Metabolism of Prostaglandin Glycerol Esters and Prostaglandin Ethanolamides In Vitro and In Vivo

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Abbreviations: COX, cyclooxygenase; AG, arachidonylglycerol; AEA, arachidonylethanolamide (anandamide); EA, ethanolamide; PG, prostaglandin; G, glycerol ester; MS, mass spectrometry; LC/MS, liquid chromatography/mass spectrometry; 15-HPGDH, 15-hydroxyprostaglandin dehydrogenase.
Summary

Prostaglandin glycerol esters (PG-G) and prostaglandin ethanolamides (PG-EA) are generated by the action of cyclooxygenase-2 (COX-2) on the endocannabinoids, 2-arachidonylglycerol (2-AG) and arachidonylethanolamide (AEA), respectively. These novel eicosanoids may have unique pharmacological properties and/or serve as latent sources of prostaglandins at sites remote from their tissue of origin. Therefore, we investigated the metabolism of PG-Gs and PG-EAs in vitro and in vivo. PGE$_2$-G was rapidly hydrolyzed in rat plasma to generate PGE$_2$ ($t_{1/2}$ = 14 s) but was only slowly metabolized in human plasma ($t_{1/2}$ » 10 min). An intermediate extent of metabolism of PGE$_2$-G was observed in human whole blood ($t_{1/2}$ ≈ 7 min). The parent arachidonylglycerol, 2-AG, and the more stable regioisomer, 1-AG, also were much more rapidly metabolized in rat plasma compared to human plasma. PGE$_2$-EA was not significantly hydrolyzed in plasma, undergoing slow dehydration/isomerization to PGB$_2$-EA. Both PGE$_2$-G and PGE$_2$-EA were stable in canine, bovine, and human cerebrospinal fluid (CSF). Human 15-hydroxyprostaglandin dehydrogenase (15-HPGDH), the enzyme responsible for the initial step in PG inactivation in vivo, oxidized both PGE$_2$-G and PGE$_2$-EA less efficiently than the free acid. The sterically hindered glyceryl prostaglandin was the poorest substrate examined in the E-series. Minimal 15-HPGDH-oxidation of PGF$_{2\alpha}$-G was observed. PGE$_2$-G and PGE$_2$-EA pharmacokinetics were assessed in rats. PGE$_2$-G was not detected in plasma 5 min following an intravenous dose of 2 mg/kg. However, PGE$_2$-EA was detectable up to 2 hours following an identical dose, displaying a large apparent volume of distribution and a half-life of over 6 min. The results suggest that endocannabinoid-derived PG-like compounds may be
sufficiently stable in humans to exert actions systemically. Further, these results suggest that the rat is not an adequate model for investigating the biological activities of 2-arachidonylglycerol or glycercyl prostaglandins in humans.
Introduction

Prostaglandins (PGs) are a class of unsaturated fatty acid metabolites with remarkably diverse and potent biological activities. Cyclooxygenases (COXs) catalyze the committed step in PG biosynthesis by oxygenating arachidonic acid to generate the hydroxy-endoperoxide, PGH$_2$ (1). Subsequent cell-type specific metabolism of PGH$_2$ results in the production of the primary prostaglandins (E$_2$, D$_2$, F$_{2\alpha}$), prostacyclin, and thromboxane A$_2$. PGs generated by COX action on arachidonic acid function as autocrine and paracrine messengers. Rapid PG catabolism in vivo prevents significant endocrine activity (2,3). For PGs present within the circulation, the initial step in metabolism is oxidation of the 15-hydroxyl group, which is catalyzed by 15-hydroxyprostaglandin dehydrogenase (15-HPGDH, EC 1.1.1.141) (4) (Eq. 1). The 15-keto derivatives that are produced exhibit dramatically reduced biological activity so their formation is considered to represent metabolic inactivation (5-8).

