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INHIBITION OF PROSTAGLANDIN E_2 CATABOLISM AND POTENTIATION OF HEPATIC PROSTAGLANDIN E_2 ACTION IN RAT HEPATOCYTES BY INHIBITORS OF OXIDATIVE METABOLISM

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Abstract—Prostaglandin E_2 (PGE_2) can modulate the actions of a number of hormones in liver. PGE_2 is rapidly metabolized in liver tissue, and thus alterations in the rate of PGE_2 catabolism might exert a short-term influence on the concentration of PGE_2 in liver. The present study examined the effects of inhibitors of oxidative metabolism on PGE_2 catabolism and action in isolated rat hepatocytes. [^{3}H]-PGE_2 was metabolized to three major products by the hepatocyte system as assessed by reverse-phase high performance liquid chromatography. Metyrapone (5 mM), aminopyrine (5 mM), SKF-525A (20 μM) and α-naphthoflavone (20 μM) each inhibited the breakdown of [^{3}H]-PGE_2. The inhibition of oxidative metabolism by these compounds was not limited to action at cytochrome P-450, and metyrapone, aminopyrine and SKF-525A each was shown to inhibit [1-^{14}C]-palmitate β-oxidation in the hepatocyte system. To determine the contribution of β-oxidation to the rapid catabolism of [^{3}H]-PGE_2, studies were performed using [1-^{14}C]-PGE_2 as substrate. Two major product peaks seen with [^{3}H]-PGE_2 as substrate lacked radioactivity when [1-^{14}C]-PGE_2 was the substrate, and thus these two products did not contain the 1-position carbon, consistent with their identity as β-oxidation products. Furthermore, [1-^{14}C]-PGE_2 also yielded $^{14}CO_2$ and a [^{14}C]-PGE_2 metabolite not seen with [^{3}H]-PGE_2. It was calculated that 60% of the rapid PGE_2 inactivation in the hepatocyte system occurred via β-oxidation. An additional, non-β-oxidation, metyrapone-sensitive, pathway accounted for 26% of PGE_2 disappearance. The effect of PGE_2 to inhibit glucagon-stimulated glycogenolysis was potentiated when metyrapone was included in the incubation, consistent with increased survival of intact PGE_2. In summary, PGE_2 was rapidly inactivated by intact hepatocytes via oxidative metabolism, primarily β-oxidation. Inhibition of prostaglandin catabolism can have short-term effects on PGE_2 concentrations and result in potentiation of PGE_2 effects on hepatic glucose metabolism.

Recent work has demonstrated that E-series prostaglandins (PGE) can modulate hormone effects in isolated rat hepatocytes [1–4] and interact with a plasma membrane PGE specific binding site [5, 6]. These observations are consistent with the concept that prostaglandins may play a physiologic role in the regulation of hepatic metabolism. Further support for this idea is provided by studies demonstrating a change in PGE specific binding site density in liver plasma membranes and on hepatocytes during the transition from the fed to the fasted state in the rat [6].

Using broken cell systems [7, 8] and isolated cells [9], liver can be shown to synthesize a variety of cyclooxygenase products, including PGE_2, prostacyclin and thromboxane. Liver tissue also has the capacity to metabolize PGE_2 to a variety of products [10–12]. Thus, prostaglandin levels will reflect the balance between prostaglandin synthesis and breakdown. Kinetic studies have demonstrated rapid clearance of added PGE_1 or PGE_2 by isolated rat hepatocytes [11]. This rapid catabolism makes demonstration of PGE_2 effects on hepatic metabolic regulation difficult after single additions of PGE_2 [2, 3].

The metabolic products generated from PGE_2 at early time points behave like compounds more polar than PGE_2 on a reverse-phase chromatographic system [11]. It is known that the liver has a high capacity for oxidative metabolism including microsomal and mitochondrial systems, and such metabolism would be expected to generate polar PGE_2 metabolites. PGE_2 has been shown to be a substrate for hepatic microsomal P-450 oxidation with the generation of ω and $\omega - 1$ hydroxylation products [13, 14]. Liver has also been shown to metabolize PGE_2 by β-oxidation in a manner analogous to other long chain fatty acids [12, 15]. The present study examined the effects of inhibition of oxidative metabolism on the kinetics of PGE_2 catabolism by intact hepatocytes. The results demonstrate that, in isolated rat hepatocytes, the concurrent inhibition of β-oxidation and microsomal P-450 significantly slows PGE_2 catabolism and potentiates the inhibitory effects of added PGE_2 on glucagon-stimulated glycogenolysis.

