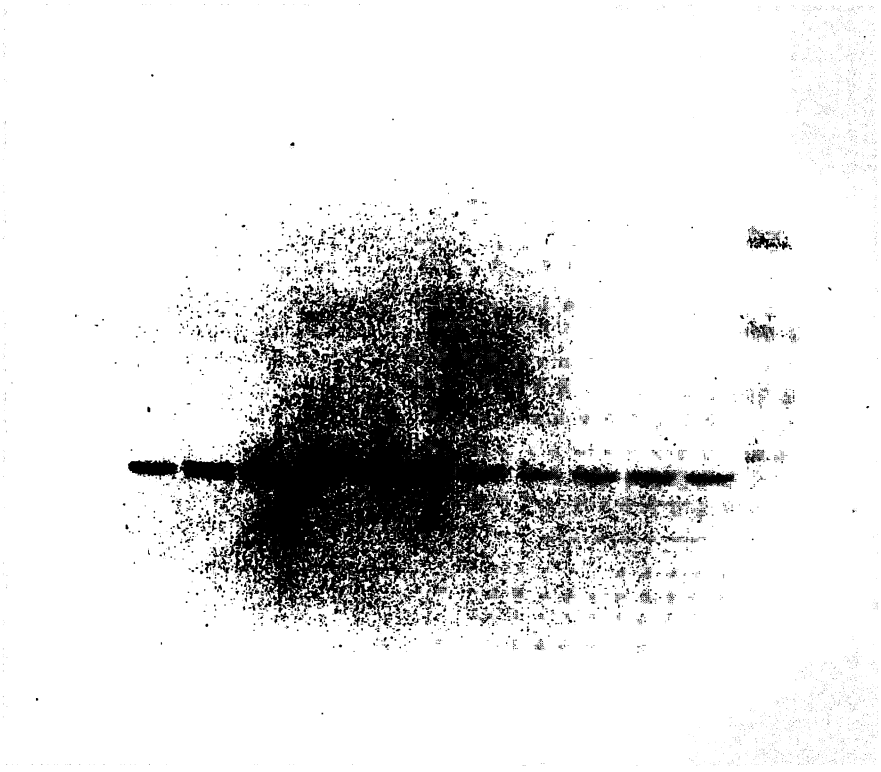

Fig. 1. Immunoblot analysis of liver microsomes from control and obese rats. Liver microsomes (3 μg) were initially subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by electrophoretic transfer to a nitrocellulose filter. The filter was stained immunochemically with anti-hamster IIE1 IgG. *Lanes 1–6*, microsomes from rats made obese by feeding with an energy-dense diet; *lanes 7–11*, microsomes from rats fed a pelleted control diet. *Far right lane*, prestained protein standards with molecular weights of 180,000, 116,000, 84,000, 58,000, 49,000, and 27,000.

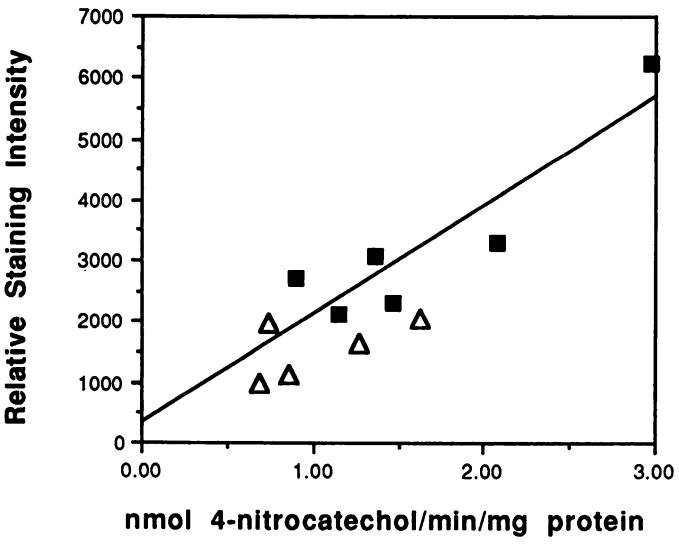

Fig. 2. Correlation between relative IIE1 concentrations in rat liver microsomes and *p*-nitrophenol hydroxylase activity. The immunoblot shown in Fig. 1 was scanned using a densitometer, and the integrated peak areas for individual microsomes were plotted versus *p*-nitrophenol hydroxylase activity. Δ, Microsomes from control rats; ■, microsomes from obese animals. A correlation coefficient of $r = 0.88$, ($p < 0.01$) was obtained by linear regression analysis.

chemical inducers of the enzyme. After short term treatment of animals with agents, including ethanol, acetone, pyrazole, and isoniazid (17), IIE1 protein levels in hepatic microsomes increased as much as 10-fold. In terms of IIE1 induction, however, it may be more valid to compare obesity with the two

TABLE 2

Inhibition of *p*-nitrophenol hydroxylation by anti-IIE1 IgG and anti-IA2 IgG

Rat liver microsomes (0.5 mg of protein, equivalent to 0.25–0.34 nmol of microsomal P450) were preincubated with either anti-IA2 IgG (1.25 mg), anti-IIE1 IgG (0.50 mg), or corresponding amounts of preimmune (control) IgG, for 3 min at 37°. *p*-Nitrophenol hydroxylation was then determined as described under Enzyme Assays. Values are expressed as the rate determined in the presence of the IgG preparations and are the mean ± standard deviation (six experiments). The numbers in parenthesis are the percentage of inhibition relative to activities determined in the presence of an equivalent amount of preimmune IgG. At preimmune IgG/microsomal P450 ratios ranging from 2 to 5 mg/nmol, inhibition of *p*-nitrophenol metabolism never exceeded 10%.

