Biochemical Pharmacology, Vol. 28, pp. 2835–2839.

0006-2952/79/0915-2835 $02.00/0

COMPARISON OF THE DRUG METABOLISING ABILITY OF RAT INTESTINAL MUCOSAL MICROSOMES WITH THAT OF LIVER

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(*Received* 28 *December* 1978; *accepted* 10 *May* 1979)

Abstract—A study of the drug metabolizing capabilities of rat intestinal microsomes was undertaken and a direct comparison made with analogous activity in hepatic microsomes. The cofactor requirements and K_m values of the cytochrome P-450 mediated 7-ethoxycoumarin *O*-de-ethylation in the epithelial cell microsomes was similar to that in hepatic microsomes, whereas the V_{max} values were 5–6 fold lower in the intestinal preparation. Greater differences were seen in other cytochrome P-450 dependent reactions. The K_m value for biphenyl-4-hydroxylation was an order of magnitude lower in the intestinal microsomes, whereas the 4-hydroxylation of aniline and acetanilide and the *N*-demethylation of ethylmorphine and aminopyrine, although very active in hepatic microsomes, were barely detectable in intestinal microsomes. The *O*-de-ethylation inhibition characteristics of metyrapone and 7,8-benzoflavone but not SKF 525 A, were similar in the liver and intestinal preparations.

These findings and the alterations in drug oxidation activity after PB and 3MC pretreatment suggest that there are both quantitative and qualitative differences in the cytochrome P-450's between liver and intestine. This suggestion is further supported by the difference in the safrole adduct binding spectrum with NADPH or with cumene hydroperoxide between the two tissues from control, phenobarbitone and 3-MC pretreated rats.

O-Deacetylation of 4-nitrophenyl acetate and indoxyl acetate in intestinal and liver preparations was very comparable while acetanilide *N*-deacetylation was three times more active in the liver. Glucuronidation of 1-naphthol by the intestinal microsomes showed a similar K_m but a lower V_{max} than the liver microsomes. No change was observed in any of these enzymes following pretreatment of rats with PB or with 3-MC.

The drug metabolising activity of the rat small intestine has been investigated by a number of workers. Glucuronyl transferases, esterases, sulphokinases, mixed function oxidases and the components of the microsomal electron transport system, i.e. cytochromes b_5 and P-450 and NADPH cytochrome *c* reductase have all been reported to be present. However reliable quantitative data of the type which has been widely reported for liver microsomes is in short supply due largely to the limitations in the sensitivity of enzyme assay methods used previously and the problems associated with the conventional preparation of a reproducible microsomal fraction from the intestinal tissue [1].

There is some controversy regarding the inducing properties of phenobarbitone (PB) on intestinal microsomal drug metabolising enzymes. PB has been reported not to stimulate significantly intestinal *C*-hydroxylation, *N*-hydroxylation, *N*-demethylation activities, cytochromes b_5 and P-450 levels [2–5] whereas some workers [6, 7] have noted induction of *N*- and *O*-dealkylations. In contrast, there is general agreement that the polycyclic hydrocarbons induce intestinal microsomal enzymes such as 3,4-benzpyrene hydroxylase, glucuronyl transferase, *O*- and *N*-dealkylation activities and cytochrome P-450 levels [7–10]. Benzpyrene hydroxylase has also been reported to be enhanced after pretreatment with phenothiazine, cigarette smoke, TCDD and isosafrole and to be regulated by dietary factors [11, 12]. There is a paucity of information on the influence of cytochrome P-450 inhibitors on intestinal mixed function oxidase activity and very little published data exist on the spectral interactions of intestinal microsomal cytochrome P-450 with model ligands [13, 14]. The lack of suitable intestinal microsomal preparations probably accounts largely for these deficiencies in information.

We have recently developed a method [1] for the preparation of microsomes from isolated rat intestinal mucosal cells. This gives a high yield of the components of the endoplasmic reticulum, relatively free of contamination by other cellular organelles. This preparation was used in the present study to evaluate thoroughly the drug metabolising capability of rat intestinal mucosa microsomes and compare it with that of liver microsomes.