METHODS

Animals and isolation of hepatocytes. Fed, male Sprague–Dawley rats (294 ± 7 g; mean ± SEM, N =

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42) were used in all studies. Rats were permitted chow (Agway Co., Longmont, CO) and water *ad lib*. Hepatocytes were prepared by a modification of the collagenase perfusion technique of Berry and Friend [16] as previously described [2]. Hepatocyte preparations used in the present studies contained 2.80 ± 0.10 mg protein/10^6 cells (mean ± SEM, N = 42), had a wet weight of 16.1 ± 0.8 mg/10^6 cells (mean ± SEM, N = 29), and were 90 ± 1% mean ± SEM, N = 42) viable on the basis of trypan blue exclusion.

Incubation. Hepatocyte incubations were conducted in 25-ml Erlenmeyer flasks as previously described [2, 6, 17]. The final composition of the incubations was hepatocytes (1.0 or 2.5 ×10^6 cells/ml), 130 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$,, 1.12 mM $CaCl_2$, 3.0 mM Na_2HPO_4, 4.0 mM glucose and 8.0 mM Tris (hydroxymethyl)amino ethane, pH 7.4, plus other compounds as indicated. Total incubation volume was 2.5 ml for studies of PGE_2 catabolism and 3.0 ml for studies of glycogenolysis.

Analysis of PGE_2 catabolism—High performance liquid chromatography (HLPC). PGE_2 was added to hepatocyte incubations at time 0 to a final concentration as indicated in figure and table legends. [^{3}H]-PGE_2 was added with a total of approximately 200,000 dpm/incubation and [^{14}C]-PGE_2 approximately 30,000 dpm/incubation at specific activities determined by the total PGE_2 concentration as indicated in individual experiments. Incubation aliquots (250 μl) were removed at time points of interest and added to an equal volume of ethanol and mixed. The ethanol/incubation mixture was then centrifuged (10,000 *g* × 2 min), and the supernatant fraction was analyzed by HPLC as previously described [11]. Briefly, 200 μl of the supernatant fraction was injected onto an HPLC system (Waters Associates 501 pump and U6K injector) coupled to a Nova-Pak C18 15-cm column equipped with a μBondapak C18 pre-column (Waters Associates). The mobile phase was acetonitrile/water/acetic acid (230:767:3, by vol.) and was pumped isocratically at a flow rate of 1.2 ml/min. Eluent fractions were collected at 1.0- or 2.0-min intervals as indicated, and radioactivity was determined by liquid scintillation counting. Previous work has validated this method with respect to recovery of total radioactivity added to the incubation, and quantitation of metabolite peaks to permit kinetic analysis of PGE_2 catabolism [11].

Metabolism of 1-^{14}C-substrates. Formation of $^{14}CO_2$ from ^{14}C-substrates (PGE_2 or palmitate) was determined as previously described [17]. Incubations were conducted in 25-ml flasks equipped with sidearms and centerwells. [1-^{14}C]-Palmitate was added to incubations complexed to defatted bovine serum albumin. Incubations for analysis of palmitate oxidation contained a final concentration of 0.3 mM palmitate (sp. act. 90 dpm/nmol) and 1% albumin in addition to the constituents noted above. At the termination of an incubation, perchloric acid was added to the incubation to a final concentration of 3%. Ethanolamine/ethylene glycol monomethyl ether (1:2 v/v) was added to the centerwell to trap $^{14}CO_2$, and the flasks were shaken for an additional 60 min. Radioactivity in the center well was then quantified by liquid scintillation counting. ^{14}C-Acid-soluble products (primarily ketone bodies, Ref. 18) generated from [^{14}C]-palmitate were quantified by determining the radioactivity in the incubation perchloric acid supernatant fraction [17, 18].

Other assays. Glucose concentration in the hepatocyte incubations was determined by a glucose oxidase method [19]. Protein concentrations were determined by the biuret method [20]. Acetoacetate and β-hydroxybutyrate concentrations were assayed using the enzymatic technique described by Olsen [21].

