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LAURIC ACID HYDROXYLATION IN HUMAN LIVER AND KIDNEY CORTEX MICROSOMES*

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Abstract—The $\omega-$ and $(\omega-1)$-hydroxylation of the medium-chain fatty acid, dodecanoic or lauric acid, was studied in liver and kidney cortex microsomes from seven human cadavers. The rates of laurate hydroxylation in human liver microsomes were found to exceed the rates recorded in human kidney cortex microsomes by 4- to 30-fold. The mean specific activity of laurate hydroxylation from the seven human kidneys was six to fourteen times lower than the specific activities found in pig, rat or hamster kidney microsomes. The effects of several known inhibitors of the liver microsomal cytochrome P-450-dependent mono-oxygenase system were also studied. Metyrapone preferentially inhibited the $(\omega-1)$-hydroxylase activity of human liver microsomes, but did not affect the ω-hydroxylation reaction. In the presence of 7,8-benzoflavone, the human liver microsomal $(\omega-1)$-hydroxylase activity was stimulated, but an inhibitory effect was observed on the ω-hydroxylation reaction. 2-Diethylaminoethyl 2,2-diphenylvalerate (SKF 525A) inhibited both hydroxylase activities in human liver microsomes. Neither metyrapone nor SKF 525A inhibited the laurate hydroxylation reactions catalyzed by human kidney microsomes. These studies indicate that the cytochrome P-450-mediated hydroxylations of medium chain fatty acids in human kidney cortex microsomes are much less active than in kidneys of other species investigated. The effects of the inhibitors, metyrapone and SKF 525A, on $\omega-$ and $(\omega-1)$-hydroxylation of laurate in human liver and kidney microsomes were similar to the effects reported in other mammalian species.

The $\omega-$ and $(\omega-1)$-fatty acid hydroxylase systems in liver [1–5] and kidney [6–8] microsomes are mediated by the cytochrome P-450 mono-oxygenase system. The renal microsomal electron transport system has been studied extensively in the rat and shows a unique substrate specificity for medium chain fatty acids [9]. Although the specific content of rat cytochrome P-450 from kidney cortex microsomes is only one-third as large as that of liver microsomes, fatty acids are metabolized by kidney microsomes with a specific activity equal to that of liver microsomes [9]. Substrates which have been used to characterize the liver microsomal cytochrome P-450 mono-oxygenase system, such as aminopyrine, benzphetamine, ethylmorphine, and steroids, are metabolized only to a small extent or not at all by the kidney microsomal system [9]. Differences have also been reported in the susceptibility of the liver and kidney laurate hydroxylase systems to inhibitors of the liver microsomal cytochrome P-450 mono-oxygenase system [10]. In addition, the absorbance maximum of the carbon monoxide–cytochrome P-450 (Fe^{2+}) complex has also been reported to be different in the two organs [10].

The physiological role of $\omega-$ and $(\omega-1)$-oxidation of fatty acids has not been elucidated. In normal livers, fatty acid hydroxylation in parenchymal cells plays only a minor role in the oxidation of fatty acids [11–14]. However, the percentage of fatty acids undergoing ω-oxidation is greatly increased in livers from diabetic or starved rats. [12, 13]. The kidney ω-oxidation system has not been examined in diabetic or starved animals to date. It is also of interest that prostaglandins have also been shown to undergo $\omega-$ and $(\omega-1)$-hydroxylation when incubated with guinea pig liver microsomes [15, 16].

In view of the high specific activity found for fatty acid hydroxylation in rat kidneys, it was of interest to compare the rates of fatty acid hydroxylation in human liver and kidney microsomes. In the present study, the $\omega-$ or 12-hydroxylase and the $(\omega-1)$- or 11-hydroxylase activities of the medium chain fatty acid, lauric acid, were studied in microsomes isolated from seven paired human livers and kidneys obtained at autopsy. The specific activities measured for human fatty acid hydroxylation were also compared with values obtained in other mammalian species. The effects of three common cytochrome P-450 inhibitors, metyrapone, 2-diethylaminoethyl-2,2-diphenylvalerate (SKF 525A), and 7,8-benzoflavone, were also studied.