Insert eq 1

The endocannabinoids, 2-arachidonylglycerol (2-AG) and arachidonylethanolamide (AEA), have recently been shown to be selective COX-2 substrates (9,10). The products of COX-2 action on these lipids have been identified as prostaglandin glycerol esters (PG-Gs) and prostaglandin ethanolamides (PG-EAs). Investigations into the biological activities of these novel lipids have only recently begun and no information is available on their metabolic disposition. Elucidation of PG-G and PG-EA metabolic routes should provide insight into the possible biological roles for these compounds and focus efforts to detect and quantify these COX-2-generated metabolites in vivo. One possible role for
PG-Gs and PG-EAs is as precursors that can be hydrolyzed to PGs in target tissues remote from their site of synthesis. In addition, PG-Gs and PG-EAs may prove to be informative biomarkers for COX-2-related pathologies such as neoplasia (11), inflammation (12), or neurodegeneration (13,14).

Consequently, we have investigated the metabolism of PG-Gs and PG-EAs in a number of biological environments. In the present study, we examined the stability of PGE2-G and PGE2-EA in plasma, whole blood, and cerebrospinal fluid (CSF) from multiple species. Examination of these fluids was driven by their accessibility in clinical settings. Prompted by dramatic species differences observed in these early studies, 2-AG stability in rat and human plasma also was investigated. Substrate structure-activity relationship studies with 15-HPGDH have provided insights into potential metabolic routes for neutral prostaglandin derivatives and support the hypothesis that these lipids may be catabolized more slowly than the free acid PGs. Finally, in vivo pharmacokinetics were determined for both PGE2-G and PGE2-EA. Our results suggest that endocannabinoid-derived prostanoids may be sufficiently stable in humans to act as systemic mediators or prodrugs. In addition, the results highlight dramatic species differences with respect to glycerol ester metabolism and indicate that rat models of 2-AG and PG-G biology will not be easily translated into the human setting.
Experimental Procedures

Materials. 2-AG, 1-AG, d8-2-AG, PGE₂, PGE₂-EA, and PGF₂α were purchased from Cayman Chemical (Ann Arbor, MI). 1(3)-PGE₂-G, d5-1(3)-PGE₂-G, and 1(3)-PGF₂α-G were synthesized as previously described (9). Synthetic PG-Gs were determined to be 9:1 mixtures of the 1(3) and 2 regioisomers by ¹H NMR. Tetradeuterated PGE₂-EA was synthesized by 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide-mediated coupling of PGE₂ with d4-ethanolamine (Cambridge Isotope Laboratories, Andover, MA) followed by silica gel purification (9). NAD⁺, NADH, and AEA were obtained from Sigma. All other chemicals were obtained from Aldrich. Male Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). Sprague-Dawley rats with jugular and femoral vein catheters were from Charles River Laboratories (Wilmington, MA). Rat plasma was obtained by abdominal aortic artery puncture of male Sprague-Dawley rats. Human whole blood and plasma were obtained by venipuncture of four healthy adult volunteers. Plasma was prepared by centrifugation and blood products were maintained on ice until use (< 2 h after collection). Blood-free canine CSF was obtained by ventricular puncture immediately post-mortem. Canine CSF was maintained on ice until use (< 1 hr after collection). Blood-free human CSF was obtained after informed consent from an otherwise healthy human undergoing neurological evaluation at Vanderbilt Hospital. Human CSF was maintained on ice until use (< 2 hr after collection). Blood-free bovine CSF was purchased from Pel-Freez Biologicals (Rogers, AR) and stored at –80˚ C. Human, recombinant 15-HPGDH was partially purified by blue agarose affinity chromatography as described previously (15).
Enzymology. 15-HPGDH activity was determined by following the formation of NADH fluorometrically as previously described with minor modifications (16). Briefly, reaction mixtures contained substrate (0.15 to 300 µM), 1.8 µg partially purified, human 15-HPGDH, and 1.5 mM NAD⁺ in 1 mL of 50 mM potassium phosphate buffer (pH 7.5) containing 0.02% BSA and 1 mM EDTA. Reactions were initiated by the addition of enzyme and allowed to proceed at room temperature. NADH formed in the reaction was recorded by the increase in fluorescence at 460 nm with excitation at 340 nm. The instrument was calibrated using different concentrations of NADH determined by direct measurements of the absorbance at 340 nm, using a molar extinction coefficient of 6.22 x 10³ M⁻¹cm⁻¹ (17). Product characterization was accomplished by treating 18 µg 15-HPGDH in 500 µL of 50 mM potassium phosphate buffer (pH 7.5) containing 0.02% BSA and 1 mM EDTA with PGE₂-G or PGE₂-EA (100 µM) in the presence or absence of NAD⁺ (1 mM). Following incubation at room temperature for 10 min, products were extracted with ice-cold EtOAc (2 x 750 µL). Organic solvent was removed under a stream of argon and the residue was redissolved in 300 µL MeCN:H₂O (1:1) and subjected to direct liquid infusion MS and UV spectroscopy (9:1 dilution in H₂O).