MATERIALS AND METHODS

Animals pretreatment and preparation of microsomes. Male Wistar albino rats 180–250 g were allowed free access to water and diet (Spillers No. 1 laboratory diet, Spillers Ltd., Croydon, Surrey, England). Pretreated animals received i.p. injections of 0.5 ml corn oil or 3-methylcholanthrene (3MC) in 0.5 ml corn oil, 25 mg/kg body weight daily for 4 days or 0.5 ml NaCl 0.9% or phenobarbitone sodium (PB) in 0.5 ml NaCl 0.9%, 80 mg/kg body wt daily for 5 days. Microsomes were prepared from the livers and intestines of these animals 16 hr after the final dosing as described previously [1].

Chemicals. Phenobarbitone sodium and ethylmorphine HCl were purchased from May & Baker Ltd., Dagenham, Essex, England. 7,8-benzoflavone and *N,N*-dimethylformamide from B.D.H. Laboratories, Poole, Dorset, England. Metyrapone and SKF 525A (diethylamino ethyl-diphenyl propyl acetate–HCl) were donated by Professor K. J. Netter, University of Marburg, Germany and Smith, Kline & French Ltd., Welwyn Garden City, Hertfordshire, England. 3-Methylcholanthrene, indoxyl acetate, 1-naphthol, glucose-6-phosphate disodium salt, glucose-6-phosphate dehydrogenase type XII, UDP glucuronic acid ammonium salt, NADPH, 1-naphthylglucuronide, 4-nitrophenol, 4 nitrophenyl acetate and Brij 35 (30% solution) were obtained from Sigma Chemical Co., Kingston-upon-Thames, Surrey, England; aminopyrine from Aldrich Chemical Co. Inc., Milwaukee, WI, U.S.A. Benzphetamine HCl was a gift from Professor A. H. Beckett, Chelsea College, London, England. Fluorescamine was donated by Roche products Ltd., Welwyn Garden City, Herts, England. Biphenyl and 4-hydroxybiphenyl were recrystallised from light petroleum and methanol respectively; 7-hydroxycoumarin (Umbelliferone) was a product of Fluka, A.G. Buch 69 Switzerland and 7-ethoxycoumarin was prepared according to the method of Ullrich & Weber [5]. Safrole was purchased from Hopkin & Williams Ltd., England, n-octylamine (puriss) from Koch-Light Laboratories Ltd., Bucks, England; cumene hydroperoxide was obtained from Peroxid Chemie (Munich, West Germany).

Spectrophotometry. All spectral measurements were made at 37° using an Amino DW2 UV Vis spectrophotometer. The determination of cytochrome b_5 and P-450 concentration, substrate binding spectra and the 427/455 nm and 438 nm absorbing complexes were as described previously [1].

Enzyme assays. 7-Ethoxycoumarin *O*-de-ethylation was determined at 37° with a Perkin Elmer MPF-3 fluorimeter using 370 nm excitation wavelength and 450 nm emission wavelength [5]. Inhibitors were added after observing the increase in fluorescence for 1 min.

Biphenyl-4-hydroxylation was determined according to the method of Creaven, Parke and Williams [15].

Ethylmorphine (10 mM), (+)-benzphetamine (1 mm) and aminopyrine (2 mM) *N*-demethylation was assayed by determination of the formaldehyde produced using the Nash method according to the modification of Cochin and Axelrod [16]. Incubations were continued for 15 min in Hepes/NaOH buffer 0.1 M pH 7.6. All values were corrected for an incubated blank which contained all components of the incubation mixture except substrate which was added at the end of the incubation. The limit of sensitivity of the assay as determined from the internally generated standard curve was approximately 30 nmoles of formaldehyde.

Aniline and acetanilide hydroxylations were assayed by the determination of the production of 4-aminophenol and 4-acetamidophenol respectively [17, 18]. The assay conditions were as described above for *N*-demethylation under which the limits of sensitivity of the assays were 10 nmoles (4-aminophenol) and 50 nmoles (4-acetamidophenol).

1-Naphthol glucuronide formation was determined fluorimetrically according to Bock [19]. Assays were performed in a total volume of 0.5 ml in the presence of 2.5 nM UDP-glucuronic acid and the substrate was dissolved in water. Detergent treated (Brij '35' 0.1%) microsomes were used. An internal standard of 1-naphthol glucuronide was employed.

Aryl esterase activity was determined by modification of methods reported previously [20–22]. The detergent and EDTA were omitted and assays were performed at pH 8.0 in Tris 0.1 M HCl buffer. The substrates, 4-nitrophenyl acetate and indoxyl acetate were added in 2.5–5.0 μl of methanol.

