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Metabolism of Prostaglandins and Xenobiotics by Adrenal Microsomal Monooxygenase in the Guinea Pig[1]

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The possibility that prostaglandins could serve as substrates for the guinea pig adrenal microsomal monooxygenase was investigated. The binding of PGE_1 to adrenal microsomes was found to exhibit a *reverse type I* spectral change. Also PGE_1 diminished the magnitude of type I spectrum elicited by cortisol binding to adrenal microsomes. The incubation of [^{3}H]PGE_1 or of [^{3}H]PGE_2 with adrenal microsomes supplemented with NADPH yielded primarily the respective 19-hydroxy metabolite. The enzymatic activity catalyzing this hydroxylation appears to be a typical monooxygenase, requiring NADPH for activity and being strongly inhibited by metyrapone, SKF 525A, and cytochrome *c*. Carbon monoxide at a ratio of 9:1 to oxygen moderately inhibited the hydroxylation of PGE_1. Whereas the liver catalyzed the hydroxylation of PGE_1 and PGA_1 equally well, the adrenal microsomes preferentially catalyzed the hydroxylation of PGE_1. This finding and the observation that α-naphthoflavone is a weak inhibitor of the adrenal PGE_1 hydroxylation points to significant differences between the adrenal and liver prostaglandin hydroxylation activities. Cortisol, which is a substrate for adrenal monooxygenase, *strongly* inhibited PGE_1 and PGE_2 hydroxylation. By contrast, certain xenobiotics (ethylmorphine, hexobarbital, benzpyrene), which are also metabolized by adrenal microsomes, only slightly inhibited the hydroxylation of PGE_1. Similarly, PGE_1 only weakly inhibited ethylmorphine and benzphetamine demethylation and hexobarbital hydroxylation. These observations suggest that adrenal microsomes contain several monooxygenases with different affinities for prostaglandins and for the different xenobiotic substrates.

The presence of a cytochrome *P*-450 monooxygenase system(s) in adrenal microsomes of the guinea pig was demonstrated several years ago. The adrenal enzyme system was found to catalyze 2α- and 6β-hydroxylation of cortisol (1–3). Subsequently it was observed that guinea pig adrenal microsomes catalyze the oxidative metabolism of various xenobiotics, such as the demethylation of *p*-chloro-*N*-methylaniline and aminopyrine (4, 5) and the hydroxylation of benzpyrene (6). In view of our earlier observations (4, 5), and the more recent findings that guinea pig adrenals metabolize ethylmorphine at about fivefold more rapid rate than the corresponding liver preparations (7), it was tempting to speculate that in the guinea pig, adrenals have a similar function in respect to drug metabolism to that of liver. Previous observations that PGA_1[4] (8, 9) and PGE_1 (9, 16, 22) are hydroxylated by guinea pig liver and kidney monooxygenase and

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[4] Abbreviations used: PGE_1, PGE_2, PGA_1, PGA_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGB_1, and PGB_2, prostaglandins E_1, E_2, A_1, A_2, $F_{1\alpha}$, $F_{2\alpha}$, B_1, and B_2; 19-OH-PG and 20-OH-PG, 19-hydroxy-PG and 20-hydroxy-PG, respectively; benzpyrene, benzo[*a*]pyrene; tlc, thin-layer chromatography; hplc, high-pressure liquid chromatography; gc, gas chromatography; ms, mass spectrometry.

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the findings that PGE_1 and $PGF_{2\alpha}$ inhibit ethylmorphine demethylation by guinea pig adrenal microsomes (27) suggested to us that PGs might serve as endogenous substrates for the adrenal microsomal monooxygenase.

In the present study we demonstrate the oxidative metabolism of PGE_1 and PGE_2 by guinea pig adrenal microsomes, describe the characteristics of the enzyme system, and compare the enzymatic activity toward PGs versus toward certain xenobiotics.

MATERIALS AND METHODS

Materials. [5,6-^{3}H]Prostaglandin E_1, 89.5 Ci/mmol and 40 Ci/mmol were purchased from New England Nuclear (Boston, Mass.) and from Amersham-Searle (Chicago, Ill.), respectively. [5,6,8,11,12,14,15-^{3}H]Prostaglandin E_2, 130–160 Ci/mmol, was purchased from New England Nuclear and Amersham. Prostaglandins E_1 and E_2 were a gift from Dr. John Pike (Upjohn Co.) and 19-hydroxy-PGE_1 methyl ester and 19-hydroxy-PGE_2 methyl ester were a gift from Dr. John Sih (Upjohn Co.). 19-Hydroxy-PGB_1 and 19-hydroxy-PGB_2 were prepared either by base hydrolysis of 19-OH-PGE_1 methylester and 19-OH-PGE_2 methyl ester, respectively, or were isolated, after base treatment of 19-OH-PGE_1 and 19-OH-PGE_2, from human semen and further purified by hplc (9, 29, 30). Dr. Kazuo Sano (Ono Pharmaceutical Co.) kindly provided 20-OH-PGE_1. Hexobarbital·Na (Evipal) was obtained from Winthrop Laboratories, Inc. [^{14}C]Hexobarbital was purchased from New England Nuclear. Glucose-6-phosphate dehydrogenase [EC 1.1.1.49], D-glucose-6-phosphate, monosodium salt, NADP, NADPH monosodium, and cytochrome *c* type III (horse heart) were purchased from Sigma Chemical Company (St. Louis, Mo.). SKF-525A·HCl (β-diethylaminoethyl diphenylpropylacetate hydrochloride) was a gift from Smith Kline and French Co. Metyrapone (metopyrone) was obtained from Ciba Company. α-Naphthoflavone was purchased from Aldrich. Thin-layer coated plates (silica gel G, E. Merck A.G., Darmstadt, West Germany) were purchased from Brinkmann Instruments. Methanol and acetonitrile for hplc were purchased from Burdick and Jackson (Muskegon, Mich.) or from Waters Associates (Milford, Mass.).