Data analysis. For studies using [^{3}H]-PGE_2 as a substrate, the percentage of originally added PGE_2 remaining at time points of interest was determined as the fraction of total radioactivity in a chromatogram associated with the PGE_2 peak. This calculation is based on the previously demonstrated calculated recovery of 100% of added radioactivity as PGE_2 or metabolites [11] and corrects for small variations in total radioactivity injected onto the HPLC system. The percentage of PGE_2 remaining could then be used as an index of PGE_2 catabolism or to estimate a first-order rate constant [11]. When [^{14}C]-PGE_2 was used as substrate, product formation was related to total radioactivity added to the incubation (this was necessitated because of the loss of radioactivity as CO_2 from the incubation prior to HPLC analysis). The radioactivity in HPLC peaks of interest was corrected for the volume of HPLC fractions collected and recovery of injected radioactivity after the chromatographic resolution (see Results and Ref. 11 for validation of recovery calculation) to obtain total radioactivity in individual peaks. This calculation is further validated in the Results (Table 4).

All data are presented as mean ± standard error of the mean. N refers to the number of studies, each performed on separate hepatocyte isolations. For the studies of PGE_2 catabolism, N refers to the number of complete HPLC analyses performed, each from a different hepatocyte preparation. Statistical analysis was performed using a Student's paired *t*-test (simultaneous incubations in the absence or presence of inhibitor of interest) with a $P < 0.05$ considered significant.

Reagents. All chemicals used were of reagent grade. Collagenase (Type II) was obtained from Worthington Diagnostic Systems, Inc., Freehold, NJ. [5, 6, 8, 11, 12, 14, 15-^{3}H(N)]PGE_2, [1-^{14}C]-PGE_2 and [1-^{14}C]-palmitate were purchased from the New England Nuclear Corp., Boston, MA. PGE_2 was purchased from Upjohn Pharmaceuticals, Kalamazoo, MI, and glucagon was the gift of the Eli Lilly Corp., Indianapolis, IN. Palmitate, 2-methyl-1,2-di-3-pyridyl-1-propanone (metyrapone), 4-dimethyl-aminoantipyrine (aminopyrine), α-naphthoflavone and defatted bovine serum albumin were obtained from the Sigma Chemical Co., St. Louis, MO. SKF-525A was obtained from Smith Kline & French Laboratories, Philadelphia, PA.

RESULTS

Consistent with previous observations [11], hepatocytes rapidly metabolized [^{3}H]-PGE_2 to compounds which acted more polar (i.e. eluted earlier)

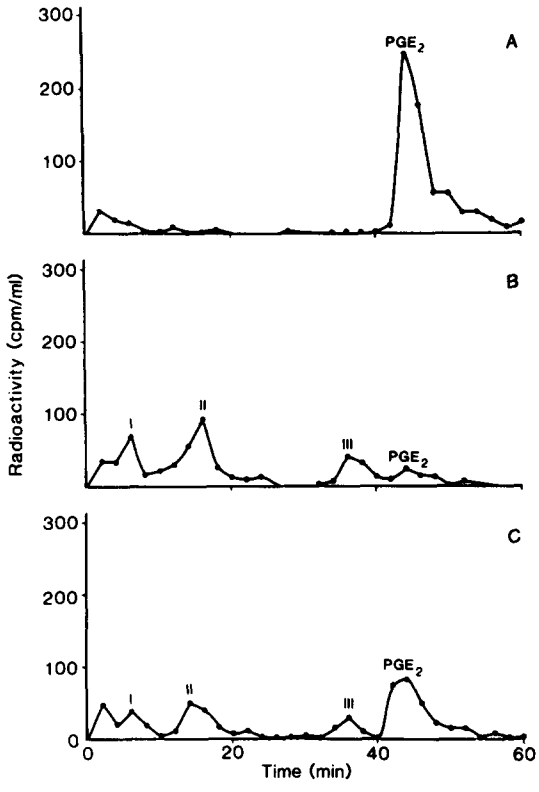

Fig. 1. Catabolism of [^{3}H]-PGE_2 by rat hepatocytes. Incubations containing 2.5×10^6 cells/ml and 10^{-7} M [^{3}H]-PGE_2 were performed as described in the text. Aliquots removed at time 0 (A) or after 5 min of incubation in the absence (B) or presence (C) of 5 mM metyrapone were analyzed by HPLC purification as described in the text. Radioactivity shown is cpm per 1.0 ml in each 2.0 min fraction collected. Data are representative of five studies summarized in Table 2.

than PGE_2 on the reverse-phase HPLC system used (Fig. 1, A and B). Recovery of total added radioactivity was not different in samples taken after 5 or 10 min as compared to the time 0 sample (time 0 = 100%; 5 min = 100 ± 3%; 10 min = 107 ± 3%, N = 13 at each time point), verifying that no important metabolites were being missed by the sample preparation and HPLC system used.

