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Regulation of arachidonic acid metabolism by cytochrome P-450 in rabbit kidney

Michal L. SCHWARTZMAN,* Nader G. ABRAHAM,†§ Mairead A. CARROLL,* Richard D. LEVERE† and John C. McGIFF*‡

Departments of *Pharmacology and †Medicine, New York Medical College, Valhalla, NY 10595, U.S.A.

Renal microsomal cytochrome *P*-450-dependent arachidonic acid metabolism was correlated with the level of cytochrome *P*-450 in the rabbit kidney. Cobalt, an inducer of haem oxygenase, reduced cytochrome *P*-450 in both the cortex and medulla in association with a 2-fold decrease in aryl-hydrocarbon hydroxylase, an index of cytochrome *P*-450 activity, and a similar decrease in the formation of cytochrome *P*-450-dependent arachidonic acid metabolites by renal microsomes (microsomal fractions). Formation of the latter was absolutely dependent on NADPH addition and was prevented by SKF-525A, an inhibitor of cytochrome *P*-450-dependent enzymes. Arachidonate metabolites of cortical microsomes were identified by g.c.–m.s. as 20- and 19-hydroxyeicosatetraenoic acid, 11,12-epoxyeicosatrienoic acid and 11,12-dihydroxyeicosatrienoic acid. The profile of arachidonic acid metabolites was the same for the medullary microsomes. Induction of cytochrome *P*-450 by 3-methylcholanthrene and β-naphthoflavone increased cytochrome *P*-450 content and aryl-hydrocarbon hydroxylase activity by 2-fold in the cortex and medulla, and this correlated with a 2-fold increase in arachidonic acid metabolites via the cytochrome *P*-450 pathway. These changes can also be demonstrated in cells isolated from the medullary segment of the thick ascending limb of the loop of Henle, which previously have been shown to metabolize arachidonic acid specifically via the cytochrome *P*-450-dependent pathway. The specific activity for the formation of arachidonic acid metabolites by this pathway is higher in the kidney than in the liver, the highest activity being in the outer medulla, namely 7.9 μg as against 2.5 μg of arachidonic acid transformed/30 min per nmol of cytochrome *P*-450 for microsomes obtained from outer medulla and liver respectively. These findings are consistent with high levels of cytochrome *P*-450 isoenzyme(s), specific for arachidonic acid metabolism, primarily localized in the outer medulla.

INTRODUCTION

The endogenous generation of prostaglandins and other oxygenated metabolites of arachidonic acid is a complex process initiated by the release of esterified arachidonic acid from cellular lipids. Once liberated from the membrane lipids by diverse stimuli (peptide hormones, neurotransmitters and mechanical disruption), the free arachidonic acid is rapidly metabolized. In addition to the well-characterized cyclo-oxygenase and lipoxygenase pathways, arachidonic acid may be oxygenated *in vivo* via the cytochrome *P*-450-dependent mono-oxygenases, a mixed-function oxidase system strictly dependent on molecular oxygen and NADPH (Capdevila *et al.*, 1981*a*). The hepatic and renal cytochrome *P*-450 systems have been shown to metabolize arachidonic acid to the mono-hydroxyeicosatetraenoic acids, ω and $\omega-1$ oxidation products and four different EETs, which can undergo hydrolysis by epoxide hydrolase to form the corresponding diol metabolites, namely the DETs (Oliw & Oates, 1981; Oliw *et al.*, 1982; Capdevila *et al.*, 1981*b*, 1982; Morrison & Pascoe, 1981). The EETs have been shown to stimulate secretion of hormones and to inhibit chloride transport in renal tubules (Capdevila *et al.*, 1983; Snyder *et al.*, 1983; Jacobson *et al.*, 1984).

Although the hepatic cytochrome *P*-450 system is well characterized, less is known concerning the renal cytochrome *P*-450 system. The renal content of the components of the cytochrome *P*-450-dependent mixed-function-oxidase system has been measured, and in most species studied it was found to be small compared with the liver (Anders, 1980). The highest levels of cytochrome *P*-450 and the components of this system were present in the renal cortex (Zenser *et al.*, 1978; Endou, 1983; Dees *et al.*, 1980, 1982).

Since cytochrome *P*-450 exists in multiple forms (Lu & West, 1980), the predominance of one of these reactions over the other may be controlled by the isoenzyme composition of each tissue or cell type. Furthermore, a particular form may be detected only after its induction with a specific drug.