Animals and adrenals. Albino–Hartley strain male guinea pigs 450–650 g were obtained from Elm Hill Farm (Chelmsford, Mass.). Animals were killed by decapitation and adrenals were removed. In certain experiments (primarily involving isolation of metabolites), adrenals from 500- to 600-g male guinea pigs obtained frozen from Rockland Farms (Gilbertsville, Pa.) were used.

Microsomes. Adrenals were homogenized in 0.25 M sucrose with a Potter–Elvehjem glass–Teflon homogenizer. Microsomes were prepared as previously described for liver microsomes (10). Immediately prior to experimentation, microsomes were suspended in ice-cold 1.15% aqueous KCl solution and used as indicated in the text.

Incubation of [^{3}H]PGE_1 or [^{3}H]PGE_2. A final volume of 1 ml contained sodium phosphate buffer (pH 7.4, 50 mM), $MgCl_2$ (12.25 mM), microsomal preparation (ca. 2–2.5 mg), and [^{3}H]PGE_1 (91 μM, 0.5–1.0 μCi; or 100 μM, 0.05 μCi) or [^{3}H]PGE_2 (100 μM, 0.05 μCi).[5] The reaction was usually started by adding the NADPH generating system consisting of glucose-6-phosphate (38.4 mM), NADP (1.3 mM), and glucose-6-phosphate dehydrogenase (3 IU). The incubations were carried out by shaking in a Dubnoff incubator at 37°C, in an atmosphere of air. To terminate the reaction and simultaneously to achieve conversion of PGEs and metabolites to the corresponding PGB derivatives (15), 0.5 ml of aqueous NaOH (4 N) was added; the resulting solution was kept at 37°C for an additional 30 min, then was acidified with HCl to pH 2–3 (*caution*: pH should not be lower than 2). Conversion of PGE to PGB, as observed by the appearance of absorbance at 278 nm, was complete within 30 min. The acidified solutions were extracted with 3 vol of ethyl acetate and the organic phase was removed, washed with minimal volume of H_2O to neutrality, and evaporated to dryness under a stream of N_2.

Detection, isolation, quantitation, and identification of PGE metabolites. Half of the ethyl acetate extract was dissolved in 2–3 ml of 0.1 N NaOH. The basic solution was extracted with 3 × 3 ml of chloroform and the organic phase was discarded (there was little or no radioactivity in the organic phase). The aqueous phase was then acidified with 1 N HCl to pH 2–3 and extracted with 3 × 4 ml of ethyl acetate. The ethyl acetate phase was washed with minimal amount of water to neutrality and evaporated to dryness under a stream of N_2 at room temperature. The residue was dissolved in methanol and the absorbance at 278 nm was measured to assess total amount of PGB derivatives (ϵ = 27.2 mM^{-1} cm^{-1}). The methanol was evaporated to dryness under N_2 at room

[5] When detection and quantitation of product formation was based entirely on radioactive measurement (tlc analysis), a high specific radioactivity of the substrate (91 μM, 0.5–1.0 μCi) was advantageous. The much lower specific radioactivity (100 μM, 0.05 μCi) was sufficient for hplc analysis. With hplc, the radioactive labeling merely served to help assess total recovery of substrate and products and quantitation was achieved by monitoring the uv absorption of products. The hplc procedure was carried out in most of the quantitation studies.

temperature and the residue was dissolved in 40–100 μl of methanol. The amount of polar metabolite(s) was determined by tlc (see Quantitation). This information was utilized to choose an appropriate aliquot from the residual half of the ethyl acetate extract for hplc analyses.[6]

Analysis of metabolites. Aliquots of methanolic solutions containing the incubation extract and PGB derivatives were subjected to hplc. Since PGB derivatives have a λ_{max} = 278 nm, monitoring in hplc was carried out at 280 and 254 nm and, occasionally, at 313 nm, and peaks having a higher extinction at 280 nm than at 254 or 313 nm were assumed to represent PGBs and PGB derivatives. Particular attention was paid to peaks with retention times which appeared to correspond to 19-OH-PGBs and 20-OH-PGBs. Coinjection of an aliquot of the extract with authentic 19-OH-PGB_1, 19-OH-PGB_2, and 20-OH-PGB_1 demonstrated identical retention times of the respective peaks (authentic 20-OH-PGB_2 was not available). Also in early experiments the metabolites in the uv-absorbing peaks were usually eluted and found to contain radioactivity. In one such experiment the radioactivity in the 19-OH-PGB_1 fraction was about 12-fold higher and in 20-OH-PGB_1 was about 2-fold higher than in equivalent volume of eluant from non-uv-absorbing fractions. In most experiments, however, collections were not made.

[6] The high-pressure liquid chromatography system (Waters Associates) was composed of ALC/GPC 204 liquid chromatograph equipped with dual wavelength uv detector (Model 440), solvent delivery systems (Model 6000A), injector (Model U6K), and solvent flow programmer (Model 660). Reverse-phase chromatography was carried out with a 4-mm × 30-cm "fatty acid analysis" column (Waters Associates) using acetonitrile/1% acetic acid in H_2O for the free acids and acetonitrile/H_2O for the methyl esters.

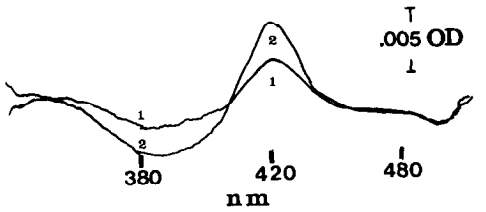

FIG. 1. Spectral interactions of PGE_1 with adrenal microsomes. Protein concentration = 0.44 mg/ml. PGE_1 was added in ethanol (2–7 μl) to the sample cuvette and equivalent volume of ethanol was added to the reference cuvette. Curve 1: $PGE_1 = 1.5 \times 10^{-4}$ M. Curve 2: $PGE_1 = 5.4 \times 10^{-4}$ M. Similar spectra were obtained with PGE_1 added in dry form (not shown).

