Metabolism of Anandamide by Human Cytochrome P450 2J2 in the Reconstituted System and Human Intestinal Microsomes

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ABSTRACT

According to the Centers for Disease Control and Prevention, the incidence of inflammatory bowel diseases (IBD) is about 1 in 250 people in the United States. The disease is characterized by chronic or recurring inflammation of the gut. Because of the localization of the endocannabinoid system in the gastrointestinal tract, it may be a potential pharmacologic target for the treatment of IBD and other diseases. Fatty acid amide hydrolase (FAAH) is a potential candidate because it is upregulated in IBD. FAAH hydrolyzes and, as a consequence, inactivates anandamide (AEA), a prominent endocannabinoid. Inhibition of FAAH would lead to increases in the amount of AEA oxidized by cytochrome P450s (P450s). CYP2J2, the major P450 epoxide nase expressed in the heart, is also expressed in the intestine and has previously been reported to oxidize AEA. We have investigated the possibility that it may play a role in AEA metabolism in the gut and have demonstrated that purified human CYP2J2 metabolizes AEA to form the 20-hydroxyeicosatetraenoic acid ethanolamide (HETE-EA) and several epoxygenated products, including the 5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acid ethanolamides (EET-EAs), in the reconstituted system. Kinetic studies suggest that the K_M values for these products range from approximately 10 to 468 μM and the K_M values from 0.2 to 23.3 pmol/min per picomole of P450. Human intestinal microsomes, which express CYP2J2, metabolize AEA to give the 5,6-, 8,9-, and 11,12-EET-EAs, as well as 20-HETE-EA. Studies using specific P450 inhibitors suggest that although CYP2J2 metabolizes AEA, it is not the primary P450 responsible for AEA metabolism in human intestines.

Introduction

More than one million people suffer from inflammatory bowel diseases (IBD) in the United States. IBD is a chronic inflammation of the lower part of the gastrointestinal tract that is caused by the immune system. Two classic types of this disease are Crohn’s disease and ulcerative colitis. IBD is characterized by severe diarrhea, abdominal pain, fatigue, nausea, and weight loss due to malnutrition. Moreover, a person diagnosed with IBD has an increased risk of developing colon cancer (Moran et al., 2013; Chang et al., 2014; Seo and Chae, 2014). Although the cause(s) of IBD is unknown, studies suggest that autoimmune reactions, genes, and the environment all may contribute to its development (Kozak et al., 2002b; Stanley and O’Sullivan, 2014; Curkovic et al., 2013; Yarlas et al., 2015).

Several therapies are currently available to treat IBD symptoms, including both medications and surgery. Medicinal interventions include aminosaliclylates (Yarlas et al., 2015), corticosteroids (Kuenzig et al., 2014), immunomodulators (Amin et al., 2015), anti-tumor necrosis factor therapies (Chang et al., 2014; Seo and Chae, 2014), as well as medications used primarily to treat accompanying symptoms, such as pain, infections, and diarrhea. Complications and failure of pharmaceuticals often make surgery a necessity for many patients with IBD (Moody et al., 2001). Removal of the colon essentially cures ulcerative colitis, but resection of the diseased segment in patients with Crohn’s disease is only a temporary treatment since the disease returns in up to 90% of cases (Moody et al., 2001). As a result, other therapeutic options need to be examined.