In vivo metabolism studies. Male Sprague-Dawley rats (200 g, n = 3) were injected with 400 µg PGE₂-G or PGE₂-EA intravenously via jugular vein catheters. PGE₂ derivatives were prepared as 37°C solutions in EtOH:PEG:saline (1:2:2) immediately prior to use. Whole blood samples (500 µL) were obtained via femoral vein catheters at 5, 15, 30, 60, 120, 240, 480 and 1440 min following dosing. Whole blood was centrifuged and plasma samples (200 µL) were isolated and stored at −80°C. Plasma
samples were thawed on ice, isotopically labeled internal standard was added, and products were extracted with EtOAc. Following removal of organic solvent under a stream of argon, the resultant residue was redissolved in MeCN:H$_2$O (1:1) and analyzed by LC/MS (see below).

**Mass spectrometry.** LC/MS was conducted essentially as described using a Waters 2690 Separations Module with a Zorbax RX-C18 narrow bore column (15 cm x 2.1 mm, 5 µm) interfaced to a Finnigan TSQ-7000 triple quadrupole mass spectrometer (9). Glyceryl prostaglandins were eluted with a gradient of 10 to 40% acetonitrile in 0.001% aqueous NaOAc in 5 min followed by isocratic elution with 40% acetonitrile. Prostaglandin ethanolamides were eluted with a gradient of 20 to 80% acetonitrile in 0.001% aqueous NaOAc in 14 min. AGs were eluted with a gradient of 60 to 100% acetonitrile in 0.001% aqueous NaOAc in 15 min followed by isocratic elution with acetonitrile. Flow rate was fixed at 0.2 mL/min in all experiments. Quantification of glyceryl and ethanolamide prostanoids was accomplished using pentadeuterated PG-G or tetradeuterated PG-EA internal standards, respectively. Quantification of AGs was accomplished using $d_{8}$-2-AG as the internal standard. Displayed mass chromatograms are normalized to a reference chromatogram defined in individual Figure legends. To assign 100% relative abundance for reference chromatograms, the area corresponding to the ion of interest was divided by the area of the deuterated internal standard employed ($d_{4}$-PG-EA, $d_{5}$-PG-G, or $d_{8}$-2-AG as appropriate) and this ratio was considered 100%. A similar analysis for each displayed chromatogram permits presentation of chromatograms with identical abundance scales as in the reference chromatogram. PGE$_{2}$
quantification was conducted by gas chromatography/negative ion chemical ionization mass spectrometry as previously described (18). All displayed mass chromatograms and spectra are representative of at least three independent experiments.
**Results**

**Plasma stability of PG-Gs, PG-EAs, and AGs.** To examine the metabolic stability of PG-Gs in plasma, synthetic PGE$_2$-G (4 μg/mL) was incubated in freshly obtained rat plasma at 37°C. As shown in Figure 1, PGE$_2$-G disappeared with a half-life of 14.4 ± 0.4 s (mean ± S.E., n = 3). Regiospecific metabolism was noted with 1(3)-PGE$_2$-G disappearing more rapidly than 2-PGE$_2$-G (Fig. 2A and Eq. 2). Metabolism was inhibited by pretreating plasma with the non-specific hydrolase inhibitor, potassium fluoride (Fig. 2B). The half-time for appearance of PGE$_2$ from PGE$_2$-G in rat plasma was 16.0 ± 0.9 s (mean ± S.E., n = 3) which corresponds closely with the half-life of PGE$_2$-G disappearance (Eq. 3). Similar experiments to probe the stability of PGE$_2$-G were conducted in human plasma. In contrast to the results with rat plasma, incubation of synthetic PGE$_2$-G (4 μg/mL) in freshly obtained human plasma at 37°C led to no detectable loss of the glyceryl prostanoid up to 10 min (Fig. 3). When human whole blood was substituted for plasma, modest PGE$_2$-G loss was observed with a half-life of approximately 7 min (Fig. 3).