One of the means by which cytochrome *P*-450 levels can be manipulated is via haem oxygenase (EC 1.14.99.3) activity. Haem oxygenase is a rate-limiting enzyme in haem degradation. Although the enzyme is normally present in liver, kidney and other organs at very low levels, the activity of haem oxygenase can be elevated by the action of an inducer such as the heavy metal cobalt (Maines & Kappas, 1975; Ibraham *et al.*, 1981). Induction of haem oxygenase is accompanied by perturbations of haem metabolism, including enhanced degradation of cytochrome *P*-450 and associated depres-

Abbreviations used: EET, epoxyeicosatrienoic acid; DET, dihydroxyeicosatrienoic acid; 3-MC, 3-methylcholanthrene; β-NF, β-naphthoflavone; TALH, medullary segment of the thick ascending limb of Henle's loop; Glu-6-*P*, glucose 6-phosphate; PBS, phosphate-buffered saline, pH 7.4, containing 2.7 mM-KCl, 1.5 mM-KH_2PO_4, 0.5 mM-$MgCl_2$, 137 mM-NaCl, 8 mM-Na_2HPO_4 and 0.9 mM-$CaCl_2$; BP, benzo[*a*]pyrene.

‡ To whom correspondence and reprint requests should be addressed.
§ Formerly Ibraham.

sion of mono-oxygenase activities, which are dependent on this haemoprotein (Maines *et al.*, 1979; Ibraham *et al.*, 1983; Bissell & Hammaker, 1976).

The aim of this present study was to define the relationship between the cytochrome *P*-450 system and the metabolism of arachidonic acid in the rabbit kidney by: (1) linking the intrarenal distribution of cytochrome *P*-450 and the metabolism of arachidonic acid via the NADPH-dependent cytochrome *P*-450 mixed-function oxidases, and (2) relating changes in arachidonic acid metabolism to manipulation of cytochrome *P*-450 levels.

MATERIALS AND METHODS

Materials

Bovine serum albumin, Glu-6-*P*, BP, NADPH, NADP and NADH were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Benzene (thiophen-free) was obtained from Fisher Scientific Co., Fair Lawn, NJ, U.S.A., and β-NF from Aldrich Chemical Co., Milwaukee, WI, U.S.A. 3-MC was purchased from Eastman Kodak Co., Rochester, NY, U.S.A. Glucose-6-phosphate dehydrogenase was purchased from Boehringer Biochemicals. [1-^{14}C]Arachidonic acid (56.1 mCi/mmol) was obtained from Amersham Co., Arlington Heights, IL, U.S.A. All other reagents were Fisher certified reagents.

Radioactive standards of EETs and DETs were prepared by non-selective epoxidation of arachidonic acid with *m*-chloroperoxybenzoic acid as described by Chung & Scott (1974). The epoxide mixture was separated by normal-phase h.p.l.c. on a μPorasil column (3.9 mm × 30 cm; Waters Associates, Milford, MA, U.S.A.) with a solvent composed of hexane/propan-2-ol/acetic acid (955:5:1, by vol.) at a flow rate of 3 ml/min. The structures of the epoxides were determined by g.c.–m.s. analysis of the methyl ester and methyl ester after hydrogenation. The corresponding DETs were obtained by acidic aqueous hydrolysis.

Animals

New Zealand White male rabbits (3.0–3.5 kg) were injected intraperitoneally with β-NF (40 mg/day per kg) and 3-MC (40 mg/day per kg) in corn oil for 3 days. Control rabbits received corn oil for 3 days. Cobalt chloride was given subcutaneously at a dose of 25 μmol/day per kg in 0.9% NaCl for 2 days. Control rabbits received equivalent volumes of 0.9% NaCl at the same time.

Preparation of tissues

Pairs of rabbits (control and treated) were anaesthetized with sodium pentobarbital (30 mg/kg) and the kidneys perfused *in situ* with 0.9% NaCl. The liver and kidneys were removed and placed in ice-cold 0.9% NaCl. Cortex, outer medulla and inner medulla were separated from each other. The excised tissues were placed in 0.15 M-KCl and homogenized (4 ml/g wet wt.) in 10 mM-Tris buffer, pH 7.5, containing 0.25 M-sucrose, by using a glass tissue grinder operated at low speed with a Con-Torque power unit. The tissue homogenates were centrifuged at 27000 ***g*** for 20 min at 4 °C. The supernatant was centrifuged at 105000 ***g*** for 1 h at 4 °C and the resulting microsomal pellet was resuspended in 0.1 M-potassium phosphate buffer, pH 7.6, and used for determination of cytochrome *P*-450 content, aryl-hydrocarbon hydroxylase activity, haem oxygenase activity and arachidonic acid metabolism. The procedure by which TALH cells were separated from the inner stripe of the outer medulla was described in detail by Ferreri *et al.* (1984).

Cytochrome *P*-450

Cytochrome *P*-450 content was measured from the reduced CO difference spectrum by using sodium dithionite as the reducing agent (Omura & Sato, 1964). The absorbance difference between 450 and 490 nm was used to calculate the cytochrome *P*-450 content, a molar absorption coefficient of 91 $mM^{-1} \cdot cm^{-1}$ being used.