TABLE I

HYDROXYLATION OF PGE_1 AND PGE_2 BY ADULT MALE GUINEA PIG ADRENAL MICROSOMES

Substrate	Hydroxylated products (nmol/mg protein/h)
PGE_1	3.8 (3.7, 3.9)
PGE_2	1.7 (1.6, 1.8)

Note. [^{3}H]PGE_1 (0.1 mM, 0.05 μCi) or [^{3}H]PGE_2 (0.1 mM, 0.05 μCi) were incubated for 60 min at 37°C with 2.2 mg adrenal microsomal protein from male guinea pigs. After base treatment the hydroxylated products were isolated and quantitated by hplc as described in Materials and Methods. 19-Hydroxylation was the major route of metabolism of both PGE_1 and PGE_2. Values represent a mean of duplicate incubations; individual values are given in parentheses. Linearity of product formation with time up to 1 h was observed. Incubations in the absence of NADPH did not yield detectable 19- or 20-hydroxy products. Nor was there a discernible peak in the region of metabolites when incubations were conducted in the absence of substrate.

Quantitation. Two procedures were utilized: (a) tlc: Extracts, which did not undergo hplc (for work-up, see above section on incubation), were dissolved in a few drops of methanol and were chromatographed on thin-layer plates (previously activated by heating at 110°C for 30 min). The solvent system consisted of ethyl acetate:acetic acid:2,2,4-trimethylpentane:water, 110:10:20:100. The plates were dried in air and were scanned on a Vangard Model 930 thin-layer scanner at high sensitivity (100D) at a rate of 20 cm/h. Usually, the zones containing the radioactivity were collected by scraping the gel with the help of a razor blade into scintillation vials. One milliliter methanol was added, the vials were swirled, and 5 ml Liquiflor (NEN) was added. The radioactivity was determined in a scintillation spectrometer and the amount of product formed in each zone was determined from the percentage of radioactivity in this zone (17). (b) hplc: Most of the quantitative determinations were not carried out by tlc, but were achieved by hplc as previously described (30, 33). The radioactivity in an aliquot from each incubation extract was determined by scintillation spectrometry.

Since the recoveries of 19-OH-PGBs and 20-OH-PGB_1 added to the incubation mixture were usually 90%+, the radioactivity provided a monitor of total recovery. A similar aliquot was subjected to hplc analysis and the amounts of products were determined from areas under the relevant uv-absorbing peaks, utilizing a previously constructed standard curve which exhibits linearity of peak area

versus amounts of PGs injected on hplc. In turn, the values obtained were normalized for the aliquot size and for recoveries of radioactivity. Control incubations lacking either NADPH, substrate, or microsomes did not exhibit in hplc uv-absorbing peaks in the metabolite(s) region. Hence there was no necessity of correction for nonenzymatic formation of product-like materials.

Identification of metabolites. To obtain sufficient quantities of metabolites, larger scale incubations of PGE_1 or PGE_2 were carried out under the above conditions with microsomes from adrenals removed by us from male guinea pigs or preferentially with adrenals purchased frozen. The eluants of peaks from hplc corresponding in retention time to 19-OH-PGB_1 or 19-OH-PGB_2 were collected in glass vials and the solvent was evaporated at room temperature under a stream of nitrogen. The residue was dissolved in methanol, converted to methyl ester with diazomethane, and the solvent was evaporated to dryness. Aliquots of the respective methyl ester were subjected to hplc. The methyl esters of the base-treated metabolites of PGE_1 and PGE_2 exhibited identical retention times by coinjections with that of methyl esters of authentic samples of 19-OH-PGB_1 and 19-OH-PGB_2, respectively; methyl esters of authentic 19-OH-PGB_1 and 20-OH-PGB_1 were found to distinctly separate (19-OH-PGB_1 having the shorter retention time) in hplc, using 25% acetonitrile/75% H_2O as eluting solvent system (9, 30). Subsequently, the methyl esters of the metabolites and of the authentic 19-OH-PGB_1 or 19-OH-PGB_2 were converted to their corresponding *t*-butyldimethylsilyl ethers, using *t*-butyldimethylsilyl chloride and imidazole (30). The resulting compounds were extracted with hexane and the extract was evaporated to dryness. The individual preparations were subjected to gas chromatography (gc) as previously described by us (9, 30). The retention time of the metabolites in gc was found to be identical by coinjection with the corresponding derivatized authentic 19-OH-PGB_1 and 19-OH-PGB_2, which in turn separate distinctly on gc. Gas chromatography/mass spectrometry (gc/ms) on the derivatized 19-OH-PGB_1 (inadequate amounts of the 19-OH-PGB_2 precluded analysis by gc/ms) was performed as previously described (9) and the fragmentation pattern of derivatized metabolites was compared with that of similarly derivatized authentic 19-OH-PGB_1 and 20-OH-PGB_1.

Metabolism of xenobiotics. Ethylmorphine (8 mM) and benzphetamine (1 mM) demethylation was determined as previously described (30) by the Nash procedure for formaldehyde (35). Benzo[*a*]pyrene (40 μM) and hexobarbital (0.67 mM) hydroxylations were determined radiometrically by assaying phenolic metabolites (36) and 3-hydroxyhexobarbital (23, 24), respectively.