**Insert eq 2**

**Insert eq 3**

The dramatic species differences observed in PGE$_2$-G plasma stability prompted an evaluation of the stability of the parent endocannabinoid, 2-AG, and the thermodynamically more stable isomer, 1-AG, in rat and human plasma. As expected, both AGs were more rapidly metabolized in rat plasma with approximate half-lives of 0.8 min for 1-AG and 1.0 min for 2-AG (Fig. 4 and data not shown). In human plasma, 1-
AG and 2-AG disappeared with half-lives of 8 min and 16 min, respectively (Fig. 4 and data not shown). Once again, regioselective metabolism of the sterically less hindered 1-glyceryl esters was observed. In fact, in human plasma, isomerization of 2-AG to 1(3)-AG competed with direct hydrolysis of 2-AG (Fig. 4D).

To examine PG-EA stability in plasma, PGE$_2$-EA (4 µg/mL) was incubated in both rat and human plasma. In contrast to the PG-Gs, no rapid hydrolysis of PGE$_2$-EA was observed. After 5 h at 37˚C, 50% and 75% of the starting PGE$_2$-EA remained in human and rat plasma, respectively (Fig. 5A). To identify the degradation product in plasma, PGE$_2$-EA (4 µg/mL) was incubated in rat plasma for 40 h at 37˚C. A product with [M + Na$^+$] = 400 amu was produced in these incubations in a time-dependent manner (Fig. 5B). This mass corresponds to a product of dehydration of PGE$_2$-EA and a similar product was generated by base-treatment of PGE$_2$-EA (Fig. 5C, Eq. 4). Base-treatment of PGE$_2$ is known to provide the dehydration/isomerization product, PGB$_2$. The degradation product found in rat plasma treated with PGE$_2$-EA, as well as base-treated PGE$_2$-EA, displayed absorption maxima at 276 nm consistent with a PGB$_2$-like structure (Fig. 5D and data not shown). Assuming an extinction coefficient similar to PGB$_2$ ($\epsilon = 26,000$ M$^{-1}$cm$^{-1}$), more than two-thirds of the starting PGE$_2$-EA was recovered from rat plasma as PGB$_2$-EA after 40 h at 37˚C. No significant PGB$_2$ free acid generation was observed in rat plasma incubations (data not shown). Thus, hydrolysis of PGE$_2$-EA to PGE$_2$ does not appear to occur at a measurable rate in rat or human plasma.

**Insert eq 4**
**CSF stability of PG-Gs and PG-EAs.** To assess the stability of glyceryl and ethanolamide prostaglandins in CSF, \( \text{PGE}_2 \)-G and \( \text{PGE}_2 \)-EA (4 \( \mu \)g/mL) were incubated at 37 °C for 5 h in freshly obtained blood-free, canine or human CSF or commercially obtained blood-free, bovine CSF. No significant degradation was observed in incubations of \( \text{PGE}_2 \)-G or \( \text{PGE}_2 \)-EA in CSF from these species (Fig. 6).