Evidence suggests that modifications of the endocannabinoid system (ECS), which consists of receptors, their ligands, and enzymes that regulate the synthesis and degradation of the endocannabinoids, may be related to several pathologic conditions, including a variety of immunologic, cardiovascular, gastrointestinal, and metabolic disorders that exhibit altered tissue concentrations of arachidonoyl ethanolamide (AEA), one of the most ubiquitous endocannabinoids (Di Marzo et al., 2004; Ashton and Smith, 2007; Bifulco et al., 2007; Lambert, 2007; Storr and Sharkey, 2007; Di Marzo, 2008; Alpini and Demorrow, 2009). Usually recognized for its expression in the brain, the ECS is also found in peripheral tissues, including those affected by IBD, such as myenteric neurons and epithelial cells of the gastrointestinal tract (Pertwee, 1999). Activation of presynaptic cannabinoid 1

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ABBREVIATIONS: AA, arachidonic acid; AEA, anandamide; AUDA, 12-(3-admantan-1-yl-ureido) dodecanoic acid; CB-1, cannabinoid receptor 1; CB-2, cannabinoid receptor 2; EA, ethanolamide; ECS, endocannabinoid system; EET, epoxyeicosatrienoic acid; ESI, electrospray ionization; FAAH, fatty acid amide hydrolase; HETE, hydroxyeicosatetraenoic acid; HIM, human intestinal microsome; HLM, human liver microsome; IBD, inflammatory bowel disease; LC/MS, liquid chromatography/mass spectrometry; P450, cytochrome P450; sEH, soluble epoxide hydrolase; sEHI, soluble epoxide hydrolase inhibitor.
receptors (CB₁Rs) in neurons and fibers of the stomach, small intestine, and colon inhibits acetylcholine release, essentially slowing digestive processes (Kulkarni-Nrala and Brown, 2000, 2001; Mascolo et al., 2002; Pinto et al., 2002; Di Carlo and Izzo, 2003). Similarly, activation of cannabinoid 2 receptors (CB₂Rs), expressed on immune and epithelial cells of the gastrointestinal tract, inhibits the release of inflammatory mediators that induce intestinal peristalsis (Izzo, 2004; Mathison et al., 2004).

The levels of AEA, which can bind to and activate both CBRs, are regulated by a number of different enzymes. AEA normally undergoes hydrolysis by fatty acid amide hydrolase (FAAH) to form arachidonic acid (AA) and ethanolamide. In patients with IBD, FAAH activity is increased, and it has been suggested as a potential target for therapy (Di Sabatino et al., 2011). It is now widely recognized that it is critically important to investigate all avenues of AEA metabolism, including oxidation by cyclo-oxygenases (Yu et al., 1997; Kozak et al., 2002a; Stanley and O'Sullivan, 2014), lipoxygenases (Hampson et al., 1995; Ueda et al., 1995; Moody et al., 2001; Kozak et al., 2002b), and cytochrome P450s (P450s) (Snider et al., 2007, 2008; Stark et al., 2008; Sridar et al., 2011; McDougle et al., 2014).

The P450s are heme-containing mono-oxygenases involved in the metabolism of a wide variety of compounds. The metabolism of AEA by human P450s has been shown to give the following metabolites: 19- and 20-hydroxyeicosatetraenoic acid ethanolamides (HETE-EA) and the 5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acid ethanolamides (EET-EA) (Alberich Jorda et al., 2004; Snider et al., 2007, 2008; Stark et al., 2008; Sridar et al., 2011; McDougle et al., 2014).

Materials and Methods

Materials. AEA, AEA-d₈, 5,6-EET-EA, 8,9-EET-EA, 11,12-EET-EA, 14,15-EET-EA, 20-HETE-EA, and 12-(3-admantan-1-yl-ureido)dodecanoic acid (AUDA) were purchased from Cayman Chemical (Ann Arbor, MI). Danazol was purchased from Steraloids, Inc. (Newport, RI). Ketoconazole and sulfaphenazole were purchased from Sigma (St. Louis, MO). All other reagents were of the highest quality and were obtained from commercial sources.

Enzymes. The human P450 CYP2J2 cDNA was a gift from Dr. Rheem Totah (University of Washington, Seattle, WA). CYP2J2 and P450 reductase were expressed in Ershicheria coli and purified as previously described (Hanna et al., 1998; Smith et al., 2008). HIMs were purchased from BD Biosciences (Woburn, MA).