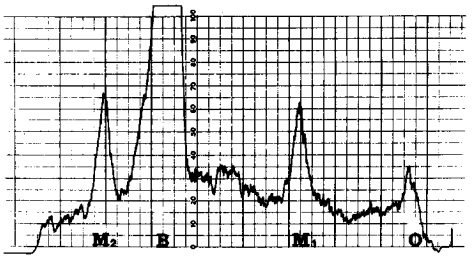

FIG. 3. Thin-layer chromatogram of a base-treated extract from an incubation of [^{3}H]PGE_1 (91 μM, 0.5 μCi) with guinea pig adrenal microsomes. O, origin; M_1, polar metabolite(s); B, PGB_1 (residual substrate); M_2, nonpolar metabolite. The small deflection prior to 0 on the abscissa indicates beginning of the 8-in. plate which was scanned at the rate of 8 in. per hour. Direction of solvent was from right to left. The R_F of M_1, B, and M_2 was 0.4, 0.7, and 0.9, respectively. The ordinate monitors radioactive counts. A chromatogram of a similarly treated extract from an incubation lacking NADPH, yielded peaks in the B and M_2 regions, but there was no peak in the M_1 region.

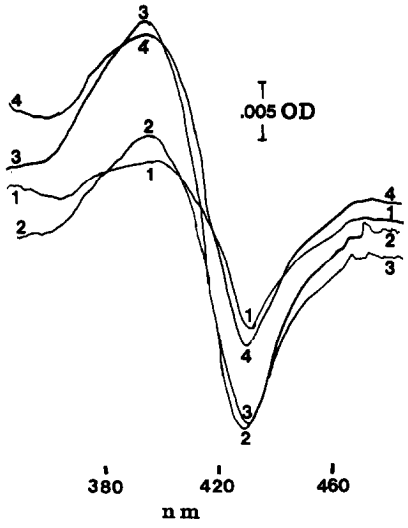

FIG. 2. Effect of PGE_1 on spectral changes elicited by addition of cortisol to adrenal microsomes. Protein concentration = 2.1 mg protein/ml. Curve 1: PGE_1 (0.8 mM) in 10 μl ethanol added to both cuvettes. Cortisol (0.046 mM) in 10 μl ethanol added to sample cuvette; vehicle added to reference cuvette. Curve 2: Cortisol (0.046 mM) in 10 μl ethanol added to sample cuvette; reference cuvette received the vehicle. Similar spectrum was obtained with cortisol added in dry form (not shown). Curve 3: Cortisol (0.096 mM) added in 20 μl ethanol as in Curve 2. Curve 4: PGE_1 (0.8 mM) in 10 μl ethanol added to both cuvettes, cortisol (0.096 mM) added to sample cuvette, vehicle added to reference cuvette.

Spectral interactions. Cortisol, PGE_1, or PGE_2 were added to adrenal microsomes in the sample cuvette as previously described (11) and the difference spectra were monitored in the DW-2 Aminco spectrophotometer in a split beam mode. When the effect of PGE_1 on cortisol-induced spectra was examined, equal amounts of PGE_1 were added to both the reference and sample cuvettes. After obtaining a baseline of equal absorbance, cortisol was added to the sample cuvette and the difference spectrum recorded.

RESULTS AND DISCUSSION

To examine whether PGE_1 exhibits binding to the adrenal microsomal cytochrome *P*-450, we determined the effect of addition of PGE_1 on the difference spectrum. The addition of PGE_1 to the adrenal microsomes yielded spectral changes which are usually referred to as *reverse type I* (12, 18), i.e., exhibiting a peak at 420 nm and a trough at about 390 nm (Fig. 1). Similar observations were obtained with bovine adrenal microsomes (13), however that study did not attempt to relate binding of PGs to metabolism. By contrast to the above observations, other investigators recently reported that PGs yield type I spectral perturbations with guinea pig adrenal microsomes (14). Currently, we have no explanation for these differences in spectral effects.

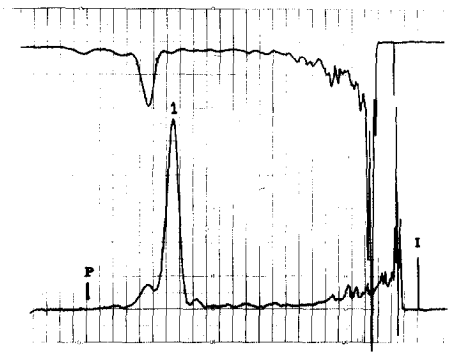

FIG. 4. hplc of a base-treated extract from an incubation of [^{3}H]PGE_1 with adrenal microsomes. I, injection point of sample; P, start of program to obtain a solvent mixture to reach 90% acetonitrile in 10 min; this procedure is carried out to elute residual substrate (PGB_1) and to wash the column of residual incubation substances. Solvent mixture: 20% acetonitrile and 80% water containing 1% acetic acid. Solvent flow rate = 2 ml/min (2500 psi); chart speed 1 cm/min. Upper scan at 254 nm and lower scan at 280 nm; the two scans are offset, hence the major peak at 254 nm corresponds to the major peak at 280 nm. Monitoring was at 0.01 AUFS (absorbance units full scale); i.e., full scale deflection represents 0.01 OD. The distance between vertical lines on scan represents 1 cm equivalent to 1 min of chromatography. The peak of interest is marked (1). The less polar peak (longer retention time) is assumed to represent a minor component (20-OH-PGB_1).