MATERIALS AND METHODS

NADPH and NADH were purchased from P-L Biochemicals (Milwaukee, WI). Lauric acid, isocitrate dehydrogenase, and DL-isocitric acid were obtained from the Sigma Chemical Co. (St. Louis, MO). Mono- and di-basic potassium phosphate, glycerol, magnesium chloride, and petroleum ether were purchased from the Mallinckrodt Co. (St. Louis, MO). Succinic acid and ethylenediaminetetraacetic acid (EDTA) were

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Table 1. Description of the age, sex, race and cause of death of the seven human cases studied

Case No.	Age	Sex	Race	Cause of death	Hours before organ removal*
1	41	M†	Caucasian	Gunshot wound	5
2	41	M	Caucasian	ASCVD†	4
3	21	M	Caucasian	Multiple injuries	7
4	52	M	Caucasian	ASCVD	2
5	54	M	Caucasian	ASCVD	3
6	26	M	Negro	Multiple injuries	3
7	42	M	Caucasian	ASCVD	3

* This is the approximate time, in hours, between death and the removal of organs from the cadaver.

† M = male; ASCVD = arteriosclerotic cardiovascular disease.

purchased from the Baker Co. (Phillipsburg, NJ). Diethyl ether was purchased from Fisher (Pittsburgh, PA). [1-^{14}C]Lauric acid and 2,5-diphenyloxazole (PPO) were purchased from Amersham-Searle (Arlington Heights, IL). Triton X-100 was obtained from Research Products Inc. (Elk Grove Village, IL). Boron trifluoride-methanol (14%) was purchased from Analabs (North Haven, CT). 7,8-Benzoflavone was purchased from Aldrich (Milwaukee, WI). SKF 525A was a gift from Smith, Kline and French Laboratories (Philadelphia, PA) and metyrapone was a gift from CIBA Pharmaceuticals (Summit, NJ).

Human livers and kidneys were obtained from seven males, 2–7 hr post-mortem. The age, race and cause of death of the subjects studied are given in Table 1.

Preparation of microsomes

Human liver. Liver homogenates were prepared by adding three parts of cold 0.25 M sucrose–0.05 M potassium phosphate buffer (pH 7.7), containing 1.0 mM EDTA, to one part of liver (wet wt) which had been perfused throughly with a 0.9% NaCl–0.05 M Tricine solution (pH 8.0). The liver was homogenized in a Waring blender using two 15-sec bursts followed by filtration through a double layer of cheesecloth. The homogenates were centrifuged at 5000 *g* for 15 min to remove cell debris and nuclei. The resulting supernatant fraction was centrifuged at 11,400 *g* for 15 min to remove mitochondria. Microsomes were obtained by centrifuging the 11,400 *g* supernatant fraction in a Spinco type 30 rotor at 78,000 *g* (average) for 60 min. Liver microsomal pellets were washed in 0.05 M potassium phosphate (pH 7.7), containing 0.1 mM EDTA and 0.02% sodium azide. All centrifugations were carried out at 4°.