Haem oxygenase assay

Haem oxygenase activity was measured in the microsomal fraction by the method of Tenhunen *et al.* (1969), as modified by Ibraham *et al.* (1979). The volume of the assay medium was 1.0 ml and consisted of the 105000 ***g*** supernatant fraction (≡ 3.0 mg of protein) as a source of biliverdin reductase, 0.8 mM-NADP, 2 mM-Glu-6-*P*, 0.5 unit of glucose-6-phosphate dehydrogenase, 5 mg of microsomal protein and 17 μmol of haemin. The reaction was terminated after 15 min by placing the tubes on ice. The bilirubin was extracted three times with 2 ml of chloroform, and scanned in an Aminco DW2C spectrophotometer.

Aryl-hydrocarbon hydroxylase activity

The aryl-hydrocarbon hydroxylase assay was determined, with BP as the substrate, by the method of Nebert & Gelboin (1968) as modified by Ibraham *et al.* (1985). Microsomes (1 mg of protein) were incubated in a 0.1 M-potassium phosphate buffer, pH 7.6, containing $MgCl_2$ (3 mM), NADP (1 mM), Glu-6-*P* (1 mM), glucose-6-phosphate dehydrogenase (0.5 unit) and NADH (1 mM), in a total volume of 1 ml. The reaction was initiated by the addition of 50 μM-BP in 20 μl of acetone to the incubation mixture and was allowed to progress for 10 min. BP metabolites were then extracted with 1 ml of hexane/acetone (4:1, v/v). The organic phase was separated and further extracted with 0.5 ml of 1 M-NaOH and its fluorescence determined at excitation and emission wavelengths of 396 and 522 nm respectively, utilizing a Hitachi/Perkin–Elmer spectrofluorimeter MPF-3. The fluorescence was quantified by comparison with the standard curve obtained with various dilutions of 3-hydroxy-BP (kindly provided as a gift from Dr. Croci Tiziano of the American Health Foundation, Valhalla, NY, U.S.A.).

Arachidonic acid metabolism

Microsomal pellets (105000 ***g***) from kidneys of control and treated rabbits were prepared as described above and resuspended in PBS, pH 7.4. Microsomal suspensions (0.3 mg of protein) were preincubated with indomethacin (10 μM) for 10 min and further incubated with 15 μM of [^{14}C]arachidonic acid, with or without NADPH (1 mM), or SKF-525A (100 μM) or both for 30 min at 37 °C. The reactions were terminated by acidification with citric acid to pH 4.5–5.0 and extracted twice with two volumes of ethyl acetate. The final extracts were evaporated to dryness under N_2 and resuspended in 100 μl of ethyl acetate. Arachidonic acid metabolites were separated by t.l.c. or by h.p.l.c.

T.l.c. separation was performed on silica-gel G plates by using the upper phase of ethyl acetate/iso-octane/

acetic acid/water (11:5:2:10, by vol.) as the solvent system. Radioactive zones were determined by autoradiography, cut out, and counted for radioactivity in a liquid-scintillation counter. Reverse-phase h.p.l.c. was performed on a C_{18} Microsorb column (250 mm × 4.6 mm; Rainin Instrument Co., Woburn, MA, U.S.A.), with a linear gradient from acetonitrile/water/acetic acid (500:500:1, by vol.) to acetonitrile/acetic acid (1000:1, v/v) at a flow rate of 1 ml/min for 40 min. Radioactivity was monitored by a flow detector (Radiomatic Instruments and Chemicals, Tampa, FL, U.S.A.). U.v. absorbance at 234 and 280 nm was monitored by a u.v. detector (model 481, Waters Associates).

Protein determination

Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin (Fraction V) as a standard.

G.c.–m.s. analysis

Radioactive peaks eluted during h.p.l.c. were extracted twice with ethyl acetate and the residue was evaporated under N_2. The methyl ester derivative was prepared by adding an ethereal solution of diazomethane for 10 min at room temperature. The trimethylsilyl derivative was then prepared by incubating the extract with 200 μl of bis(trimethylsilyl)trimethylfluoroacetamide for 2 h at room temperature. G.c.–m.s. analysis was performed on an HP model 5985A instrument (Hewlett–Packard, Palo Alto, CA, U.S.A.), equipped with HP models 7906 and 7920 data systems. The g.c. column contained 1% SE-30 (Supelco, Bellefonte, PA, U.S.A.) and was operated between 180 to 270 °C at a temperature-increase rate of 8 °C/min. The ion-source temperature was 200 °C and the electron energy was 70 eV.

RESULTS

Cytochrome *P*-450 distribution and activity in renal and hepatic microsomes (microsomal fractions)

Cytochrome *P*-450 was present in spectrally detectable amounts in hepatic and renal tissues. It should be noted that the 'outer medulla' referred to in the Tables and the Figure represents the inner stripe of the outer medulla, the zone from which we isolated the TALH cells (Ferreri *et al.*, 1984). The comparative distribution and content of liver and renal cytochrome *P*-450 and the effects of agents which either induce or suppress it are shown in Table 1. Within 48 h after cobalt treatment, cytochrome *P*-450 content in the liver and cortex was significantly decreased. The pattern of the response of outer-medullary cytochrome *P*-450 to cobalt treatment was similar to that of the cortex, a decrease of about 70–80% in cytochrome *P*-450 levels being observed. In both tissues the decrease in cytochrome *P*-450 was accompanied by the expected increase in haem oxygenase activity (Table 3). After cobalt treatment cytochrome *P*-450 was not detectable in the inner medulla. Treatment with 3-MC and β-NF resulted in an increase of almost 2-fold in liver and kidney cytochrome *P*-450. Moreover, as shown in Table 1, 72 h after 3-MC and β-NF treatment, the percentage increase in cytochrome *P*-450 content was similar in all renal zones.