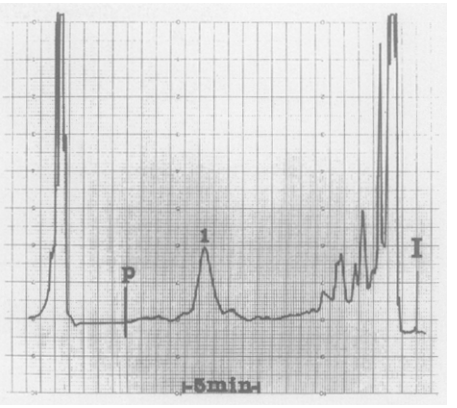

FIG. 5. hplc of a base-treated extract from incubation of [^{3}H]PGE_2 with adrenal microsomes. Injection point (I). Conditions of hplc: Solvent, 20% acetonitrile/80% H_2O (1% acetic acid) at 2 ml/min; chart speed, 1 cm/min; absorbance units full scale (AUFS) = 0.01. The scan depicts absorption at 280 nm. The metabolite of interest with higher uv absorption at 280 nm than at 254 nm is marked (1). The minute component with slightly longer retention time is assumed to represent 20-OH-PGB_2. (P) marks the initiation of 100% acetonitrile to elute residual unmetabolized PGB_2 and other incubation contaminants. The markings on the baseline delineate 5 min of chromatography.

Surprisingly, PGE_2 produced little or no spectral perturbations with guinea pig adrenal microsomes (not shown). The subsequent observation that PGE_2 is a much poorer substrate than PGE_1 for the monooxygenase-catalyzed hydroxylation (see below, Table I) supports the notion that PGE_2 has a low affinity for the adrenal *P*-450.

Since cortisol exhibits typical type I spectral changes in guinea pig adrenal microsomes (5), we examined the effect of PGE_1 on the cortisol spectral changes. The addition of PGE_1 diminished the magnitude of type I spectral changes of cortisol (Fig. 2). Examination of this effect by a

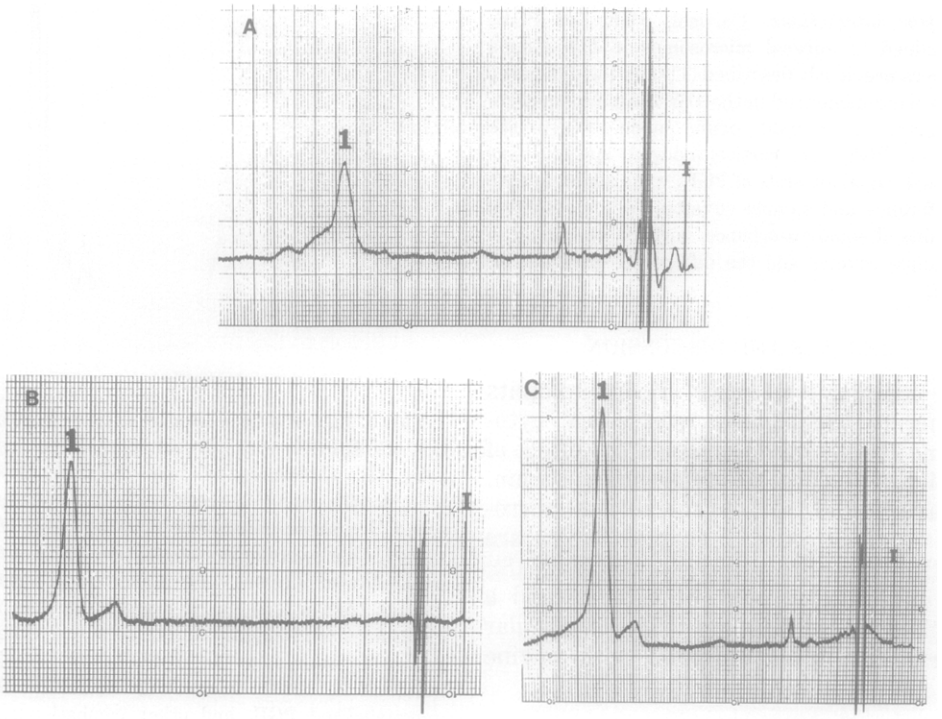

FIG. 6. (A) hplc of a portion of the isolated major polar metabolite (peak 1) from hplc of several incubations of PGE_2 which has been rechromatographed (note the less polar, longer retention time shoulder, possibly 20-OH-PGB_2). Conditions as in Fig. 5. (B) hplc of *authentic* 19-OH-PGB_2 (contains a more polar unknown component). (C) hplc of coinjection of polar metabolite. Same amount as in (A) and authentic 19-OH-PGB_2 (same amount as in (B)).

Lineweaver–Burk plot analysis demonstrated lines intersecting in the first quadrant (not shown) and suggested that the interference by PGE_1 was of a complex nature.

To determine whether PGE_1 is metabolized by the guinea pig adrenal microsomal system, radiolabeled PGE_1 was incubated with a 10,000*g* supernate of an adrenal homogenate or with a 105,000*g* pellet (microsomes) in the presence of an NADPH generating system. Base was added to stop the reaction and to simultaneously convert the residual PGE_1 and its metabolites to the corresponding PGB_1 derivatives (λ_{max} = 278 nm). A portion of the ethyl acetate extract of the incubation mixture with adrenal microsomes was subjected to tlc (Fig. 3). A similar chromatogram was obtained from incubations with 10,000*g* supernate. The chromatographic mobility of the polar metabolite (M_1), not significantly formed in the absence of NADPH, was similar to that observed with 19-hydroxy-PGB_1 (19-OH-PGB_1) and 20-hydroxy-PGB_1 (20-OH-PGB_1);[7] the tlc system used does not separate the two compounds (9), hence in most studies we utilized hplc. Currently we have no information on the nature of the nonpolar product (M_2), which is also formed in the absence of NADPH; the possibility that M_2 represents a dehydration product of PGB_1, i.e., 13-14,15-16 diene, occasionally formed during acidification and extraction was not excluded.