The assay system for acetanilide *N*-deacetylation was developed from a fluorimetric method [23] for the determination of primary amines as their fluorescamine derivatives. Acetanilide (1–20 mM final concentration) and microsomal protein (0.8 mg) were incubated in 0.1 M Hepes–NaOH buffer pH 8.0 for 4–8 min. The reaction was quenched with sodium hydroxide (0.5 ml of 1 N). Aniline standards were added to the appropriate tubes and all solutions were extracted with 7 ml of butyl acetate [24]. The organic phase (5 ml) was extracted with 4 ml 0.02 N HCl. An aliquot (3 ml) of the aqueous layer was adjusted to approx pH 7.4 with 1.0 N phosphate buffer, pH 8.0 (0.5 ml) and 0.1 ml of 'aged' fluorescamine solution (2 mg/ml in acetone, stored for 24 hr at 4°) added rapidly with vigorous mixing. The solutions were then allowed to stand for 1 hr in the dark until the fluorescence stabilized before reading in a spectrofluorimeter using 400 nm excitation wavelength and 500 nm emission wavelength.

RESULTS

A comparison of the apparent K_m values of 7-ethoxycoumarin *O*-deethylation of liver with intestinal mucosa indicates a slightly lower affinity of intestinal microsomes for the substrate (Table 1). A markedly lower (ten fold) K_m for biphenyl 4-hydroxylation (was observed) in intestinal microsomes is found compared with that obtained for liver microsomes.

Attempts to obtain apparent Michaelis constants for other mono-oxygenase substrates in the intestine using assay methods developed for liver were not successful. The hydroxylation of aniline and acetanilide and the *N*-demethylation of ethylmorphine and aminopyrine were barely detectable using the methods normally used to assay these enzymes in liver. In contrast, the intestinal mucosa showed a reasonable capacity to *N*-demethylate benzphetamine, the activity being approx 10 per cent of that found in the liver.

The results obtained with two substrates for aryl esterase, i.e. 4-nitrophenyl acetate and indoxyl acetate show a close similarity between liver and intestine.

Amidase activity has been extensively studied in the liver in which acetanilide and phenacetin deacetylation appear to be primarily of microsomal origin. In the present study acetanilide *N*-deacetylation was found to be readily detectable in microsomes from both rat liver and intestinal mucosa. Attempts to determine activity in both tissues at a fixed enzyme concentration and several substrate concentrations resulted in similar, curvilinear Michaelis–Menten plots with an apparent K_m of 5×10^{-3} M. The use of a detergent (0.1% Brij-35) was without effect on either the V_{max} or on the shape of this double reciprocal plot.

The 1-naphthol glucuronidation enzyme system

Table 1. Kinetic values of various drug metabolising enzymes in intestinal mucosa and liver microsomes

Substrate	Intestinal mucosa K_m	Intestinal mucosa V_{max}	Liver K_m	Liver V_{max}
7-Ethoxycoumarin-*O*-deethylation	6.2 (5.6–7.0)	0.123 (0.114–0.139)	1.9 (1.4–2.5)	0.8 (0.72–0.86)
Biphenyl-4-hydroxylation	1.7 (1.5–2.0)	0.24 (0.21–0.32)	17 (15–18)	3.3 (2.9–3.8)
4-Nitrophenylacetate-de-esterification	5.2 (5.0–5.4)	2400 (2000–2700)	6.0 (5.5–6.7)	5700 (5000–6500)
Indoxylacetate de-esterification	2.6 (2.5–2.9)	3800 (3400–4400)	4.1 (3.1–5.2)	3400 (2900–4000)
Acetanilide deacetylation	480 (440–540)	10,100 (7200–13,300)	540 (480–670)	32,200 (24,500–39,000)
1-Naphthol glucuronidation	7.4 (6.8–8.5)	20.2 (18.5–21.7)	10 (9–12)	48.9 (38.8–54.3)
Benzphetamine-*N*-demethylation	—	1.3 (0.9–1.7)	—	13 (11.5–14.8)

K_m values are $\times 10^{-5}$ M. Maximum activities are expressed in nmoles/min/mg microsomal protein. Figures in parentheses are ranges. Each figure represents the mean of at least 3 separate experiments.

showed a somewhat greater affinity (K_m, approx. 7×10^{-5} M) in microsomes of the intestinal mucosa than in the liver (K_m approx. 1×10^{-4}). The apparent V_{max} for detergent treated (0.1% Brij-35) 'normal' intestinal microsomes was approx. 40 per cent of the liver value.