Determination of cytochrome *P*-450 function was assessed by measuring aryl-hydrocarbon hydroxylase activity. Aryl-hydrocarbon hydroxylase activity was detectable in all zones of the kidney, with the highest in the cortex, the renal activity ranging from 3 to 10% of that of the liver (Table 2). Treatment with 3-MC and β-NF caused a marked increase in aryl-hydrocarbon hydroxylase activity, which correlated with the increase in cytochrome *P*-450 content (Table 1). The hepatic cytochrome *P*-450 content and aryl-hydrocarbon hydroxylase activity were increased about 65%, whereas the renal cytochrome *P*-450 and aryl-hydrocarbon hydroxylase activity increased by 2-fold or more (Tables 1 and 2). The decrease in hepatic and renal cytochrome *P*-450 produced by cobalt treatment was accompanied by a substantial decrease in aryl-hydrocarbon hydroxylase activity. Although we were unable to detect cytochrome *P*-450 spectra in the inner medulla after cobalt treatment, we could make a good assessment of aryl-hydrocarbon hydroxylase activity because of the sensitive fluorometric method used for this assay. The inner stripe of the outer medulla possesses a cytochrome *P*-450-dependent aryl-hydrocarbon hydroxylase activity more than half that of the renal cortex. Further, this activity can be manipulated by changing the tissue content of cytochrome *P*-450. This finding substantiates our original finding on the presence and induction of

Table 1. Effects of cobalt and 3-MC+β-NF on microsomal cytochrome *P*-450 content in liver and renal microsomes

Hepatic and renal microsomes were prepared from control rabbits and rabbits treated with either $CoCl_2$ (25 μmol/day per kg body wt.) for 2 days or 3-MC and β-NF (40 mg each/day per kg body wt.) for 3 days, and the cytochrome *P*-450 content was measured spectrophotometrically as described in the Materials and methods section. Values are means±S.E.M. of duplicate determinations of five separate experiments, $n = 10$. $P < 0.001$ when compared with control values for all values obtained from treated rabbits. Abbreviation used: ND, not detectable.

Source of microsomes	Treatment . . .	Cytochrome *P*-450 content (nmol/mg of protein)		
		Control	3-MC+β-NF	$CoCl_2$
Liver		0.82±0.03	1.37±0.05	0.34±0.05
Kidney				
Cortex		0.15±0.01	0.29±0.03	0.07±0.01
Outer medulla		0.06±0.01	0.12±0.01	0.02±0.01
Inner medulla		0.03±0.01	0.06±0.01	ND

Table 2. Effects of cobalt and 3-MC+β-NF on aryl-hydrocarbon hydroxylase activity in hepatic and renal microsomes

Hepatic and renal microsomes were prepared from control rabbits and rabbits treated with either cobalt (25 μmol/day per kg body wt.) for 2 days or 3-MC+β-NF (40 mg each/day per kg body wt.) for 3 days, and the activities of aryl-hydrocarbon hydroxylase, using BP as the substrate, were measured as described in the Materials and methods section. Each point represents the average of two measurements on five rabbits for each treatment. Values are the means±S.E.M. $P < 0.005$ when compared with control values for all values obtained from treated rabbits.

Source of microsomes	Aryl-hydrocarbon hydroxylase activity (pmol of 3-hydroxybenzo[a]pyrene/min per mg of protein)		
Treatment ...	Control	3-MC+β-NF	$CoCl_2$
Liver	235.43±8.15	388.18±35.21	77.01±14.81
Kidney			
Cortex	22.81±1.97	53.47±8.40	9.50±0.85
Outer medulla	13.25±0.79	40.05±3.92	6.61±1.62
Inner medulla	8.38±0.08	17.53±1.68	3.49±0.81

Table 3. Effect of cobalt on haem oxygenase in hepatic and renal microsomes

Rabbits were treated subcutaneously with $CoCl_2$ (25 μmol/kg body wt.) once a day for 2 days. At 24 h after the second injection, hepatic and renal microsomes were prepared as described in the Materials and methods section and were used as the source of haem oxygenase. 3-MC and β-NF (40 mg each/kg body wt.) were injected intraperitoneally for 3 days as described in the Materials and methods section. Values are the means±S.E.M. of duplicate determination on five different rabbits for each treatment. $P < 0.001$ when compared with control values. Abbreviation used: NS, not significant.