Previous studies have demonstrated that the time course of PGE_2 metabolism in the hepatocyte system could be approximated and characterized by first-order kinetics [11]. Therefore, in the current study the catabolism of PGE_2 in the hepatocyte system has been expressed as the percentage of PGE_2 remaining after 5 or 10 min of incubation, and by the apparent first-order rate constant (k). In incubations containing 10^{-5} M PGE_2 and 1×10^6 hepatocytes/ml, addition of the cytochrome P-450 inhibitors 5 mM metyrapone, 20 μM SKF-525A and 20 μM α-naphthoflavone caused significant inhibition of [^{3}H]-PGE_2 metabolism (Table 1). Metyrapone (5 mM) inhibited [^{3}H]-PGE_2 metabolism by 68%, 20 μM SKF-525A by 53%, and α-naphthoflavone by 51% (Table 1). This inhibition of metabolism resulted in greater concentrations of unmetabolized PGE_2 remaining in the incubation 5 or 10 min after the addition of [^{3}H]-PGE_2. Similar inhibition of [^{3}H]-PGE_2 metabolism was demonstrated in incubations containing 10^{-7} M PGE_2 and either 1×10^6 or 2.5×10^6 hepatocytes/ml using 5 mM aminopyrine (Table 2) or 5 mM metyrapone (Fig. 1C, Table 2). Thus, all four cytochrome P-450 inhibitors tested significantly decreased the rate of PGE_2 catabolism by intact hepatocytes under a variety of conditions.

Previous studies have demonstrated the presence of β-oxidation products following the incubation of PGE with hepatic tissue [10, 12, 15]. Compounds classically characterized as P-450 inhibitors have also shown effects on fatty acid β-oxidation [22]. Therefore, it was important to verify in our system whether the P-450 inhibitors used also inhibited β-oxidation. Metyrapone (5 mM), 5 mM aminopyrine and 20 μM SKF-525A each inhibited the conversion of [1-^{14}C]-palmitate to ^{14}C-products by 58, 31 and 14% respectively (Table 3). To distinguish the contributions of β-oxidation and microsomal P-450 oxidation to the catabolism of [^{3}H]-PGE_2 seen above (Fig. 1), the metabolism of [1-^{14}C]-PGE_2 was studied in the hepatocyte system. The amount of unchanged [1-^{14}C]-PGE_2 (10^{-7} M) remaining in the incubation after 5 min was similar to that seen with [^{3}H]-PGE_2 (Table 4 vs Table 2). However, the metabolite profile generated from [1-^{14}C]-PGE_2 differed significantly from

Table 1. Effects of inhibitors of oxidative metabolism on [^{3}H]-PGE_2 (10^{-5} M) breakdown in hepatocytes

Additions	Percent PGE_2 remaining after: 5 min	10 min	k (min^{-1} 10^{-6} cells)
Control	59 ± 7 (15)	42 ± 9 (14)	0.129 ± 0.026 (15)
5 mM Metyrapone	83 ± 3* (5)	62 ± 4† (4)	0.041 ± 0.007* (5)
20 μM SKF-525A	78 ± 5* (9)	62 ± 10† (9)	0.060 ± 0.007* (9)
20 μM α-Naphthoflavone	78 ± 6* (8)	61 ± 10 (8)	0.063 ± 0.020* (8)

Incubations containing 1×10^6 hepatocytes/ml and PGE_2 added to a final concentration of 10^{-5} M were performed as described in the text. Aliquots were removed after 0, 5 and 10 min of incubation of HPLC analysis of PGE_2 and metabolites. The percentage of PGE_2 remaining was calculated at each time point, and these values used to calculate an apparent first-order rate constant (k). Values are means ± SEM with N in parentheses.

* $P < 0.05$ vs control.

† $0.05 < P < 0.10$ vs control.