AEA Metabolism Assays. CYP2J2 was reconstituted with reductase (1:2 ratio) in lipid (L-α-dilauroyl-phosphocholine) on ice for 60 minutes as described previously (von Weymarn et al., 2004; Snider et al., 2007). The metabolism of AEA was determined using incubation mixtures (0.5 ml) containing 100 mM potassium phosphate buffer (pH 7.4), catalase (500 U), AEA (1.25–50 μM), and reconstituted CYP2J2 (50 pmol). For microsomal studies, 200-μl aliquots of microsomal protein were combined with 100 mM potassium phosphate buffer, pH 7.4, 3.3 mM MgCl₂, and AEA in the presence or absence of the P450 inhibitors, danazol (100 nM) for CYP2J2, ketoconazole (1 μM) for CYP3A4, and sulfaphenazole for CYP2C9 (10 μM), in a final volume of 0.25 ml. Reactions were initiated by the addition of 1 mM (recombinant) or 1.3 mM (microsomes) NADPH and allowed to continue with shaking for 25–30 minutes at 37°C. Control reactions were performed in the absence of NADPH. The reactions were terminated by the addition of 1–2 ml of cold ethyl acetate. After the addition of the internal standard, AEA-d₈, the samples were vortexed for 2 minutes and centrifuged at full speed using a desktop centrifuge for 5 minutes. The organic layers were collected and dried down under a constant stream of nitrogen gas. The dried samples were resuspended in 100 μl of methanol, and 10-μl aliquots were subjected to electrospray ionization-liquid chromatography/mass spectrometry (ESI-LC/MS) analysis. The standard curves for the various metabolites that were used for the quantification and determination of the Kₘ and kₑₐₜ values were generated by subjecting various known amounts of authentic standards to the same sample workup and ESI-LC/MS analysis.

ESI-LC/MS Analysis. Samples (10 μl) were injected onto a Hypersil ODS column (5 μm, 4.6 × 100 mm; Thermo Fisher Scientific, Waltham, MA) that had been equilibrated with 25% solvent A (0.1% acetic acid in water) and 75% solvent B (0.1% acetic acid in methanol). The metabolites were resolved using the following gradient: 0 minutes, 75% B; 20 minutes, 100% B; 25 minutes, 100% A; 30 minutes, 75% B; 5 minutes, 75% B; 20–25 minutes, 100% B; 14 minutes, 75% B; 15 minutes, 25% B; 16 minutes, 100% B; 20 minutes, 75% B; 25 minutes, 100% B. The extracted metabolites were extracted and analyzed as described in Materials and Methods. The extracted ion chromatograms for the mono-oxygenated products (m/z 364, top) and the diols formed by the hydrolysis of the epoxides (m/z 382, bottom) are shown. The dashed lines represent the chromatograms observed in the presence of 10 μM of AUDA, a soluble epoxide hydrolase inhibitor (sEH), and the solid lines represent the products formed in the absence of the sEH.

Fig. 1. AEA metabolism by HIMs in the presence and absence of AUDA. HIMs (200 μg) were incubated with AEA (25 μM) for 30 minutes at 37°C, and the metabolites were extracted and analyzed as described in Materials and Methods. The extracted ion chromatograms for the mono-oxygenated products (m/z 364, top) and the diols formed by the hydrolysis of the epoxides (m/z 382, bottom) are shown. The dashed lines represent the chromatograms observed in the presence of 10 μM of AUDA, a soluble epoxide hydrolase inhibitor (sEH), and the solid lines represent the products formed in the absence of the sEH.
B; 25–26 minutes, 100%–75% B; and 26–30 minutes, 75% B. The flow rate was 0.3 ml/min. The column effluent was directed into the LCQ mass analyzer (Thermo Fisher Scientific). The ESI conditions were as follows: sheath gas, 90 arbitrary units; auxiliary gas, 30 arbitrary units; capillary temperature, 200°C; and spray voltage, 4.5 V. Data were acquired in positive ion mode for AEA and its metabolites using the Xcalibur software package (Thermo Fisher Scientific) with one full scan from 300 to 500 mass/charge ratio (m/z) followed by one data-dependent scan of the most intense ion.