Source of microsomes	Haem oxygenase activity (nmol of bilirubin/h per mg of protein)		
Treatment ...	Control	$CoCl_2$	3-MC+β-NF
Liver	1.29±0.09	10.11±1.50	1.02±0.08
Kidney			
Cortex	1.87±0.22	6.13±0.90	1.20±0.10
Outer medulla	1.90±0.28	8.02±1.72	1.30±0.12
Inner medulla	1.03±0.09	1.20±0.15 (NS)	0.80±0.07

cytochrome *P*-450 in the inner stripe of the outer medulla (Ferreri *et al.*, 1984).

Distribution and comparative activities of haem oxygenase in renal and hepatic microsomes

The basal level of haem oxygenase was studied, for the first time, regionally in the kidney (Table 3). After treatment of rabbits with cobalt, the liver haem oxygenase was increased up to 8-fold. In some experiments, the increase of hepatic haem oxygenase reached 20-fold. Zonal variations were seen in the kidney: namely, in the cortex and outer medulla, haem oxygenase activity increased 3–4-fold in response to $CoCl_2$, whereas it was unaltered in the inner medulla. The outer medulla displayed an exceedingly high level of haem oxygenase activity, the specific activity being similar to that of the cortical enzyme. Therefore, the kidney may be considered another site for haem degradation in addition to the liver, an organ well known for its prominent haem degradative activity. Administration of 3-MC and β-NF caused a consistent decrease of 20–30% in haem oxygenase activity when compared with that of the control (Table 3).

Arachidonic acid metabolism by renal microsomes via cytochrome *P*-450-dependent mono-oxygenase

Metabolites of arachidonic acid formed by cytochrome *P*-450-dependent enzyme(s) were defined as those metabolites whose formation was absolutely dependent on NADPH addition, inhibited by SKF-525A, an inhibitor of cytochrome *P*-450-dependent enzymes via a type-I binding mechanism (Parnham *et al.*, 1981), and unaffected by indomethacin. Arachidonic acid was converted into cytochrome *P*-450-derived metabolites by microsomes obtained from hepatic and renal tissues. Figs. 1(*a*) and 1(*b*) represent h.p.l.c. separation of arachidonic acid metabolites formed by cortical and outer-medullary microsomes respectively in the presence of NADPH. In the cortex, most of the radioactivity associated with metabolites eluted between 18 and 22 min and consisted of a major peak at 19.8 min, compound II, and two smaller peaks at 18 min and 21 min, compounds I and III respectively. In addition, there was a radioactive peak at 28.5 min, compound IV, with a similar retention time to that of the 11,12-EET standard. Further, these radioactive metabolites did not demonstrate u.v. absorbance at 234 (Fig. 1*a*) or at 280 nm

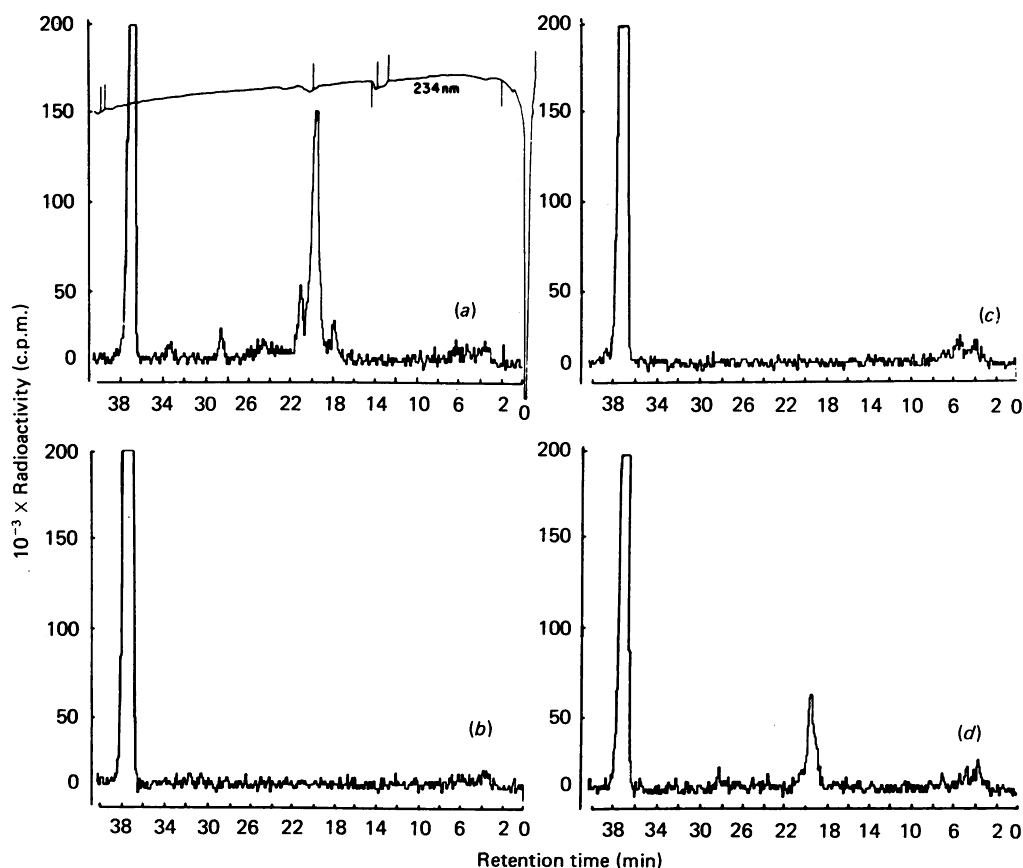

Fig. 1. Reverse-phase h.p.l.c. of arachidonic acid metabolites formed by renal microsomes