Human kidneys. Kidneys were perfused with 1 liter of 0.9% NaCl–0.05 M Tricine (pH 8.0), and the cortex was then dissected from the medulla. Kidney homogenates were prepared by adding four parts of cold 0.05 M potassium phosphate (pH 7.7), containing 1.0 mM EDTA and 20% glycerol, to one part of cold kidney cortex tissue (wet wt). Homogenization of the kidney tissue was first performed in a Waring blender and then repeated in a teflon–glass homogenizer. The homogenate was filtered through a double layer of cheesecloth and centrifuged at 5000 *g* for 30 min to remove cell debris and nuclei. The resulting supernatant fraction was centrifuged at 22,000 *g* for 15 min to remove mitochondria. The supernatant fraction was transferred and recentrifuged at 78,000 *g* (average) for 60 min. The microsomal pellets were washed in 0.05 M potassium phosphate buffer (pH 7.7), containing 0.15 M KCl and 20% glycerol, and recentrifuged. It was found that recovery of the kidney microsomal enzyme activities was improved if glycerol was used in the buffers during the preparation of the kidney cortex microsomes. Microsomes were resuspended in 0.05 M potassium phosphate (pH 7.7) containing 0.1 mM EDTA, 0.02% sodium azide, and 20% glycerol.

Pig liver. Microsomes from pig liver were prepared by a modification of the procedure used for human liver microsomes. The livers were perfused with 0.9% NaCl and homogenized in three parts of 0.25 M sucrose–0.01 M Tris–HCl (pH 7.8), containing 1.0 mM EDTA, to one part of liver (wet wt). The remainder of the procedure was identical for the preparation of human liver microsomes.

Pig kidney. The cortex was dissected away from the medulla, cut into thin slices, and placed in cold 0.9% NaCl. Kidney homogenates were prepared by adding four parts of 0.02 M Tris–HCl (pH 7.8), containing 1.0 mM EDTA, to one part of kidney cortex tissue (wet wt). Homogenizations were performed in a Waring blender, followed by filtration through a double layer of cheesecloth. The homogenates were centrifuged at 5000 *g* for 30 min to remove cell debris and nuclei. The resulting supernatant fraction was centrifuged at 22,500 *g* for 15 min to remove mitochondria. The supernatant fraction, following removal of mitochondria, was centrifuged at 78,000 *g* (average) for 60 min. The microsomal pellet was washed in 0.15 M KCl and recentrifuged. The microsomal pellet was resuspended in 0.05 M potassium phosphate buffer (pH 7.7), containing 0.1 mM EDTA and 0.02% sodium azide.

Rodent liver and kidney microsomes. Kidney cortex and liver microsomes from Sprague–Dawley rats and Syrian hamsters were prepared by the method of Remmer *et al.* [17].

Enzymes assays

Laurate hydroxylation was determined by a modification of the method of Prough *et al.* [18]. Final incubations contained 1 mg of liver microsomes and 1.5–4.0 mg of kidney microsomes in 2 ml of 50 mM potassium phosphate (pH 7.5). [1-^{14}C]Potassium laurate (0.5 μCi/μmole) was added at a final concentration of 100 μM to the reaction mixture. Metyrapone was dissolved in methanol and 7,8-benzoflavone in acetone.

Table 2. Comparison of the specific activities of laurate hydroxylation in the seven human livers and kidneys studied

Case No.	Liver Sp. act.*	Liver 12:11†	Kidney Sp. act.	Kidney 12:11	Liver:kidney‡
1	1.94	0.8	0.20	9.4	9.7
2	3.52	4.0	0.10	3.7	35.2
3	2.07	4.2	0.28	15.4	7.4
4	2.18	2.0	0.16	10.8	13.6
5	2.45	1.7	0.22	15.9	11.1
6	1.34	2.4	0.13	12.5	10.3
7	0.86	1.7	0.22	6.2	3.9
Mean	2.05 ± 0.84	2.4 ± 1.3	0.19 ± 0.06	10.6 ± 4.5	13.0 ± 10.2

* Specific activity is expressed as nmoles of 11- and 12-hydroxylaurate formed/min/mg of microsomal protein. Values represent the means of duplicate experiments.

† Ratio of the specific activity of 12-hydroxylaurate to 11-hydroxylaurate in human liver or kidney microsomes.

‡ Ratio of the specific activity of 11- and 12-hydroxylaurate formed in liver to the specific activity of 11- and 12-hydroxylaurate formed in kidney.