**15-HPGDH oxidation of PG-Gs and PG-EAs.** 15-HPGDH was examined for its ability to oxidize \( \text{PGE}_2 \), \( \text{PGE}_2 \) methyl ester, \( \text{PGE}_2 \)-EA, and \( \text{PGE}_2 \)-G. \( \text{PGE}_2 \) and derivatives were evaluated because previous reports indicate that \( \text{PGE}_2 \) is the best natural substrate for 15-HPGDH (15,16,19). The steady-state kinetic parameters for each substrate were determined using a fluorimetric assay for NADH generation and are summarized in Table 1. All of the \( \text{PGE}_2 \)-related substrates were oxidized by the enzyme. The experimentally determined \( K_m \) for 15-HPGDH with \( \text{PGE}_2 \) as substrate is in good agreement with previous reports (15,19-21). In addition, the lower \( K_m/V_{max} \) for \( \text{PGE}_2 \) methyl ester as compared to \( \text{PGE}_2 \) conforms to studies demonstrating that 15-HPGDH is less active with methyl ester substrates than with free acids, and, more importantly, that a substrate carboxylate is not required for oxidation by the enzyme (19,22). As shown in Table 1, the ability of 15-HPGDH to oxidize \( \text{PGE}_2 \) and derivatives decreased with increasing steric bulk at carbon 1. The enzyme was nearly fivefold less active with \( \text{PGE}_2 \)-G, the most sterically hindered substrate examined. The products generated by 15-HPGDH oxidation of \( \text{PGE}_2 \)-G and \( \text{PGE}_2 \)-EA were characterized by UV spectroscopy and MS. Enzymatic oxidation of both \( \text{PGE}_2 \)-G and \( \text{PGE}_2 \)-EA generated products with absorption maxima near 230 nm, consistent with an \( \alpha,\beta \)-unsaturated carbonyl moiety. This
functional group could only have arisen by oxidation of the alcohol at carbon 15 (Fig. 7B and 7D) (23). Mass spectral analysis of the 15-HPGDH products revealed the loss of two amu relative to the substrates PGE₂-G and PGE₂-EA (Fig. 7A and 7C). Thus, although both PGE₂-G and PGE₂-EA contain less hindered alcohols in the ester and amide functional groups than the 15(S)-hydroxyl group, only one oxidation event per substrate molecule was observed and oxidation occurred only at carbon 15.

PGF₂α and PGF₂α-G oxidation by 15-HPGDH was also evaluated. PGF₂α was oxidized by 15-HPGDH with \( K_m \) and \( V_{max} \) values of 21 ± 3 \( \mu \)M and 820 ± 30 nmoles/min/mg protein, respectively. Again, these values are in good agreement with previous reports (15,16,22). In contrast, the steady state kinetic values for 15-HPGDH oxidation of PGF₂α-G could not be determined due to the extremely low activity observed with this substrate at all concentrations examined (Fig. 8).

**In vivo pharmacokinetics of PGE₂-G and PGE₂-EA.** In vivo catabolism of endocannabinoid-derived PGE₂ derivatives was investigated by injecting rats with these lipids (2 mg/kg, i.v.) and quantifying plasma concentrations over a 24 h period. No detectable starting material was evident in plasma from PGE₂-G-treated animals at 5 min after dosing (\( n = 3 \)). In contrast, PGE₂-EA was detectable in plasma up to 2 hours after treatment. Plasma levels decreased monoexponentially with an apparent half-life of 380 ± 50 s (mean ± S.E., \( n = 3 \)). The calculated \( t = 0 \) concentrations were quite low (1.1 ± 0.2 \( \mu \)g/mL) indicating a large apparent volume of distribution (approximately 360 mL).
Discussion

The present findings establish the metabolic stability of endocannabinoid-derived PG-like lipids in a variety of biological settings. The oxygenated 2-AG product, PGE$_2$-G, proved highly labile in rat plasma (Fig. 1). Hydrolytic metabolism was demonstrated by preferential catabolism of the less sterically hindered 1(3)-glyceryl esters when compared to the 2-glyceryl ester, sensitivity to potassium fluoride inhibition, and a half-time for PGE$_2$ generation essentially indistinguishable from the half-life observed for PGE$_2$-G hydrolysis (Fig. 2). In stark contrast, PGE$_2$-G was stable in human plasma. The dramatic species differences observed between human and rat plasma are consistent with previous metabolism studies of non-natural PG esters (24,25). Although stable in human plasma, PGE$_2$-G was catabolized in human whole blood with a half-life of approximately 7 min. Enhanced ester lability in human whole blood when compared to plasma has been reported for both prostanoid and non-prostanoid esters and suggests a role for blood cells in glyceryl PG metabolism in humans (25-31).