Microsomes (300 μg of protein) were incubated with [^{14}C]arachidonic acid (0.4 μCi) plus unlabelled arachidonic acid to a final concentration of 15 μM for 10 min at 37 °C. (*a*) Control incubation in the presence of 1 mM-NADPH; (*b*) outer-medullary microsomes in the presence of NADPH-boiled microsomes; (*c*) cortical or outer-medullary microsomes in the absence of NADPH; and (*d*) cortical microsomes were preincubated with SKF-5235A (100 μM) for 10 min and then incubated in the presence of 1 mM-NADPH. The reactions were terminated by acidification, and arachidonic acid metabolites were extracted and separated by reverse-phase h.p.l.c. as described in the Materials and methods section.

(result not shown), indicating the lack of diene or triene structures which are typical of lipoxygenase metabolites of arachidonic acid. The formation of these arachidonic acid metabolites was enzyme-dependent, since they are not generated by boiled microsomes (results not shown). When NADPH was omitted from the incubation medium, these metabolites were not formed (Fig. 1*c*). Preincubation of microsomes with 100 μM-SKF-525A inhibited the conversion of arachidonic acid into compounds I–IV by more than 70% (Fig. 1*d*). For example, the production of compound II was reduced after preincubation with SKF-525A from 0.58 to 0.16 μg/30 min per mg of protein.

The mass spectra of the methyl ester trimethylsilyl derivative of compounds II and III had many features in common with a *c* value of 20.9 and 21.3 respectively. Ions in the upper mass range were noted at m/z 406 (M^+), 391 (M^+-15), 375 (M^+-31), and 359 ($M^+-15-31$) for both compounds. After hydrogenation, a major ion at m/z 117 [base peak fragment of $CH_3CHOSi(CH_3)_3$] was noted for compound II, suggesting the structure of 19-hydroxyeicosatetraenoic acid. The hydrogenated methyl ester trimethylsilyl derivative of compound II showed a major ion at m/z 103 [fragment of $CH_2OSi(CH_3)_3$], suggesting the structure of 20-hydroxy-eiocosatetraenoic acid. These results are consistent with those reported by Oliw & Oates (1981), Oliw *et al.* (1982) and Morrison & Pascoe (1981). The methyl ester derivative of compound IV showed characteristic ions of an epoxide metabolite with fragments at m/z 334 (M^+), 316 (M^+-18) and 303 (M^+-31). The mass spectra of the hydrogenated methyl ester derivative of compound IV showed fragments at m/z 340 (M^+), 322 (M^+-18) and 309 (M^+-31) and ions of high intensity at m/z 155 [$M^+-(CH_2)_9CO_2CH_3$] and 227 [$M^+-(155-C_2H_2O)$], suggesting the structure of 11,12-EET. The *c* value for compound IV was 20.2. The amount of compound I was not sufficient for obtaining its mass spectrum; however, its retention time on h.p.l.c. was similar to that of 11,12-DET. The patterns of arachidonic acid conversions via an NADPH-dependent mechanism, under the experimental conditions we have used, were similar in cortical and outer-medullary microsomes, as shown in Figs. 2(*a*) and 2(*b*).

Relationship of renal microsomal arachidonic acid metabolism to the activity of the cytochrome *P*-450-dependent pathway

The formation of cytochrome *P*-450-dependent arachidonic acid metabolites by renal microsomes corresponds

Table 4. Effect of cobalt and 3-MC+β-NF treatments on arachidonic acid metabolism by hepatic and renal microsomes

Microsomes were prepared from control rabbits and rabbits treated with either cobalt or 3-MC+β-NF, and microsomal metabolism of arachidonic acid was assayed as described in the Materials and methods section. Values are the means±S.E.M. for three separate experiments. $P < 0.01$ when compared to control values for all values obtained from treated rabbits.