Control experiments containing an equal volume of methanol or acetone were performed when inhibitors were used. Samples were methylated with boron trifluoride–methanol (14%) and extracted with petroleum ether. Samples were subjected to a stream of nitrogen or air until dried. This process must be carefully monitored to prevent excessive loss of the unmetabolized methyl ester derivative of lauric acid; samples were immediately removed after the last traces of ether were evaporated because continued exposure of the dried residue to the gas stream increased losses of the radioactive-containing material. Total radioactive recovery was 60–80 per cent. Liver and kidney microsomal cytochrome b_5 and cytochrome P-450 activities, except for human kidney cytochrome P-450, were determined by the method of Omura and Sato [19] using extinction coefficients of 185 $mM^{-1}cm^{-1}$ and 91 $mM^{-1}cm^{-1}$ for cytochrome b_5 and cytochrome P-450 respectively. Human kidney microsomal cytochrome P-450 content was determined by a modification of the procedure of Jakobsson and Cinti [20] using 50 mM potassium phosphate buffer, containing 0.1 mM EDTA (pH 7.7). An extinction coefficient of 104 $mM^{-1}cm^{-1}$ was used to calculate human kidney cytochrome P-450 content [21]. Protein determinations were measured by the procedure of Lowry *et al.* [22] using human serum albumin as standard.

RESULTS

The specific activities of the human liver and kidney ω– and (ω–1)-laurate hydroxylase systems are shown in Table 2. The specific activities of total laurate hydroxylation (11- and 12-hydroxylated products) in liver microsomes were four to thirty-five times greater than the values found in the homologous kidney preparation. The range of specific activities in liver was 0.86 to 3.52 nmoles/min/mg of microsomal protein with a mean of 2.05 ± 0.84. The ratio of 12-hydroxylaurate to 11-hydroxylaurate formed in liver ranged from 0.8 to 4.2 with a mean of 2.4 ± 1.3.

The specific activity of laurate hydroxylation by human kidney microsomes ranged from 0.10 to 0.28 nmoles of 11- and 12-hydroxylaurate produced/min/mg of microsomal protein, and the mean value was approximately 10-fold less than the mean liver value. The ratio of 12-hydroxy- to 11-hydroxylaurate specific activities in the kidney microsomes was 10.6 ± 4.5.

A comparison of the specific activities of laurate hydroxylation in human liver and kidney with values

Table 3. Comparison of laurate hydroxylation and cytochrome P-450 values in human, pig, rat and hamster liver and kidney microsomes*

Species	Liver Sp. act.†	Liver 12:11	Liver Cyt. P-450§	Kidney Sp. act.	Kidney 12:11	Kidney Cyt. P-450
Human	2.05 ± 0.84	2.4	0.38 ± 0.16	0.19 ± 0.06	10.6	0.04‖
Pig	1.84 ± 0.70	1.5	0.71 ± 0.18	1.13 ± 0.60	3.0	0.28 ± 0.06
Rat	3.33 ± 0.63	1.5	0.73 ± 0.06	2.77 ± 0.50	3.0	0.33 ± 0.01
Hamster	4.20 ± 0.18	1.3	0.77 ± 0.05	2.40 ± 0.30	4.3	0.40 ± 0.06

* The human data represent the mean values obtained from seven cases. The data for pig represent the mean values of four cases. Rat and hamster data were collected from pooled microsomal preparations of six to twenty livers or kidneys repeated in triplicate.

† Specific activity is expressed in nmoles of 11- and 12-hydroxylaurate formed/min/mg, of microsomal protein.

‡ Ratio of the specific activity of 12-hydroxy- to 11-hydroxylaurate formation in liver or kidney microsomes.

§ Nanomoles of cytochrome P-450/mg of microsomal protein.

‖ The human kidney cytochrome P-450 value represents the mean of two cases.