**Data Analysis.** Nonlinear regression analyses of the data were performed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA).

**Results**

**AEA Metabolism by HIMs.** To investigate the ability of HIMs to metabolize AEA, incubations containing 200 μg of HIMs were used to metabolize AEA for 30 minutes at 37°C as described in Materials and Methods. Figure 1 (top) shows the extracted ion chromatogram at m/z 364 for the mono-oxygenated metabolites from this reaction, with peaks eluting at 15.9, 18.4, 19.2, 19.8, and 20.7 minutes corresponding to 20-HETE-EA, 14,15-, 11,12-, 8,9-, and 5,6-EET-EAs, respectively. Although no standard is commercially available for positive identification of the product eluting at 17.3 minutes, it is thought to be 19-HETE-EA (Snider et al., 2007, 2009; Pratt-Hyatt et al., 2010). The peaks eluting at 11.6, 13.4, and 15.6 minutes observed at m/z 382 correspond to the diols formed when the epoxides (m/z 364) are hydrolyzed (Fig. 1, bottom).

Figure 2 shows the kinetic curves for the products formed by human intestine microsomal metabolism of AEA. Two different kinetic models were used to determine the K_m and V_max values for the formation of 20-HETE-EA, 5,6-, 8,9-, and 11,12-EET-EAs. Michaelis-Menten kinetics was used to determine the formation rate of 20-HETE-EA. The presence of sEH altered the formation of all four EET-EAs. As a result, double reciprocal plots were used to estimate the kinetic constants for three of the EET-EAs. The levels of formation of the 14,15-EET-EA, under these conditions, were too low to calculate accurate values for the kinetic constants. The K_m values for 20-HETE-EA, 5,6-, 8,9-, and 11,12-EET-EAs were 145, 37, 850, and 337 μM, respectively. The estimated V_max values were 578, 2, 7, and 12 pmol/min per milligram of protein for 20-HETE-EA, 5,6-, 8,9-, and 11,12-EET-EAs, respectively, giving V_max/K_m values of 50, 0.3, 0.1, 0.3 μM⁻¹min⁻¹ for 20-HETE-EA, 5,6-, 8,9-, and 11,12-EET-EAs, respectively. The kinetics for the formation of the 19-HETE-EA could not be calculated.
because there is no standard available for quantitation of this metabolite.

**Metabolism of AEA by Human Recombinant CYP2J2.**

Several P450s are known to be expressed in the intestine, including CYP3A4, CYP2C9, CYP2C19, and CYP2J2 (Paine et al., 2006). Although the expression levels of CYP2J2 in the intestine are reported to be less than 2% of the total P450, the physiologic relevance of 2J2 in the intestine has previously been suggested by reports of its contribution to the intestinal metabolism of certain drugs, such as ebastine and astemizole (Hashizume et al., 2002; Matsumoto et al., 2002). AA is metabolized by recombinant CYP2J2 to form four regioisomeric EETs (Wu et al., 1996). As shown in Fig. 3, because of the similarities between the structures of AEA and AA, and the fact that AEA is a substrate for several other P450s, including CYP2D6, CYP3A4, and CYP2B6 (Omura and Sato, 1964; Snider et al., 2007, 2008; Sridar et al., 2011), we investigated the possibility that AEA could also be a substrate for CYP2J2.