The effect of PB and 3MC pretreatment on 7-ethoxycoumarin deethylation in liver and intestine is summarised in Table 2. The response to PB and 3MC in the small intestine was similar but in liver 3MC has the greatest effect. NADPH-cytochrome *c* reductase activity was not increased in the intestinal mucosa by either pretreatment while that of the liver responded (185 per cent increase) to PB but not 3MC. The increase in intestinal cytochrome P-450 paralleled that of the deethylation of 7-ethoxycoumarin after 3MC but monooxygenase induction was greater than cytochrome P-450 enhancement after PB treatment. Cytochrome b_5 did not appear to respond to PB or 3MC pretreatments in liver or intestine. No induction of 1-naphthol glucuronidation was noted in the intestinal epithelium following either pretreatment, while the hepatic enzyme was more responsive to 3MC (3-fold increase) than to phenobarbitone (2-fold increase). Aryl esterase activity of liver and intestine mucosa was unresponsive to either inducing agent.

Qualitative spectral changes in intestinal microsomes were also observed as a result of 3MC treatment. A shift was seen in the absorption peak of the reduced cytochrome P-450–carbon monoxide complex from 450.7 to 449.1 nm and the absorption maximum of the n-octylamine difference spectrum shifted from 434 to 431 nm. The NADPH-safrole dependent 427/455 spectrum in the intestine [1] remained unaltered by PB but after 3MC the 427 nm peak persisted in the intestinal microsomes but not in those of liver. The cumene hydroperoxide mediated safrole 438 nm spectrum previously observed [1] with control intestinal microsomes was also observed in microsomes from PB and 3MC-treated animals, as was the appearance of the 427/455 nm spectrum upon the subsequent addition of sodium dithionite to all preparations. In contrast, in liver microsomes these spectra were observed to be a property of control and PB-treated preparations but they were barely detectable following 3MC treatment.

The effects of three inhibitors of hepatic drug meta-

Table 2. Effects of various inducers and inhibitors on 7-ethoxycoumarin deethylation of intestinal mucosa and liver microsomes

		Intestinal mucosa	Liver
(a) Inducers			
% induction by	phenobarbitone	225 ± 58	410 ± 67
	3-methylcholanthrene	390 ± 180	1110 ± 223
(b) Inhibitors			
K_is or % inhibition in control microsomes	SKF 525A	46% (NC) (at 5×10^{-4} M)	0.93×10^{-5} M(C)
	7,8 benzoflavone	1.3×10^{-5} M(C)	3.2×10^{-5} M(C)
	metyrapone	48% (Mixed) (at 1×10^{-6} M)	46% (Mixed) (at 2×10^{-5} M)
K_is in microsomes from 3MC preteated rats	SKF 525A	1.1×10^{-5} M(C)	1.2×10^{-5} M(c)
	7,8 benzoflavone	4.3×10^{-8} M(C)	0.82×10^{-7} M(C)

C = competitive inhibition, Mixed = mixed inhibition and NC = non-competitive inhibition.

bolising enzymes on microsomal 7-ethoxycoumarin *O*-deethylation in rat liver and intestinal mucosa are summarised in Table 2. SKF 525A was found to be equally effective as a competitive inhibitor in control and 3-methylcholanthrene treated liver microsomes with an apparent inhibition constant (K_i) of approx. 1×10^{-5} M. An identical action was also obtained in the intestinal microsomes after 3MC pretreatment. Differences were seen however in intestinal control microsomes, where the inhibitor was slightly less effective, a concentration of SKF 525A of 5×10^{-4} M producing 46% inhibition. At a 7-ethoxycoumarin concentration of 1×10^{-4} M a non-competitive type of inhibition was noted as judged by Michaelis–Menten plots, the apparent V_{max} being lowered while the K_m remained unchanged. 7,8-Benzoflavone was found to be a potent competitive inhibitor of *O*-dealkylation in the intestine and liver from both control and 3MC treated animals the effect being more marked in the microsomes from the 3MC treated animals. The intestinal and hepatic *O*-dealkylases exhibited similar sensitivity toward 7,8-benzoflavone. The inhibition in 3MC-induced preparations, especially of liver, was short-lived. *O*-Dealkylation returning to control rates within about 3 min, possibly due to the rapid metabolism of the small quantities of 7,8-benzoflavone (10^{-7} M) to a non-inhibitory metabolite(s).