Table 2. Effects of inhibitors of oxidative metabolism on [^{3}H]-PGE_2 (10^{-7} M) breakdown in hepatocytes

Cell concentration (10^6 cells/ml)	Additions	Percent PGE_2 remaining after 5 min	k (min^{-1} 10^{-6} cells)
1.0	Control	49 ± 7 (7)	0.156 ± 0.035 (7)
1.0	5 mM Metyapone	79 ± 6* (5)	0.050 ± 0.015* (5)
1.0	5 mM Aminopyrine	69 ± 11* (5)	0.087 ± 0.043* (5)
2.5	Control	22 ± 3 (5)	0.123 ± 0.009 (5)
2.5	5 mM Metyrapone	48 ± 10* (5)	0.068 ± 0.021* (5)

Incubations were conducted as in Table 1, except a final PGE_2 concentration of 10^{-7} M was used and cell concentrations were as indicated. Values are means ± SEM with N in parentheses.
* $P < 0.05$ vs control at same cell concentration.

that seen with [^{3}H]-PGE_2 (Fig. 2B vs Fig. 1B). The metabolites eluting at approximately 6 (Peak I) and 16 (Peak II) min seen when [^{3}H]-PGE_2 was the substrate (Fig. 1) were not seen when [1-^{14}C]-PGE_2 was the substrate (Fig. 2). However, a new metabolite peak was seen eluting with the solvent front at approximately 2 min (Peak A). The metabolite peak eluting at approximately 36 min (Peak III) was seen with both [^{3}H]-PGE_2 and [1-^{14}C]-PGE_2 as substrates. These results indicate that peaks I and II seen with [^{3}H]-PGE_2 as substrate are not seen with the [1-^{14}C]-PGE_2 substrate and thus do not contain the l-position carbon. Peak A seen only with [1-^{14}C]-PGE_2 must contain the 1-position carbon but not the majority of the PGE_2 molecule carbons as it was not seen with [^{3}H]-PGE_2 as substrate in which the radioactivity was present beginning with the 5-position carbon. Thus, Peak A is consistent with the ketone body products of β-oxidation (acetoacetate and β-hydroxybutyrate). Standard solutions of both β-hydroxybutyrate and acetoacetate co-eluted with Peak A under the HPLC conditions used (data not shown). Additionally, radioactivity from Peak A was rechromatographed on the same reverse-phase system but with a mobile phase of 50 mM KH_2PO_4 (pH 2.01) at a flow rate of 1.2 ml/min. Under these conditions, acetoacetate and β-hydroxybutyrate displayed retention times of 2.8 and 3.3 min, respectively, and the radioactivity from Peak A again coeluted with the ketone body standards (Table 5). $^{14}CO_2$ was also generated during 5-min incubations of 10^{-7} M [1-^{14}C]-PGE_2 in the hepatocyte system (Table 4). $^{14}CO_2$ and ^{14}C-ketone bodies represent the major final oxidation products generated from 1-^{14}C-fatty acids and are generated in a ratio favoring ketone bodies approximately six to one (see Table 3, Refs. 17 and 18). The ratio of $^{14}CO_2$ generated to ^{14}C-ketone bodies (peak A) from [1-^{14}C]-PGE_2 was similar to that seen with [1-^{14}C]-palmitate oxidation (Table 3) and provides further evidence that β-oxidation is occurring in the system. Based on the ^{14}C-product recoveries in Table 4, β-oxidation products accounted for approximately 60% of the metabolized [1-^{14}C]-PGE_2. Metyrapone (5 mM) inhibited the formation of β-oxidation products and the Peak III metabolite when [1-^{14}C]-PGE_2 was the substrate (Table 4, Fig. 2).

To determine if the inhibition of PGE_2 catabolism by inhibitors of oxidative metabolism increased the concentration of active PGE_2, the effect of metyrapone on PGE_2 inhibition of glucagon-stimulated hepatic glycogenolysis was studied in the hepatocyte model (Table 6). Metyrapone had no effect on basal rates of glycogenolysis. In the absence of metyrapone, PGE_2 at concentrations of 10^{-7} M did not inhibit glucagon-stimulated glycogenolysis over the 10 min following hormone addition, whereas 10^{-6} M PGE_2 inhibited glucagon-stimulated glycogenolysis by 52%. In contrast, in the presence of 5 mM metyrapone PGE_2 significantly inhibited glucagon-stimulated glycogenolysis at concentrations as low as 10^{-7} M (5% vs 32% inhibition in the absence and presence of metyrapone respectively). The concentration dependence of metyrapone's potentiation of inhibition of glucagon-stimulated glycogenolysis by PGE_2 roughly paralleled the concentration depen-

Table 3. Effects of inhibitors of oxidative metabolism on [1-^{14}C]-palmitate oxidation in hepatocytes