Figure 4 shows the extracted ion chromatograms for the metabolism of AEA by purified human CYP2J2. Figure 4 (top) is the extracted ion chromatogram at m/z 348, and it shows the positive ion formed by AEA in the mass spectrometer, which elutes at 23.6 minutes using the LC/MS conditions described in Materials and Methods. The bottom chromatogram shows that five mono-oxygenated products with m/z values of 364 were formed which eluted at 15.2, 18.1, 19.1, 19.6, and 20.5 minutes. Based on their retention times and fragmentation patterns compared with authentic standards (data not shown), the metabolites were identified as 20-HETE-EA, 14,15-, 11,12-, 8,9-, and 5,6-EET-EA, respectively.

**Kinetic Studies on the Metabolism of AEA by Human Recombinant CYP2J2.**

The reaction conditions used to determine the kinetic constants, $K_M$ and $k_{cat}$, for the hydroxylation and epoxygenation of AEA by purified CYP2J2 were initially shown to be linear with respect to protein concentration (up to 25 pmol) and time of incubation (up to 25 minutes) (data not shown). As shown in Fig. 5, the metabolism of AEA exhibited typical Michaelis-Menten kinetics for the formation of 20-HETE-EA and 5,6-, 8,9-, 11,12-, 14,15- EET-EAs. The extrapolated $K_M$ values calculated from the data in Fig. 5 using GraphPad Prism 6 software were 10, 468, 104, 101, and 103 μM, and the $k_{cat}$ values were 0.2, 23.3, 3.6, 4.1, and 6.9 pmol/min per picomoles of P450, respectively. The efficiency of CYP2J2 for the formation of all five metabolites, as measured by the $k_{cat}/K_M$ values, was relatively the same for all products formed with values of 0.02, 0.05, 0.03, 0.04, and 0.07 μM$^{-1}$min$^{-1}$ for the 5,6-, 8,9-, 11,12-, 14,15-EET-EAs, and 20-HETE-EA, respectively.

**AA as a Competitive Inhibitor of AEA Metabolism.**

The formation of AA metabolites by cytochromes P450 and their involvement in intestinal vasodilatation was suggested some time ago (Proctor et al., 1987). Since it is known that CYP2J2 catalyzes the metabolism of AA in the intestine (Delozier et al., 2007) and that AA is a relatively potent inhibitor of several P450s, including CYP2C9 and CYP2C19 (Yao et al., 2006), we investigated the ability of AA to inhibit the CYP2J2-catalyzed metabolism of AEA in the recombinant system. The results show that AA inhibits the formation of all five AEA metabolites to varying extents. Adding 20 μM AA, twice the amount of AA compared with AEA, inhibited the formation of 20-HETE-EA, 5,6-, 8,9-, 11,12-, and 14,15 EET-EA.
Fig. 5. Kinetic analysis of AEA metabolism by purified CYP2J2. Varying concentrations of AEA (1.25–50 μM) were metabolized by reconstituted CYP2J2 (25 pmol) in the reconstituted system for 25 minutes at 37°C. The symbols for the metabolites are 14,15-EET-EA (∗), 11,12-EET-EA (△), 8,9-EET-EA (▲), 5,6-EET-EA (●), and 20-HETE-EA (♦). Standard curves were generated for each metabolite to determine the amount of product formed. Experiments were done in duplicate and repeated four times (n = 8). Error bars represent S.E.M. values.

Fig. 6. AA inhibition of the metabolism of AEA by CYP2J2. Recombinant CYP2J2 (25 pmol), CPR reductase (50 pmol), and DLPC (10 μg) were reconstituted on ice for at least 30 minutes. The reconstituted enzyme complex was added to a reaction tube containing 0.1 M KPi (pH 7.4), catalase, and varying concentrations of AA (0–20 μM), and the mixtures were incubated for 5 minutes at 37°C. AEA (10 μM) was added to the reaction mixtures and incubated for 5 minutes at 37°C. The reactions were initiated with 1 mM of NADPH and allowed to continue for 30 minutes at 37°C. The reactions were quenched and the products isolated and analyzed as described in Materials and Methods. Each bar represents the average amount of product formed ± S.E.M. Statistical significance was determined by the Student’s t test. *P < 0.05.