To determine the nature of the polar metabolite(s) of PGE_1, a portion of the

[7] The chromatographic data of the free acid-M_1 (tlc and hplc) and of the methyl ester of M_1 (hplc) support the assignment of structure as 19-OH-PGB_1. The unequivocal assignment of metabolite was obtained by gc/ms of derivatized metabolite (see Materials and Methods and text).

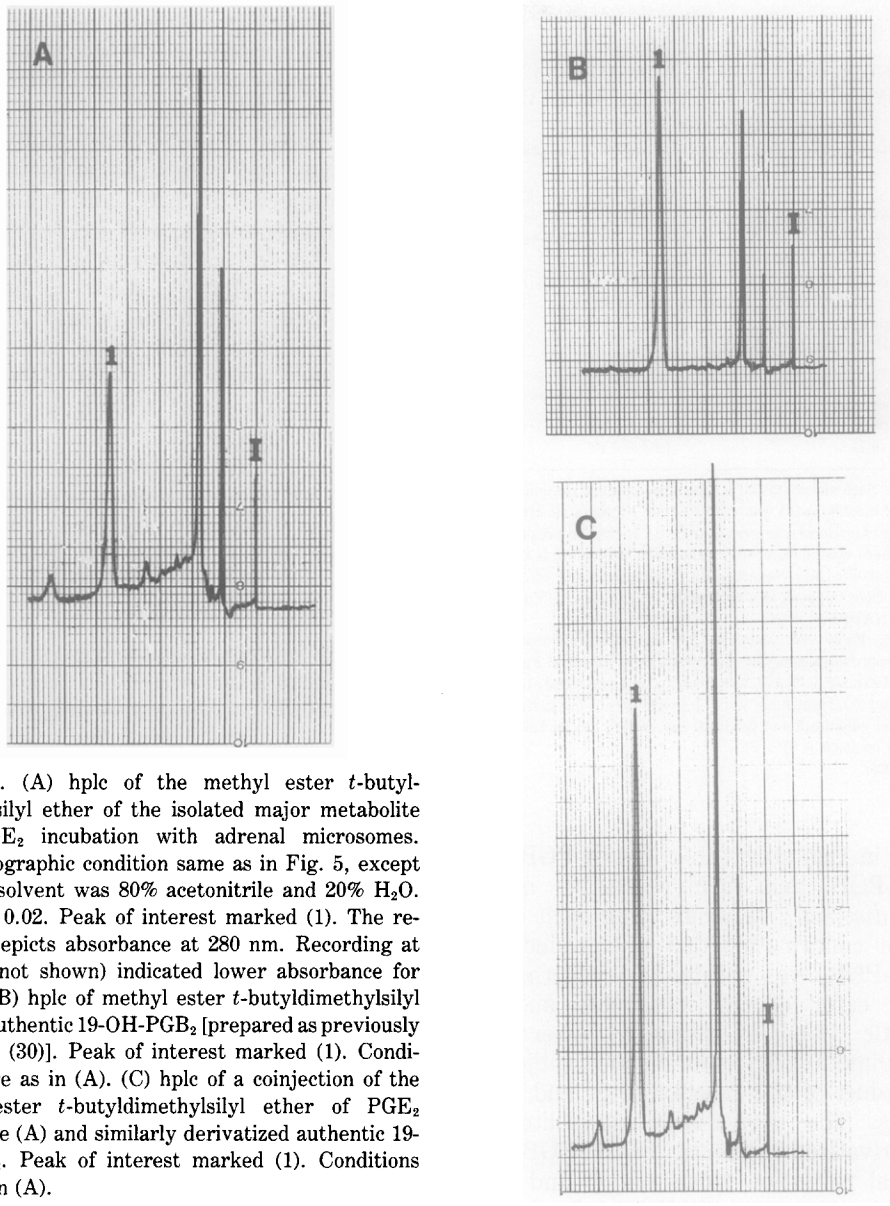

FIG. 7. (A) hplc of the methyl ester *t*-butyldimethylsilyl ether of the isolated major metabolite from PGE_2 incubation with adrenal microsomes. Chromatographic condition same as in Fig. 5, except that the solvent was 80% acetonitrile and 20% H_2O. AUFS = 0.02. Peak of interest marked (1). The recording depicts absorbance at 280 nm. Recording at 254 nm (not shown) indicated lower absorbance for peak 1. (B) hplc of methyl ester *t*-butyldimethylsilyl ether of authentic 19-OH-PGB_2 [prepared as previously described (30)]. Peak of interest marked (1). Conditions were as in (A). (C) hplc of a coinjection of the methyl ester *t*-butyldimethylsilyl ether of PGE_2 metabolite (A) and similarly derivatized authentic 19-OH-PGB_2. Peak of interest marked (1). Conditions were as in (A).

base-treated ethyl acetate extract was subjected to reverse-phase hplc (Fig. 4). The major polar radioactive metabolite, with uv absorption at 280 nm higher than at 254 nm, had a similar retention time as 19-OH-PGB_1; identical retention time of this metabolite to that of 19-OH-PGB_1 was also established, in hplc, by coinjection with authentic 19-OH-PGB_1. Also, the methyl ester of the isolated major metabolite, prepared with diazomethane and purified by hplc, yielded a product which cochromatographed in hplc with the methyl ester of authentic 19-OH-PGB_1. About 2% of the less polar peak with a retention time corresponding to 20-OH-PGB_1 was also detected.[8] Under these hplc conditions, we previously demonstrated (9, 22) and con-

[8] We observed that adrenal cytosol does not catalyze the NAD-dependent oxidation of 20-OH-PGE_1 to the corresponding dicarboxylic acid (37). Hence the possibility that significant 20-hydroxylation did occur, but was not detected because of further oxidation, is unlikely.