Additions	Rate of formation (nmol palmitate converted/10^6 cells/10 min)		
	$^{14}CO_2$	^{14}C-Acid-soluble products	Total (CO_2 + acid-soluble products)
Control	2.6 ± 0.3	15.4 ± 1.5	18.0 ± 1.7
5 mM Metyrapone	1.3 ± 0.1*	6.1 ± 0.5*	7.5 ± 0.5*
20 μM SKF-525A	3.2 ± 0.7	12.4 ± 1.3†	15.5 ± 1.1†
5 mM Aminopyrine	2.5 ± 0.5	10.0 ± 1.0*	12.5 ± 3.0*

Incubations containing 2.5 × 10^6 hepatocytes/ml and 0.3 mM palmitate (90 dpm/nmol) were performed as described in the text. Values are means ± SEM, N = 5 in all cases.
* $P < 0.05$ vs control.
† $0.05 < 0.10$ vs control.

Table 4. Effects of inhibitors of oxidative metabolism on [1-^{14}C]-PGE_2 breakdown in hepatocytes

	Percentage of radioactivity added recovered as:				
	$^{14}CO_2$	Peak A	Peak III	PGE_2	Total
Control	6.9 ± 0.8	41.6 ± 3.1	21.7 ± 3.1	16.0 ± 3.1	86 ± 4
5 mM Metyrapone	1.8 ± 0.5*	28.7 ± 3.7*	5.0 ± 1.4*	59.8 ± 6.6*	95 ± 2

Incubations containing 2.5×10^6 hepatocytes/ml and 10^{-7} M PGE_2 were performed as described in the text. Peak A and peak III refer to the peaks identified in Fig. 2. Total dpm added/incubation (100%) were 26,300 ± 300 (N = 4). $^{14}CO_2$ was collected in centerwell traps, while peak A, peak III and PGE_2 were determined based on HPLC separations. Values are normalized to total counts in particular experiments and are means ± SEM; N = 4 in all cases.
* $P < 0.05$ vs control.

dence for metyrapone inhibition of PGE_2 catabolism (Table 7). Thus, inhibition of PGE_2 catabolism by metyrapone was associated with potentiation of the inhibitory effect of exogenous PGE_2 on glucagon-stimulated glycogenolysis in the hepatocyte system.

DISCUSSION

The level of prostaglandins in liver tissue will reflect the balance between prostaglandin synthesis and prostaglandin catabolism. The rapid rate of PGE catabolism by liver tissue [11] suggests that alterations in the rate of PGE_2 catabolism could have short-term effects on hepatic PGE levels. Because PGE has been shown to modulate the actions of a number of hormones on the regulation of hepatic metabolism [1–4], such alterations in PGE levels may alter the state of hepatic carbohydrate metabolism. The present study demonstrates that inhibition of oxidative metabolism in intact hepatocytes significantly decreased the rate of PGE_2 catabolism. Further, this inhibition of PGE_2 catabolism was associated with a potentiation of the action of PGE_2 to inhibit glucagon-stimulated glycogenolysis.

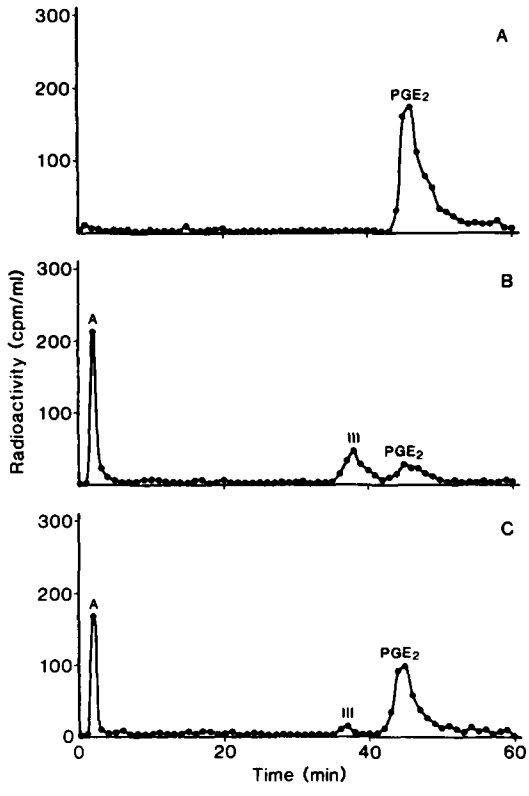

Fig. 2. Catabolism of [1-^{14}C]-PGE_2 by rat hepatocytes. Incubations containing 2.5×10^6 cells/ml and 10^{-7} M [1-^{14}C]-PGE_2 were performed as described in the text. Aliquots removed at time 0 (A) or after 5 min of incubation in the absence (B) or presence (C) of 5 mM metyrapone were analyzed by HPLC purification as described in the text. Radioactivity shown is cpm per 1.0 ml in each 1.0-min fraction collected. Data are representative of four studies summarized in Table 4.