The intestinal microsomes were more sensitive than those of liver to the action of metyrapone, the concentrations of metyrapone producing 50% inhibition of 7-ethoxycoumarin de-ethylation (at a substrate concentration of 10^{-4} M) being 1×10^{-6} M for intestine and 2.5×10^{-5} M for liver. The inhibition in each case appeared to be of a mixed type; the substrate affinity and apparent V_{max} were both reduced.

DISCUSSION

In contrast to low 7-ethoxycoumarin *O*-dealkylation activity in mouse jejunum, the specific activity of the mixed function oxidase which carries out this reaction in rat intestinal microsomes is approx. 15 per cent of that in rat liver. The K_m of the 7-ethoxycoumarin de-ethylase in these two tissues is very similar. Biphenyl 4-hydroxylase in the intestinal microsomes has a much lower K_m than is observed in liver, a phenomenon which is also seen with benzpyrene hydroxylation [10] and might indicate a particular ability of the intestinal tissue to metabolise aromatic hydrocarbons occuring at low concentrations in the diet. In contrast, 4-hydroxylation of aniline was barely detectable. Previous reports have described levels of aniline hydroxylase in rat intestine ranging from zero to greater than half the value of liver [2, 3, 25]. This anomaly may be attributable to the presence in the homogenate, obtained by the mucosal scrapings but not in the preparation used in the present work, of various ferri-haemoproteins and non-haem compounds capable of replacing cytochrome P-450 as the terminal oxidase in the aniline hydroxylation [26]. The ability of the small intestine to metabolise benzphetamine, was about 10 per cent of that of liver, whereas, the *N*-demethylation of aminopyrine and ethylmorphine was barely detectable in the intestinal preparations. These findings would imply distinct differences in the type and/or relative concentrations of cytochrome P-450 species in liver and intestinal microsomes. The intestinal hydrolytic enzymes are chiefly located in the microsomal fraction [1]. Our results indicate that these enzymes closely resemble those of liver in their apparent K_m values. It is interesting to note that the esterase purified by Lundqvist and Augustinsson [27] has a K_m (4-nitrophenyl acetate) of 6×10^{-5} M which is identical to that obtained in the present study. The K_m of the intestinal and liver microsomal *N*-deacetylation of acetanilide was also very similar. The affinity of the enzyme for acetanilide was lower than that found with other substrates for microsomal enzymes such as *p*-nitrophenylacetate, indoxyl acetate or 7-ethoxycoumarin. Our findings on the properties of rat liver 1-naphthol glucuronyl transferase are generally in agreement with previous findings [28] but, in the present study detergent-activation was observed in both liver and intestinal tissues which is contrary to published reports [29, 30]. This discrepancy may be related to different degrees of unmasking of microsomal glucuronyl transferase due to variation in the procedure used for preparing microsomes. Intestinal microsomes, showed a greater affinity for 1-naphthol than those for liver. No induction of aryl esterase by Pb or 3MC was observed in either rat liver or intestine. In contrast, pretreatment of rabbits with PB has been shown to cause a significant increase in hepatic hydrolysis of procaine [31]. Whether this discrepancy reflects a species difference or a differential induction of microsomal esterases remains to be established. Both PB and 3MC stimulated the glucuronyl transferase activity of the liver microsomes, but the intestinal enzyme remained almost unaltered.

The reduced CO spectrum and the safrole adduct binding spectrum with NADPH or with cumene hydroperoxide from intestinal microsomes obtained from rat pretreated with phenobarbitone indicate that the induced form of cytochrome P-450 is similar to that observed in the livers of these animals. Following 3MC pretreatment the blue spectral shift of the absorption maxima of the n-octylamine and carbon monoxide spectra in intestinal microsomes indicated the induction of a form of cytochrome P-450 similar to that induced by 3MC in liver. However, the liver and intestinal mixed function induction patterns were sufficiently different to suggest that there may be some differences in the induction mechanisms or in the form of cytochrome induced.

The findings using the inhibitors SKF 525A metyrapone and α-naphthoflavone also suggest that the cytochrome pattern in control and induced liver compared with intestine is not identical. Characterization of these differences must await studies with solubilized and/or purified enzymes and investigation with specific antibodies.

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