Marked species differences in plasma PGE$_2$-G stability suggested that the parent endocannabinoid also might be metabolized differently in rat and human plasma. As seen in Figure 4, 2-AG and 1-AG were significantly more stable in human plasma than in rat plasma. The demonstrated lability of AGs in rat plasma is consistent with previous findings indicating that 2-AG is rapidly hydrolyzed in mouse whole blood (32). Taken together, these results suggest that rodent models may not be wholly adequate for investigating the human biology of glycerol esters of both PGs and arachidonic acid and
highlight possible complications in AG and PG-G quantification from rodent tissue (e.g. blood contamination).

In contrast to esterified PGs, PGE\textsubscript{2}-EA was slowly metabolized in plasma from both rats and humans. Plasma slowly promotes the dehydration/isomerization of PGE\textsubscript{2}-EA to provide PGB\textsubscript{2}-EA. The more rapid degradation of PGE\textsubscript{2}-EA in human plasma is consistent with earlier demonstrations that human albumin促进s the conversion of PGE\textsubscript{2} to PGB\textsubscript{2} more efficiently than rat albumin (33). However, in contrast to glycerol esters, this species difference was very minor and unlikely to affect the overall in vivo disposition of PGE\textsubscript{2}-EA, suggesting that rodents may serve as adequate models for investigating the human biology of PG-EAs. This conclusion is supported by recent evidence that the cardiovascular effects of exogenous AEA, but not 2-AG, are mediated by cannabinoid receptors and not through arachidonic acid or subsequent metabolites in anesthetized mice (32).

The demonstrated stability of PGE\textsubscript{2}-G and PGE\textsubscript{2}-EA in the CSF of three mammalian species indicates that this biological fluid has no significant metabolic capacity for neutral PG derivatives. This finding suggests that in vivo detection and quantification of endocannabinoid-derived PGs may be possible by careful sampling of CSF. Rigorous exclusion of blood and the use of higher sensitivity detection methods will be required to test this possibility and several approaches are currently under evaluation. High sensitivity PG detection methods typically rely on immunological techniques, which are
unlikely to distinguish PG free acids from PG-Gs or PG-EAs. Thus, the simple transfer of available PG detection technologies will most likely not prove useful.

The cytosolic, NAD$^+$-dependent 15-HPGDH is the key enzyme in PG inactivation in vivo. In fact, resistance to 15-HPGDH oxidation has been used as a rational drug design strategy in the development of long-lasting, non-natural prostaglandin analogues (e.g. misoprostol). The demonstration that both PGE$_2$-G and PGE$_2$-EA are oxidized by 15-HPGDH confirms that carboxylic acid-enzyme interactions are not critical for turnover. Despite the tolerance for uncharged moieties at carbon 1, a stepwise increase in $K_m$ was observed with increasing steric bulk at the carboxylate. As determined by $^1$H NMR and as illustrated in Figure 2, the tested synthetic PGE$_2$-G is a 9:1 mixture of 1(3)-PGE$_2$-G and 2-PGE$_2$-G (data not shown). Our results suggest that the sterically more hindered 2-PGE$_2$-G, the expected initial product of 2-AG oxygenation by COX-2, would be an even worse 15-HPGDH substrate than the PGE$_2$-G employed in these studies. More importantly, the decreased activity of 15-HPGDH toward PG-Gs and PG-EAs suggests that these lipids may be longer lasting in vivo than the PGs. This raises the possibility that COX-2-generated PG esters or amides exert biological actions in tissues remote from their site of generation thereby extending the range of action of this class of eicosanoids.