It is recognized that many tissues have the capacity to metabolize PGE_2. Formation and accumulation of 13,14-dihydro-15-keto-PGE_2 has been used as an index of PGE_2 formation [22–25], and this metabolite is generated from PGE_2 by a number of tissues [26, 27]. Unlike many other tissues, liver also has a high capacity for oxidative metabolism, and the generation of β-oxidation and ω-oxidation products of hepatic PGE_2 metabolism has been demonstrated [10, 12–14, 26]. The products of PGE_2 metabolism generated during short-term incubation with intact hepatocytes appear to be more polar than PGE_2 [11], and thus 13,14-dihydro-15-keto-PGE_2. which eluted after PGE_2 under the HPLC conditions employed (data not shown), was not thought to be a significant product of hepatic PGE_2 catabolism. Therefore, the present study employed inhibitors of oxidative metabolism to perturb PGE_2 metabolism in the hepatocyte system.

The four compounds used in the present study (metyrapone, aminopyrine, SKF-525A and α-naphthoflavone) all dramatically decreased the rate of PGE_2 catabolism in the hepatocyte system (Tables 1 and 2). Microsomal cytochrome P-450 hydroxylation of PGE_2 is known to occur [13, 14], and all the compounds used are recognized inhibitors of cytochrome P-450. However, these compounds also have the capacity to inhibit other pathways of oxidative metabolism [22]. Because β-oxidation products of PGE_2 metabolism have been isolated from hepatocyte systems [12], the contribution of β-oxidation to the rapid catabolism of PGE_2 was examined in further detail.

Metyrapone, aminopyrine and SKF-525A all inhibited the oxidation of [1-^{14}C]-palmitate (Table 3) at concentrations used to inhibit PGE_2 catabolism. Thus, the compounds could clearly affect cellular β-oxidation in addition to inhibiting cytochrome P-450-mediated reactions. The use of [1-^{14}C]-PGE_2

Table 5. Chromatography of acetoacetate, β-hydroxybutyrate and peak A

Fraction	Radioactivity (cpm/fraction)	Acetoacetate (nmol/fraction)	β-Hydroxybutyrate (nmol/fraction)
1	0.0	0.0	0.1
2	0.0	1.5	0.8
3	0.0	1.2	1.0
4	0.0	1.2	1.4
5	3.5	0.4	2.2
6	0.0	1.0	0.5
7	0.0	1.9	0.9
8	0.0	3.3	0.5
9	1.1	6.9	0.1
10	9.2	6.7	0.6
11	44.6	4.9	3.2
12	12.7	2.7	2.1
13	0.0	3.8	0.5
14	0.0	2.1	0.6
15	0.0	2.3	0.8

Radioactivity collected with Peak A (Fig. 2) was rechromatographed after the addition of authentic acetoacetate (30 nmol) and β-hydroxybutyrate (30 nmol). HPLC conditions were as described in the text. Eluent fractions (0.3 min) were collected and assayed for radioactivity, acetoacetate and β-hydroxybutyrate. Data are representative of three experiments.

permitted the contribution of β-oxidation to be studied quantitatively. Several observations support the conclusion that β-oxidation contributes significantly to the rapid PGE_2 catabolism by hepatocytes. First, significant amounts of $^{14}CO_2$ were generated, demonstrating loss of one or more carbons from the carboxyl-terminal end of the PGE_2 molecule. Second, peaks I and II which represent major products of [^{3}H]-PGE_2 catabolism (Fig. 1) were not seen when [1-^{14}C]-PGE_2 was the substrate (Fig. 2). Thus, peaks I and II do not contain the original 1-position carbon. Third, a new product peak was seen when [1-^{14}C]-PGE_2 was the substrate. This peak co-eluted with β-hydroxybutyrate and acetoacetate (both in the HPLC system used for PGE analysis, and a more polar system which moves the ketone bodies away from the solvent front, Table 5), the normal products formed from acetyl-CoA generated by hepatic β-oxidation. Fourth, the ratio of $^{14}CO_2$ to ^{14}C-peak A (presumptively ketone bodies) formed is consistent with the normal relative amounts of $^{14}CO_2$ and ^{14}C-ketone bodies formed from [^{14}C]-acetyl-CoA generated during hepatic β-oxidation (Table 3; Refs. 17 and 18). It is concluded, therefore, that β-oxidation (either mitochondrial and/or peroxisomal, Ref. 28) accounts for approximately 60% of early PGE_2 catabolism in the liver, and that this β-oxidation was inhibited by the compounds used in the present study.