TABLE II

EFFECT OF INHIBITORS OF MONOOXYGENASE ON HYDROXYLATION OF PGE_1 BY ADRENAL MICROSOMES IN THE GUINEA PIG

Additions (+) or deletions (−)	Product (nmol/mg protein/1 h)	Percentage inhibition
Expt 1[a]		
Complete	4.2	—
+metyrapone (5 mM)	0.8	81
+cytochrome *c* (0.5 mM)	0.8	81
−NADPH generating system	0	100
Expt 2[b]		
Atmosphere		
N_2:O_2 (9:1)	2.35 ± 0.01	—
CO:O_2 (9:1)	1.62 ± 0.22[c]	31

[a] Expt 1: Each vial contained 1.9 mg of microsomal protein and [^{3}H]-PGE_1 (91.2 nmol,0.5 μCi) was incubated for 1 h in an air atmosphere; quantitation of products was carried out by tlc. In the absence of NADPH (control), there was no discernible radioactive peak on tlc in the region of the metabolite M_1. Nevertheless, the background counts were subtracted from the peak in a comparable region on tlc of incubations containing NADPH.

[b] Expt 2: Each vial contained 2.6 mg of microsomal protein prepared from frozen adrenals (purchased from Rockland Farms) and [^{3}H]PGE_1 (100 nmol, 0.05 μCi) was incubated in a controlled atmosphere of O_2, N_2, and CO. Incubations were carried for 60 min in quadruplicates. Analysis and quantitation of products was carried out by hplc. Values represent a mean (n = 4) ± SE.

[c] $P \leq 0.025$.

firmed in this study that 19-OH-PGB_1 and 20-OH-PGB_1 and their respective methyl esters *distinctly* separate from each other. Further confirmation that the product was 19-OH-PGB_1 was obtained by reacting the methyl ester of the metabolite and the authentic 19-OH-PGB_1-methyl ester with *t*-butyldimethylsilyl chloride and subjecting the product of this reaction to gc and gc/ms analysis. The derivatized metabolite and the derivatized authentic 19-OH-PGB_1 had identical retention times on gc and fragmentation patterns, yielding molecular ion at m/e = 594 and a major fragment at 537 [M^+-(*t*-butyl)].

It is of interest that PGE_2 is metabolized by adrenal microsomes at a much lower rate than PGE_1 (Table I); similarly guinea pig liver microsomes also metabolize PGE_2 less effectively than PGE_1 (30). The characterization of the polar metabolites from PGE_2 incubations was carried out essentially as described above for PGE_1. The more polar metabolite, which consists of 95%+ of the total NADPH-dependent metabolism (Fig. 5), was isolated by hplc and characterized in the form of 19-OH-PGB_2. Namely the free acid (19-OH-PGB_2), the corresponding methyl ester, and the methyl ester *t*-butyldimethylsilyl ether derivatives had identical retention times when coinjected in hplc with similarly derivatized authentic 19-OH-PGB_2 (Figs. 6A–C and 7A–C; coinjection of the methyl esters is not shown). Also the metabolite methyl ester-*t*-butyldimethylsilyl ether had identical retention time in gc as a similarly derivatized authentic 19-OH-PGB_2. Insufficient amounts of the isolated PGE_2 metabolite precluded the use of electron impact mass fragmentation pattern analysis. The almost insignificant formation of a less polar metabolite and unavailability of authentic 20-OH-PGE_2 or 20-OH-PGB_2

TABLE III

EFFECT OF ALTERNATE SUBSTRATES AND INHIBITORS OF MONOOXYGENASE ON HYDROXYLATION OF PGE_1 AND PGE_2 BY ADRENAL MICROSOMES IN THE GUINEA PIG

Additions (mM)	Percentage inhibition				
	Expt 1[a]	Expt 2[b]		Expt 3[c]	
	PGE_1	PGE_1	PGE_2	PGE_1	PGE_2
SKF 525A (0.1)	45	41	24		
Ethylmorphine					
(0.1)	9				
(1.0)	28	14	26		
Cortisol					
(0.01)	42				
(0.04)				100	100
(0.10)	88				
ANF					
(0.0001)	14				
(0.1)	26				

Note. Value represents the mean of percentage inhibition of duplicate samples agreeing within 5%. Microsomal preparations in experiments 2 and 3 are from the same animals used in Table I.

[a] Expt 1: Each incubation contained 3.8 mg microsomal proteins; [^{3}H]PGE_1 (20 nmol, 0.5 μCi); controls yielded 1.5 nmol hydroxylated products analyzed by tlc. Cortisol and α-naphthoflavone (ANF) were added in 5 μl ethanol; also 5 μl ethanol was added to other incubations in this experiment. Ethylmorphine·HCl and SKF 525·HCl were added in water.

[b] Expt 2: Each incubation contained 2.2 mg microsomal protein and [^{3}H]PGE_1 (103 nmol, 0.05 μCi) or [^{3}H]PGE_2 (106 nmol, 0.05 μCi); controls yielded with PGE_1 3.8 nmol products/h, with PGE_2 1.7 nmol/h (analyzed by hplc).

[c] Expt 3: Each incubation in duplicate contained 1.1 mg microsomal protein and [^{3}H]PGE_1 or [^{3}H]PGE_2 (100 nmol, 0.05 μCi) and 5 μl ethanol ± cortisol; controls yielded with PGE_1 3.5 nmol products/h with PGE_2 1.4 nmol/h (products analyzed by hplc).

derivatives did not permit the determination whether the minor, less polar, metabolite of PGE_2 metabolism was the expected 20-OH-PGE_2.

By contrast to the effective metabolism of PGE_1, PGA_1 was metabolized only at about one-third the rate of PGE_1 (not shown). This finding was surprising since guinea pig liver microsomes hydroxylate equally well both PGE_1 and PGA_1 (9, 30).