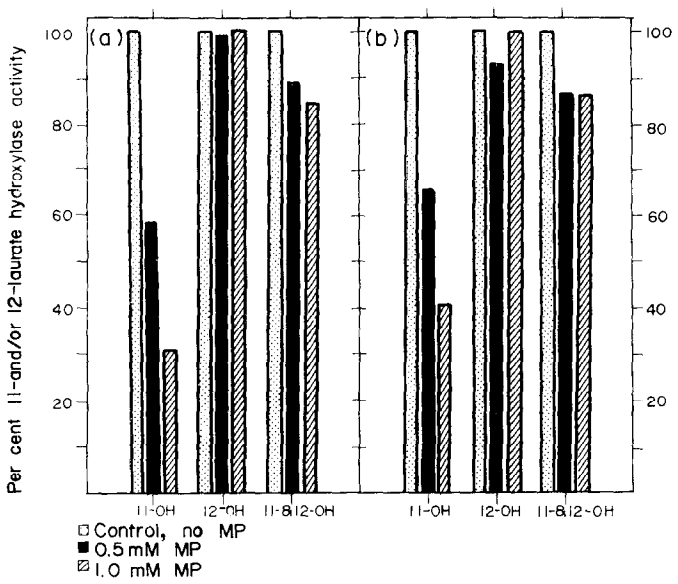

Fig. 1. Effects of metyrapone on liver 11- and 12-hydroxylaurate formation in Case 6 (panel a) and Case 7 (panel b). The specific activities of 12-hydroxylaurate formation in a and b are 0.94 and 0.53 nmole/min/mg of microsomal protein respectively. The rates of 11-hydroxylaurate formation in a and b are 0.39 and 0.31 nmole/min/mg of microsomal protein respectively.

obtained from other species is shown in Table 3. The specific activities of laurate hydroxylation were six to fourteen times greater in pig, rat or hamster kidney cortex microsomes than in human kidney microsomes. On the other hand, the values obtained in liver microsomes were similar to rates obtained in other species examined. The ratio of 12-hydroxy- to 11-hydroxylaurate formed in human liver microsomes was also similar to values obtained in pig, rat and hamster livers. The ratio of 12-hydroxy- to 11-hydroxylaurate was significantly higher in human kidney than in the other species examined.

The cytochrome P-450 contents of liver and kidney microsomes of the various species are also shown in Table 3. The low laurate hydroxylase activity in human kidney microsomes can be explained in part by the low content of cytochrome P-450 present in this tissue. Cytochrome P-450 content could not be measured accurately in all of the human kidney microsomal preparations because of mitochondrial cytochrome and

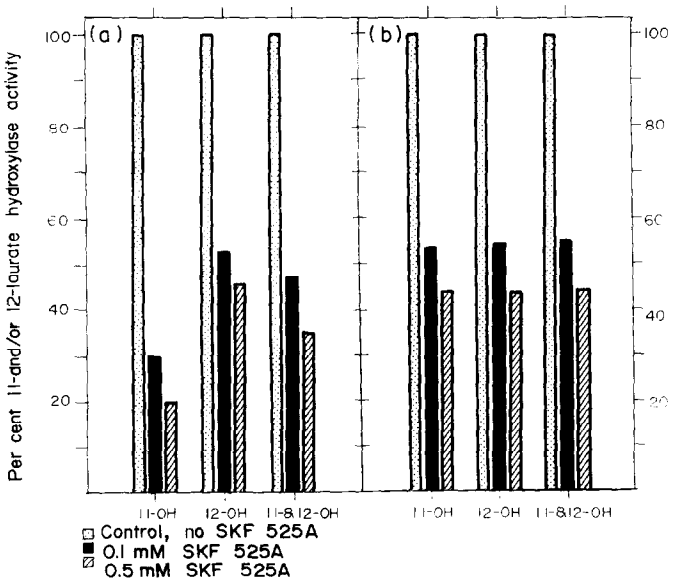

Fig. 2. Effects of SKF 525A on liver 11- and 12-hydroxylaurate formation in Case 6 (panel a) and Case 7 (panel b). The specific activities of 12-hydroxylaurate formation in a and b are 0.94 and 0.53 nmole/min/mg of microsomal protein respectively. The specific activities for the 11-hydroxylase in a and b are 0.39 and 0.31 nmole/min/mg of microsomal protein respectively.