Peak III seen as a product of both [^{3}H]-PGE_2 and [^{14}C]-PGE_2 catabolism represents approximately 26% of the total PGE_2 catabolized. While this product has not been identified, it is more polar than PGE_2 and its formation was greatly reduced by the P-450 inhibitors used in the present study. This peak may represent an ω or $\omega - 1$ hydroxylation product. Such products are generated rapidly when PGE_2 is incubated with purified hepatic microsomes [13, 14].

Table 6. Effect of metyrapone on PGE_2 inhibition of glucagon-stimulated glycogenolysis

Additions	Rate of glucose production (nmol/2.5 × 10^6 cells/10 min)	
	No metyrapone	5 mM Metyrapone
Basal	0	76 ± 52 (5)
Glucagon	405 ± 59 (11)	378 ± 64 (11)
Glucagon + 10^{-7} M PGE_2	384 ± 57 (6)	276 ± 66* (6)
Glucagon + 10^{-6} M PGE_2	194 ± 88* (7)	178 ± 60* (7)

Incubations containing 2.5 × 10^6 cells/ml were conducted as described in the text. Glucagon (10^{-6} M) and PGE_2 were added at time 0 as indicated. Glucose concentrations in the incubation at 0 and 10 min were determined and the difference was calculated. Data presented are the rates of glucose production from 0 to 10 min corrected for basal glucose production. Basal rates of glucose production were 665 ± 74 nmol/2.5 × 10^6 cells/10 min. Values are means ± SEM, with N shown in parentheses.

* $P < 0.05$ vs glucagon alone.

Table 7. Concentration dependence of metyrapone action

Metyrapone concn (mM)	Glycogenolysis (nmol/2.5 × 10^6 cells/10 min)	% PGE_2 remaining after 5 min
5.0	240	24.5
4.0	277	29.3
3.0	219	12.6
2.0	252	3.1
0.5	351	0.0
0.0	300	9.0
Glucagon alone	352	

The effects of metyrapone at the concentrations indicated on the inhibition of glucagon-stimulated glycogenolysis (10^{-6} M glucagon, glycogenolysis determined as glucose production over 0–10 min) by 10^{-7} M PGE_2, and on the catabolism of [1-^{14}C]-PGE_2 (10^{-7} M) were determined. Incubations contained 2.5 × 10^6 hepatocytes/ml. Values are means of two to four experiments.

Importantly, inhibition of PGE_2 degradation in the hepatocyte system led to increased PGE_2 concentrations which were manifested by increased PGE_2 action. By using metyrapone to inhibit PGE_2 degradation, concentrations of PGE_2 too low to have effects on glucose-stimulated glycogenolysis over a 10-min incubation could be shown to have the predicted inhibitory effects (Tables 6 and 7). These observations suggest that alterations in PGE_2 catabolism may have short-term effects on the action of PGE_2 generated in liver. The results also imply that the catabolic products of PGE_2 degradation do not affect hepatic glucose inhibition, though no studies have yet been performed with purified metabolites to verify this point.

The effects of the inhibitors used in the present studies do not represent non-specific chemical toxicity on the hepatocytes, but are secondary to inhibition of oxidative metabolism. This conclusion is based on the lack of effect of metyrapone on basal and glucagon-stimulated glycogenolysis (Table 6), the ability of glucagon to stimulate hepatocyte cAMP accumulation in the presence of 5 mM metyrapone, 20 μM SKF-525A, 5 mM aminopyrine and 20 μM α-naphthoflavone (data not shown), and the maintenance of viability based on trypan blue exclusion (data not shown).

Thus, these results demonstrate that the rapid inactivation of PGE_2 by hepatocytes is due to oxidative metabolism, largely β-oxidation. The rate of PGE_2 catabolism can be decreased by inhibition of oxidative metabolism, resulting in higher PGE_2 concentrations, and increased hepatic PGE_2 effects. Alterations in PGE_2 catabolism can therefore contribute to the short-term regulation of hepatic PGE_2 concentrations and the effect of PGE_2 on hepatic glucose metabolism.

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