These results together with those observed by us in incubations of PGE_1, PGE_2, PGA_1, and PGA_2 with guinea pig liver and kidney microsomes (9, 16, 22, 30) indicate that ω-1 hydroxylation is a prime pathway in the guinea pig, independent of the tissues examined.

To examine whether the enzymatic activity which catalyzes the (ω-1)-hydroxylation of PGE_1 [formation of M_1 (19-OHPGB$_1$)] is a monooxygenase, cofactor requirements and the effects of recognized inhibitors of hepatic monooxygenase on M_1 formation were examined. Results show that NADPH generation is required for metabolism and that metyrapone, cytochrome *c*, and SKF 525A markedly inhibit the formation of the polar metabolite (Tables II, III). Of interest is that α-naphthaflavone which markedly inhibits liver microsomal benzpyrene hydroxylation in 3-methylcholanthrene-treated rats and mice but not in control or phenobarbital-treated animals (25, 26) produced a weak inhibition of PGE_1-hydroxylation. By contrast, previously we have noted a pronounced inhibition by α-naphthoflavone of PGA_1 and PGE_1 hydroxylation in guinea pig liver microsomes (9, 30). Thus it appears that the guinea pig adrenal monooxygenase is markedly less sensitive to this inhibitor than the liver enzyme. Carbon monoxide at a ratio of CO/O_2 of 9:1 moderately inhibited PGE_1 hydroxylation (Table II).[9] This finding is similar to our observation with liver microsomal metabolism of PGE_1, where at a ratio of CO/O_2 of 5:1 there was inhibition of the hydroxylation of PGE_1 (9).

Our observation on the differences in spectral interactions of cortisol versus that of PGE_1 may suggest that adrenal micro-

TABLE IV

OXIDATIVE METABOLISM OF XENOBIOTICS BY ADRENAL MICROSOMES IN ADULT MALE GUINEA PIGS

	Metabolic products assayed		
Substrate (mM)	HCHO (nmol/20 min/ mg protein)	Phenolic (nmol/15 min/ mg protein)	3-OH-hexobarbital (nmol/30 min/ mg protein)
Expt 1			
Ethylmorphine (8)	166.4		
Ethylmorphine (0.5)	112.5		
Benzphetamine (1.0)	143.8		
Benzphetamine (0.2)	106.0		
Benzo[α]pyrene (0.042)		2.2	
Expt 2			
Hexobarbital (0.1)			1.7

Note. In Expt 1, the microsomal preparation was from the same guinea pigs used in the experiment in Table I. Each value represents a mean of duplicate incubations.

TABLE V

EFFECTS OF POTENTIAL ALTERNATE SUBSTRATES (PGE_1 AND CORTISOL) ON OXIDATIVE METABOLISM OF XENOBIOTICS

	Percentage inhibition		
Additions (mM)	Hexobarbital	Ethylmorphine	Benzphetamine
PGE_1 (0.1)	25.6		
PGE_1 (1.0)		9.1	10.7
Cortisol (0.1)	86.6		

Note. Substrate concentrations were: Hexobarbital (0.1 mM), ethylmorphine (0.5 mM) and benzphetamine (0.2 mM). Nonsaturating concentrations were used to facilitate detection of potential inhibition by PGE_1 and cortisol. Values represent mean of duplicates agreeing within less than 5%. Control values of products of incubation of hexobarbital, ethylmorphine, and benzphetamine were: 1.7 nmol 3-OH-hexobarbital/30 min/mg protein, 112.5 nmol HCHO/mg protein/20 min, and 106 nmol HCHO/mg protein/20 min, respectively.

[9] Considerable variability in rates of hydroxylation of PGE_1 was observed with various batches of adrenal microsomes obtained frozen (Table II). Hence it was essential to carry out inhibition studies with microsomes from the same adrenal preparations.

somes, similarly to liver, contain several cytochrome *P*-450s. Also the large differences in rates of demethylation activities versus hydroxylation activities (Table IV) might be taken to suggest that different monooxygenases are involved in catalyzing the various reactions, though other reasons might be the cause for these observations. Furthermore, that adrenal microsomes contain several monooxygenases (cytochrome *P*450s) is also supported by our findings on the low inhibition of PGE_1 hydroxylation by ethylmorphine (Table III), and on the low inhibition by PGE_1 of hexobarbital hydroxylation, and of demethylation reactions (Table V).[10] By contrast, cortisol markedly inhibits both PGE_1 and hexobarbital hydroxylation (Tables III, V). Also our preliminary kinetic studies, based on Lineweaver–Burk plot analysis (not shown), suggest that cortisol inhibits PGE_1 hydroxylation noncompetitively; in turn this finding further supports the possibility that PGE_1 and cortisol are metabolized by different monoxygenases.

It is of interest that whereas kidney cortex microsomes catalyze both the ω- and ω-1 hydroxylation of PGE_1 (16, 22), both liver (9, 30) and adrenal microsomes catalyze primarily the (ω-1)-hydroxylation. This finding together with the observations on the unusually high potency of guinea pig adrenal microsomal monooxygenase toward xenobiotics and steroids, tempt a speculation that in this species, adrenals and liver may have similar monooxygenase catalytic functions. Furthermore, as suggested by previous findings, guinea pig adrenals might be able to serve as a model of a human adrenal monooxygenase system in an attempt to understand the unusually high monooxygenase activity in human fetal adrenals (6, 27). Of interest requiring further exploration is the possibility that PGs present in adrenals (19–21, 28, 29, 34) are substrates of adrenal monooxygenase(s) and that (ω-1)-hydroxylation provides an *in situ* deactivation step for endogenous PGs.

[10] A somewhat higher inhibition of ethylmorphine demethylation by PGE_1 (1 mM produced 13% inhibition and 5 mM produced 30% inhibition) in guinea pig adrenals was reported by other investigators (32).

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