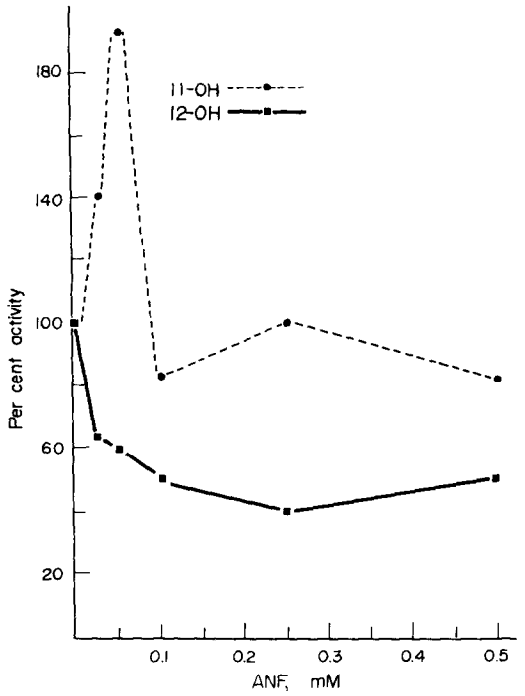

Fig. 3. Effects of 7,8-benzoflavone on liver 11- and 12-hydroxylaurate formation in one human liver microsomal preparation (Case 7). The specific activities of the 11- and 12-hydroxylase are 0.39 and 0.53 nmole/min/mg of microsomal protein respectively.

methemoglobin contamination. In the two preparations where cytochrome P-450 could be examined spectrally, an average of 0.04 nmole of the hemoprotein was present per mg of microsomal protein. This value is approximately eight to ten times lower than the cytochrome P-450 content in pig, rat and hamster kidney microsomes.

The effects of metyrapone and SKF 525A on human liver and kidney laurate hydroxylation activities were examined in cases 6 and 7. Metyrapone at 0.5 and 1.0 mM inhibited the liver laurate 11-hydroxylation activity 35 and 60 per cent respectively in the two separate cases (Fig. 1, Panels a and b). The laurate 12-hydroxylase activity was unaffected by either concentration of metyrapone. The ratio of 12-hydroxy- to 11-hydroxylaurate increased from 2.9 to 5.0 and 7.7 for case 6, and from 3.2 to 4.5 and 8.3 in case 7, at 0.5 and 1.0 mM metyrapone respectively. Metyrapone had no inhibitory effect on the laurate hydroxylase activity in human kidney microsomes (data not shown).

SKF 525A inhibited both laurate 11- and 12-hydroxylase activities in human liver microsomes as shown in Fig. 2, panels a and b). In one case, as shown in Fig. 2b, the 11-hydroxylase activity was inhibited to the same extent as the 12-hydroxylase activity, but in the other case studied (Fig. 2a), the 12-hydroxylation reaction was less sensitive to the inhibitory action of SKF 525A than was the 11-hydroxylase activity; however, in both instances, SKF 525A inhibited the 12-hydroxylase activity. SKF 525A had no effect on the kidney microsomal laurate hydroxylase activities, similar to the effect of metyrapone (data not shown).

Laurate hydroxylation was also measured in the presence of 7,8-benzoflavone in one case, and the results are shown in Fig. 3. At 0.025 and 0.05 mM 7,8-benzoflavone, the 11-hydroxylaurate was stimulated 41 and 93 per cent respectively. At higher concentrations the 11-hydroxylated derivative was inhibited 10–20 per cent. The 12-hydroxylase activity was inhibited at all concentrations used. The effect of 7,8-benzoflavone was not tested on kidney microsomes.