Source of microsomes	Treatment ...	Cytochrome *P*-450-dependent arachidonic acid metabolism (μg of arachidonic acid converted/30 min per mg of protein)		
		Control	3-MC+β-NF	$CoCl_2$
Liver		2.02±0.31	2.79±0.14	1.11±0.03
Kidney				
Cortex		0.68±0.05	1.63±0.26	0.29±0.10
Outer medulla		0.49±0.06	1.06±0.40	0.24±0.09
Inner medulla		0.10±0.02	0.29±0.12	0.06±0.03

to the distribution of the cytochrome *P*-450 throughout the kidney (Table 4). The specific activity of the cytochrome *P*-450-dependent arachidonate metabolites formed by renal microsomes was relatively higher than that of liver microsomes. For example, whereas outer-medullary cytochrome *P*-450 activity is 7.5% of the hepatic cytochrome *P*-450, the extent to which arachidonic acid is metabolized by the outer-medullary cytochrome *P*-450-dependent enzymes is 24% of that metabolized by the hepatic system (Table 4). This may reflect the presence of a high level of a specific renal cytochrome *P*-450 isoenzyme(s) responsible for arachidonic acid metabolism.

The formation of arachidonic acid metabolites via the cytochrome *P*-450-dependent pathway was affected by the level of cytochrome *P*-450. Thus induction of cytochrome *P*-450 by 3-MC and β-NF caused about a 2-fold increase in the amount of arachidonic acid converted into these metabolites (Table 4). Similarly, induction of haem oxygenase reduced the ability of these tissues to convert arachidonic acid to cytochrome *P*-450-dependent metabolites by 50% (Table 4). Addition of $CoCl_2$ *in vitro* at concentrations of 1–100 μM did not affect arachidonic acid metabolism.

The data in Tables 1 and 4 further demonstrate that, under any experimental conditions, the amount of arachidonic acid converted via the NADPH-dependent pathway in relation to the amount of cytochrome *P*-450 is not changed; i.e., for the cortex, the values are: 4.6, 5.5 and 4.0 μg of arachidonic acid converted/30 min per nmol of cytochrome *P*-450 for the control, 3-MC+β-NF- and cobalt-treated rabbits respectively. Moreover, the specific activity of the cytochrome *P*-450-dependent arachidonic acid metabolism is higher in renal microsomes than in the liver, with the highest activity in the outer medulla rather than in the liver (Table 5).

Relationship between TALH-cell arachidonate metabolism and the activity of the cytochrome *P*-450-dependent pathway

The inner stripe of the outer medulla is the zone from which we isolated epithelial cells obtained primarily from TALH segment (Ferreri *et al.*, 1984; Schwartzman *et al.*, 1985*a*). Metabolism of arachidonic acid by TALH cells responded to induction and depletion of cytochrome *P*-450 much as did hepatic and renal microsomes. TALH cells isolated from rabbits treated with 3-MC and β-NF showed a 2-fold increase in the conversion of arachidonic acid into biologically active compounds, as has been recently described (Schwartzman *et al.*, 1985*b*), namely 0.08±0.01 as against 0.14±0.02 μg of arachidonic acid converted/min per mg of protein ($n = 3$, $P < 0.01$) for control and treated rabbits respectively. Depletion of cytochrome *P*-450 with $CoCl_2$ resulted in a 60% decrease in the conversion of arachidonic acid by TALH cells, namely 0.11±0.05 as against 0.05 μg/min per mg ($n = 3$, $P < 0.01$) for control and treated rabbits respectively.

DISCUSSION

The medulla as well as the cortex contain a significant amount of cytochrome *P*-450 which is involved, not only in detoxification of foreign compounds such as benzopyrene, but also in the generation of metabolites from arachidonic acid. The present study reveals a close relationship between induction of cytochrome *P*-450 content and enzyme activity and metabolism of arachidonic acid. Further, the outer medulla possessed half of the cortical cytochrome *P*-450-mediated aryl-hydrocarbon hydroxylase activity, a finding that accords with

Table 5. Specific activities of the NADPH-dependent cytochrome *P*-450 pathway of arachidonic acid

Microsomes were prepared from liver and kidneys of untreated rabbits and assayed for arachidonic acid metabolism and cytochrome *P*-450 content as described in the Materials and methods section. Values are means±S.E.M., $n = 3$.

Source of microsomes	Cytochrome *P*-450-dependent arachidonic acid metabolism (μg of arachidonic acid converted/30 min per nmol of cytochrome *P*-450)
Liver	2.48±0.31
Kidney	
Cortex	4.63±0.31
Outer medulla	7.94±0.71
Inner medulla	3.01±0.54

NADPH-dependent arachidonic acid metabolism by this zone. Zenser *et al.* (1978) have reported that the cortex was the only zone of the kidney containing cytochrome *P*-450 and aryl-hydrocarbon hydroxylase activity in untreated rabbits, although it was possible to induce medullary activity after treatment of rabbits with 3-MC. In addition, they showed that the inner medulla possessed high activity of dodecanoic (lauric) acid hydroxylase, which is also a cytochrome *P*-450-dependent enzyme. Thus restriction of the renal cytochrome *P*-450 system to structures having primarily cortical representation, such as the proximal convoluted tubules (Endou, 1983), is untenable.