A rigorous test of the hypothesis that endocannabinoid-derived PGs may be metabolically more stable than their free acid counterparts was conducted using a rat model. Thirty years ago, Hamberg and Samuelsson demonstrated that, in humans, intravenously administered PGE$_2$ was rapidly cleared from blood with essentially no parent PG
remaining after just 4.5 min (34). The importance of 15-HPGDH in PGE$_2$ clearance was supported by the identification of the primary metabolite as a 15-keto-derivative. Although 15-HPGDH is expressed throughout the body, tissues with the highest levels of activity include lung and kidney (35). Consequently, pulmonary and renal blood flow are critical determinants of rapid PG inactivation in vivo. The enhanced renal and pulmonary blood flow in rats compared to humans predisposes to rapid PG clearance.

In rats, PGE$_2$-G was rapidly cleared after intravenous administration with no detectable parent compound present in plasma at 5 min. The rapid catabolism of PGE$_2$-G is consistent with the hydrolytic lability of the glycerol moiety in rat plasma. Given the hydrolytic stability of PGE$_2$-G in human plasma, further investigations on the metabolism of glycercyl PGs in species with less active plasma esterases should prove informative. In contrast to the glycerol ester, PGE$_2$-EA was detectable in rat plasma up to 2 h after dosing suggesting that this AEA-derived prostanoid is significantly longer lasting than PGE$_2$. In addition, a half-life greater than 5 min theoretically permits significant blood transport prior to inactivation. Conceivably, locally produced COX-2 metabolites of AEA could act on a distant target organ equipped with an appropriate PG-EA receptor or amidase.

These findings provide the first insights into the catabolism of COX-2-generated, oxygenated endocannabinoid products. The dramatic species differences observed with glycerol esters indicate that the rat is not a suitable model for studying the in vivo human biology of 2-AG and PG-Gs. Less efficient 15-hydroxyl oxidation by 15-HPGDH
suggests that COX-2 metabolites of 2-AG and AEA may be significantly more stable than the corresponding arachidonic acid metabolites (PGs). This is supported by the observed in vivo pharmacokinetics of PGE\textsubscript{2}-EA. The rapid metabolism of PGE\textsubscript{2}-G in rat does not preclude an extended lifespan for this ester in humans particularly considering the marked differences in plasma esterase activity between the two species. Taken together, the present findings support the possibility that endocannabinoid-derived PG-like compounds may be sufficiently stable in humans to exert actions systemically either as PG precursors or as unique signal mediators.

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References


Table 1: Steady-state kinetic values of 15-HPGDH

<table>
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<th>Substrate</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (nmoles/min/mg)</th>
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<tr>
<td>PGE$_2$</td>
<td>2.7 ± 0.4</td>
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<tr>
<td>1(3)-PGE$_2$-G</td>
<td>18 ± 1</td>
<td>770 ± 10</td>
<td>43</td>
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</table>

*Kinetic values were determined using the fluorimetric assay as described in Experimental Procedures. The maximum rates of reaction were obtained in quadruplicate with substrate concentrations varying from 0.15 to 300 µM. Substrate stocks were prepared in ethanol and diluted 500-fold to yield final concentrations. Values are the mean ± S.E.*
Figure Legends

Figure 1. **Kinetics of PGE\textsubscript{2}-G disappearance in rat plasma.** Freshly obtained rat plasma was treated with synthetic PGE\textsubscript{2}-G (4 µg/mL) and incubated at 37 °C for the indicated times. The plasma was extracted with ethyl acetate and the extract was subjected to LC/MS analysis with selected ion monitoring (m/z 449). (A). Selected ion mass chromatograms normalized to the 15 s sample. (B). Kinetics of PGE\textsubscript{2}-G disappearance (mean ± S.E., n = 3)

Figure 2. **PGE\textsubscript{2}-G metabolism in rat plasma.** (A). Metabolism of PGE\textsubscript{2}-G in rat plasma is regioselective. Freshly obtained rat plasma was treated with synthetic PGE\textsubscript{2}-G (4 µg/mL), incubated at 37° C for the indicated times, and an organic extract was subjected to LC/MS analysis with selected ion monitoring (m/z 449). Mass chromatograms are normalized to the largest peak detected in each chromatogram to highlight the regioselective metabolism of PGE\textsubscript{2}-G. Consequently, individual panels are not on identical scales. For quantitative comparisons, please see Figure 1A. (B) Metabolism of PGE\textsubscript{2}-G in rat plasma is fluoride-sensitive. Freshly obtained rat plasma was treated with synthetic PGE\textsubscript{2}-G (4 µg/mL) and incubated at 37° C in the presence or absence of 20 mM KF for the indicated times and subjected to LC/MS analysis with selected ion monitoring (m/z 449). Individual chromatograms are normalized to the 30 s chromatogram.