Microsomes	*p*-Nitrophenol hydroxylation			
	+Preimmune	+Anti-IA2	+Anti-IIE1	Sum[a]
	nmol of product formed/min/mg of protein			
Control	0.96 ± 0.32	0.56 ± 0.49 (42%)	0.38 ± 0.22 (60%)	0.94
Obese	2.59 ± 0.66	1.92 ± 0.86 (26%)	0.59 ± 0.27 (77%)	2.51

[a] Sum of the anti-IA2 and anti-IIE1 IgG-inhibitable rates.

other pathophysiological conditions known to affect hepatic IIE1 concentrations, namely diabetes (either spontaneous or chemically promoted) and starvation. In the uncontrolled diabetic or fasted states, rats exhibit no more than a 1.4–3.0-fold increase in microsomal IIE1 content and a moderate enhancement of low-K_m NDMA demethylation (15, 16, 38), which are comparable to those found here in the obese rat. In contrast to diabetes or starvation, however, obesity always results in an increase in liver mass (37% in this study), which would further magnify any toxicological consequences of the enhanced hepatic IIE1 concentrations.

In considering the mechanism of IIE1 induction, obesity may also have more in common with diabetes and/or starvation

than with the chemical inducers. It was recently reported that the elevated levels of hepatic IIE1 found in spontaneously diabetic BB rats were highly correlated with plasma concentrations of the ketone β-hydroxybutyrate and that IIE1 induction in diabetes is associated directly or indirectly with increased plasma levels of ketone bodies (39). A role for ketones has also been proposed in the starvation-mediated increase in microsomal IIE1 content (15). Whereas the IIE1 substrate acetone, a ketone, also serves as an efficacious inducer of the enzyme (17), exogenously administered β-hydroxybutyrate does not (40). Furthermore, even in the absence of increased plasma ketones, ethanol remains a potent IIE1 inducer (31). Because the energy-dense diet used here to produce obesity receives the majority of its calories from fat (21), it is likely that our obese rats had elevated serum ketone levels as well.[3] However, factors associated with the obese state itself and unrelated to diet must also play a role in IIE1 induction in obesity. For instance, acetaminophen disposition in rats that were fed the high fat diet but failed to become obese more closely resembled that of pellet-fed control than obese animals (21); acetaminophen activation is one of the microsomal drug-metabolizing reactions shown in this study to be enhanced by obesity. Rats made ketotic by the administration of medium chain triacylglycerides exhibit, in addition to enhanced microsomal aniline and NDMA metabolism, large increases in a IIB1-catalyzed activity, pentoxyresorufin dealkylation, and in P450 reductase activity (41), neither of which was observed here in obese overfed rats.

Hydroxylation of *p*-nitrophenol has been proposed as a specific marker of IIE1 induction (42). We studied the effect of obesity on this reaction in detail. As with acetaminophen, aniline, and NDMA metabolism, rates of *p*-nitrophenol metabolism were higher in liver microsomes from obese rats (Table 1). A comparison of the kinetics of this microsomal reaction in obese and control animals revealed a doubling of the V_{max} (from 1.00 to 2.50 nmol of product formed/min/mg of protein) but no change in K_m (80 μM), indicating that only a single rat enzyme (or two enzymes with similar kinetic properties), elevated by obesity, catalyzed *p*-nitrophenol hydroxylation. Our results are consistent with those of Kim *et al.* (43), who examined *p*-nitrophenol hydroxylation kinetics in microsomes from untreated and pyridine-treated rats and found a substantial increase in V_{max} but no alteration of K_m values. The significant correlation ($r = 0.88$, $p < 0.01$) obtained between microsomal IIE1 contents and *p*-nitrophenol reaction rates implies that IIE1 was the predominant, if not sole, catalyst of this reaction in rat liver microsomes. Yet, antibodies against IIE1 did not completely inhibit microsomal *p*-nitrophenol hydroxylase activity in either obese or control rats. Whereas one possible explanation for this incomplete inhibition may be antibody inaccessibility to membrane-bound antigen, the pronounced inhibition of microsomal *p*-nitrophenol hydroxylation by antibodies against another P450 enzyme, IA2, suggests that IA2 also plays a role in this reaction. We have recently described IA2 participation in other microsomal activities attributed solely to IIE1, namely acetaminophen activation (28), ethanol oxidation, and aniline hydroxylation.[2] Thus, caution should be exercised when using *p*-nitrophenol, ethanol, aniline, or acetaminophen oxidation as marker activities to assess IIE1 induction, particularly when substantiating immunochemical data are lacking.

Previous studies with rats (1) have demonstrated a higher incidence of acetaminophen-promoted liver and kidney damage with obesity. The elevated content of IIE1 in liver microsomes from obese rats and the resultant increase in microsomal acetaminophen bioactivation shown here provide a logical explanation for the enhanced susceptibility of these animals to acetaminophen toxicity. The capacity of obesity produced by a high fat diet to increase hepatic IIE1 concentration could have significant implications for humans with this most prevalent disease. Considering that the rat model of obesity mimics many key biochemical changes associated with the human disease (44) and that the obesity-producing diet used here reflects to some degree the human diet, IIE1 induction may accompany the obese state in humans. Indeed, enhanced bioactivation by IIE1 of therapeutic agents (e.g., acetaminophen, enflurane) and environmental pollutants may contribute to the higher incidence of occult liver disease and certain cancers observed in obese individuals.

Acknowledgments

The authors would like to gratefully acknowledge Mrs. Michelle Mouck and Mr. Christopher L. Sorge for skillful technical assistance.

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Send reprint requests to: Judy L. Raucy, University of New Mexico, Toxicology Program, College of Pharmacy, Albuquerque, NM 87131.