Induction of haem oxygenase after cobalt treatment produced profound and protracted alterations in several key aspects of the mono-oxygenase systems, specifically the content and functional activity of cytochrome *P*-450. These alterations are of particular interest in that the perturbation produced in cytochrome *P*-450 resulted in similar directional alterations of arachidonic acid metabolism, reaffirming the close relationship that exists in the kidney between *P*-450-dependent mono-oxygenases and metabolism of arachidonic acid. The inability of cobalt to induce inner-medullary haem oxygenase activity suggests that the activity of the enzyme may be controlled by (a) factor(s) that differs from those regulating the enzyme activity in the liver and the renal cortex and outer medulla, or that the amount of cobalt reaching the inner medulla is insufficient to induce haem oxygenase but able to interfere with cytochrome *P*-450 synthesis and activity (Tables 1 and 2). Haem oxygenase activity was decreased in response to administration of 3-MC and β-NF. As haem oxygenase is known to be induced by its own substrate, haem (Ibraham *et al.*, 1983), the decrease in haem oxygenase activity after 3-MC and β-NF pretreatment may be due to a reduced endogenous haem pool as a result of accelerated incorporation of haem into the apoprotein of cytochrome *P*-450 (Table 3).

Microsomal preparations of renal tissues, especially the cortex, have been shown to metabolize arachidonic acid in the presence of NADPH to several oxygenated metabolites, namely the *vic*-diols, trihydroxyeicosatrienoic acids and ω and $\omega-1$ hydroxylation metabolites (Oliw & Oates, 1981; Oliw *et al.*, 1982; Morrison & Pascoe, 1981). In these studies, cytochrome *P*-450-dependent mixed-function oxidases appeared to be involved in the formation of these metabolites, because of the dependency on NADPH, the inhibitory effect of CO and the inability of EDTA or Fe^{2+} to alter the formation of these oxygenated metabolites.

In the present study we have demonstrated that, in addition to the cortical microsomes, the outer-medullary microsomes converted arachidonic acid in the presence of NADPH mainly via ω and $\omega-1$ hydroxylation pathways to form the 20- and 19-hydroxyeicosatetraenoic acids. This is in agreement with the results reported by other investigators (Oliw & Oates, 1981; Morrison & Pascoe, 1981). In addition, renal microsomes, as shown in the present study and first reported by Oliw & Oates (1981) and Oliw *et al.* (1982), possess epoxygenase activity as well as epoxide hydrolase activity and, thereby, can convert arachidonic acid into epoxides and *vic*-diols. The formation of these metabolites was dependent on NADPH addition and was inhibited by SKF-525A, but was unaffected by indomethacin, thus suggesting the involvement of cytochrome *P*-450. The inner-medullary microsomes contain small amounts of cytochrome *P*-450, which is reflected by low conversion of arachidonic acid into the NADPH-dependent arachidonate metabolites (Tables 1 and 4). Indeed, the formation of these metabolites was closely related to the activity of cytochrome *P*-450 in a specific tissue. Thus induction of cytochrome *P*-450 content increased the formation of NADPH-dependent metabolites of arachidonic acid to the same extent (2-fold) as aryl hydrocarbon hydroxylase activity, which is a cytochrome *P*-450-dependent enzyme. Similarly, depletion of cytochrome *P*-450 by inducing haem oxygenase activity caused a corresponding decrease in arachidonic acid metabolite formation (about 50%). The close relationship between cytochrome *P*-450 and arachidonic acid metabolism was strongly supported by the demonstration that the amount of arachidonic acid converted into these metabolites when expressed on a per-nmol-of-cytochrome-*P*-450 basis, under any experimental condition (control induction or depletion), was not changed, on the basis of the results of Tables 1 and 4.

The finding that outer-medullary cytochrome *P*-450 isoenzyme(s)-specific activity directed towards arachidonic acid metabolism is much higher than that observed in the liver (Table 5) is important in terms of the potential contribution of these arachidonic acid metabolites to renal function. The cytochrome *P*-450 species that is specific for arachidonic acid metabolism appears to predominate in the kidney, an interpretation based on the comparison in the present study of the capacities of liver and kidney for the metabolism of arachidonic acid by cytochrome *P*-450-dependent enzymes. Moreover, the highest concentration of this cytochrome *P*-450 species is present in the inner stripe of the outer medulla, the zone from which TALH cells are isolated. TALH cells have been demonstrated to metabolize arachidonic acid specifically via cytochrome *P*-450-dependent enzymes to biologically active compounds (Schwartzman *et al.*, 1985*a*). Therefore it is important to study the means by which we can increase this cytochrome *P*-450 species by various inducers or drugs. This may lead to a more complete understanding of the mechanism(s) and the role of cytochrome *P*-450-dependent enzyme(s) in generating biologically active arachidonic acid metabolites (Capdevila *et al.*, 1983; Snyder *et al.*, 1983; Jacobson *et al.*, 1984; Schwartzman *et al.*, 1985*a*). In the present study we have demonstrated that the flux of arachidonic acid through the *P*-450-dependent enzyme can be controlled by manipulating the level of cytochrome *P*-450 in a given tissue.

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