Figure 3. **PGE\textsubscript{2}-G metabolism in human plasma and whole blood.** Kinetics of PGE\textsubscript{2}-G disappearance following treatment of freshly obtained human plasma or whole blood
with synthetic PGE$_2$-G (4 µg/mL) and incubation at 37˚ C for the indicated times. Samples were subjected to LC/MS analysis with selected ion monitoring (m/z 449). Values represent the mean ± S.E. (n = 4).

Figure 4. **Arachidonylglycerol ester stability in plasma.** LC/MS selected ion mass chromatograms (m/z 401) of 1-AG (A, C) and 2-AG (B, D) incubated at 37˚ C in rat (A, B) or human (C, D) plasma for the indicated times. Chromatograms are normalized to the 15 s sample.

Figure 5. **PGE$_2$-EA stability in plasma.** (A). PGE$_2$-EA (4 µg/mL) remaining after incubation in rat or human plasma for 5 h at 37˚ C (mean ± S.E., n ≥ 3). (B). PGE$_2$-EA (4 µg/mL) was incubated in rat plasma for the indicated times and subjected to LC/MS analysis with selected ion monitoring for the mass corresponding to the dehydration product of PGE$_2$-EA (m/z 400). Chromatograms are normalized to the 2400 min sample. (C) PGE$_2$-EA (4 µg/mL) was treated as indicated and samples were analyzed for the dehydration product by LC/MS (m/z 400). Chromatograms are normalized to the base treated sample (lowest panel). (D). Absorption spectra of PGE$_2$-EA (20 µg/mL) incubated in rat plasma for the indicated times.

Figure 6. **Stability of PGE$_2$-G and PGE$_2$-EA in CSF.** PGE$_2$-G (■) or PGE$_2$-EA (□) (4 µg/mL) was incubated in blood-free bovine, canine, and human CSF at 37˚ C for 5h. Quantification was accomplished by selected ion monitoring LC/MS. Values represent the mean ± S.E. (n ≥ 3).
Figure 7. **PGE$_2$-G and PGE$_2$-EA Oxidation by 15-HPGDH.** Products of 15-HPGDH action on PGE$_2$-G (A, B) and PGE$_2$-EA (C, D) in the presence and absence of NAD$^+$ were isolated as described in Experimental Procedures and subjected to direct liquid infusion MS (A, C) and absorption spectroscopy (B, D).

Figure 8. **PGF$_2$$_\alpha$ and 1(3)-PGF$_2$$_\alpha$-G Metabolism by 15-HPGDH.** Oxidation of 100 μM (A), 30 μM (B), and 10 μM (C) PGF$_2$$_\alpha$ (solid line) and 1(3)-PGF$_2$$_\alpha$-G (dashed line) by 15-HPGDH. Reactions were monitored as described in Experimental Procedures.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6

PGE$_2$-X Remaining at 5h (%)

- Bovine
- Canine
- Human

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**Figure 7**

A. 

- NAD$^+$

+ NAD$^+$

B. 

$\lambda_{\text{max}} = 231 \text{ nm}$

C. 

- NAD$^+$

+ NAD$^+$

D. 

$\lambda_{\text{max}} = 228 \text{ nm}$
Figure 8
\[ \text{PGE}_2 \xrightarrow{15\text{-HPGDH}} \text{15-keto-PGE}_2 \]  
(Eq. 1)
2-PGE$_2$-G $\xrightarrow{\text{Isomerization}}$ 1(3)-PGE$_2$-G

(Eq. 2)
(Eq. 3)
Dehydration

(Peq. 4)
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