DISCUSSION

A comparison of the laurate hydroxylation system between human kidney and liver microsomes revealed a large difference in their specific activities. This finding is in contrast to the laurate hydroxylase system in the rat, hamster and pig where the specific activities in liver and kidney microsomes are very similar. Our results confirm the earlier findings of Jakobsson and Cinti [20] that lauric acid hydroxylation in human kidney cortex microsomes is considerably lower than the specific activities obtained in rat kidneys. In addition, we found that human liver microsomes were able to hydroxylate laurate four to thirty times faster than the kidney cortex microsomes prepared from the same human. The specific activities of laurate hydroxylation found in human liver are similar to the specific activities found in livers of other mammalian species for laurate hydroxylation, and were also similar to the aminopyrine *N*-demethylase activity recorded in these human liver microsomes [23].

The low specific activity of laurate hydroxylation in kidney cortex microsomes could be attributed in part to the lower specific content of cytochrome P-450 present. Although the microsomal cytochrome P-450 content in kidney was less than that found in liver in all species examined, the ratio of the specific content of human kidney cortex cytochrome P-450 to human liver cytochrome P-450 was substantially lower than the ratios found in the other mammalian species examined. The NADPH-cytochrome *c* reductase activities measured in the human kidney cortex microsomes were five to ten times lower than reductase activities found in pig or rat kidney cortex microsomes [23]. This may suggest that the microsomal electron transport system in the adult human kidney cortex is less developed than in other mammalian species. The lower activity of laurate hydroxylation in human kidney may also have been the result of autolytic damage to the microsomal components occurring between the time of death and the preparation of microsomes. This time period ranged from 2 to 7 hr for human tissue. The acidic nature of the renal milieu may have enhanced the activities of proteolytic and lipolytic enzymes and led to inactivation of the enzyme systems under study.

SKF 525A and metyrapone failed to inhibit laurate hydroxylase activity in human kidney microsomes. This insensitivity toward these known cytochrome P-450 inhibitors was demonstrated previously in rat kidney microsomes [16]. Metyrapone inhibited the 11-hydroxylase activity in human liver microsomes but failed to inhibit the 12-hydroxylase activity. This is in slight contrast to its action in rat liver where both hydroxylase activities were reported to be inhibited by metyrapone although the 12-hydroxylase activity was

less sensitive to the inhibitory action than the 11-hydroxylase [24]. SKF 525A inhibited both the ω- and $(\omega-1)$-laurate hydroxylase activities in the human livers studied.

A unique inhibitory effect was observed when 7,8-benzoflavone was incubated in the one human liver tested with this compound. The 11-hydroxylaurate activity exhibited a bimodal pattern toward 7,8-benzoflavone. At low concentrations of 7,8-benzoflavone (0.025 and 0.050 mM), the 11-hydroxylase activity was stimulated, but was inhibited slightly at the higher concentrations of 7,8-benzoflavone. Kapitulnik *et al.* [25] have previously reported a bimodal effect of 7,8-benzoflavone on benzo[*a*]pyrene, zoxazolamine, and antipyrine hydroxylation in human liver. The 12-hydroxylase activity was inhibited at all concentrations of 7,8-benzoflavone used.

Kupfer *et al.* [16] have recently reported an inhibition of guinea pig liver microsomal cytochrome P-450-mediated prostaglandin 19- and 20-hydroxylase activity by 7,8-benzoflavone. The 19- and 20-hydroxylated metabolites of prostaglandins and their dicarboxylic acid derivatives have been recovered from human urine and seminal fluid [26, 27]. It is an interesting possibility that the natural substrate for the cytochrome P-450-mediated ω- and $(\omega-1)$-fatty acid hydroxylase system in liver and kidney is prostaglandins.

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