Discussion

IBDs are characterized by chronic inflammation of all or part of the digestive tract. Patients suffer intestinal cramps and spasms, severe diarrhea, rectal bleeding, nausea and vomiting, and loss of appetite and weight that can lead to malnutrition (Britton and Peppercorn, 1997). Some reports indicate that people suffering from Crohn’s disease get symptomatic relief by smoking marijuana (Di Carlo and Izzo, 2003). This effect has been attributed to the ability of marijuana to stimulate appetite, alleviate nausea, control spasms, and potentially reduce inflammation (Grinspoon and Bakalar, 1997; Nocerino et al., 2000). Although all the components of the ECS have been detected in the gastrointestinal system (Wright et al., 2008), evidence suggests that the levels of endocannabinoids, particularly AEA, are significantly increased during intestinal inflammation (Izzo et al., 2001; D’Argenio et al., 2006).

In inflammatory disorders, the ECS is overstimulated in the small and large intestine (Di Marzo and Izzo, 2006). Our laboratory has previously studied the metabolism of AEA by human kidney and human liver microsomes (HLMs) (Snider et al., 2007). The metabolite profile for HLM is similar to that of HLM with the formation of the 20- and 19-HETE-EAs and the 11,12-, 8,9-, and 5,6-EET-EAs. Although human liver microsomes readily produced the 14,15-EET-EA metabolite, its formation from HLMs was observed only at concentrations of AEA exceeding 100 μM or in the presence of a soluble epoxide hydrolase inhibitor. Incubation of AEA with human kidney microsomes yielded only the 20-HETE-EA (Snider et al., 2007). The efficiencies or Vmax/Km values for the formation of 20-HETE-EA, 5,6-, 8,9-, and 11,12-EET-EAs by HLMs were 50, 0.3, 0.1, 0.3 μM⁻¹ min⁻¹. Because the addition of the epoxide hydrolase inhibitor AUDA increased the levels of formation of the EET-EAs, these findings suggest the presence of epoxide hydrolase in HLM preparations. As a result, the kinetic constants calculated may be significantly lower than the actual values since the EET-EAs are constantly being hydrolyzed by epoxide hydrolase. Moreover, under the reaction conditions used to minimize solvent effects, the highest concentration of substrate that could be reached was 1.4 mM. Even at this high AEA concentration, Vmax could not be obtained for the EET-EAs, which is likely due to the presence of sEH. Based on the Vmax/Km values, liver microsomes are approximately 2 and 25 times more efficient at catalyzing the formation of 20-HETE-EA than are the kidney and intestine microsomes, respectively. The 20-HETE-EA metabolite is the major product of P450-catalyzed AEA metabolism by HLM and HIMs (Snider et al., 2007). In relation to the epoxides, the 8,9-EET-EA was the preferred product formed by HLM, but the 11,12-EET-EA seems to be the preferred product formed by HIM based on the Vmax and the catalytic efficiency (Vmax/Km).

To date, the P450s known to metabolize AEA are reported to be found primarily in the liver, kidney, and brain (Snider et al., 2007, 2008; Sridar et al., 2011). CYP2J2 is the most abundantly expressed P450 in the cardiovascular system (Wu et al., 1996), but it is also expressed in other tissues, such as the intestines (Wu et al., 1996; Zeldin et al., 1997b; Paine et al., 1996; Zeldin et al., 2001; D'Argenio et al., 2006).
and CYP2C families (Wu et al., 1996; King et al., 2002; El-Sherbeni and El-Kadi, 2014). Although CYP2J2 is known for its racemic production of the four epoxides (1:1:1:1) from AA (Wu et al., 1996), the four EET-EAs are produced in a ratio of 2:1:1:2 for the 5,6-, 8,9-, 11,12-, and 14,15-EET-EA.

The kinetic constants, $K_M$ and $k_{cat}$, were determined for each of the five major metabolites formed by CYP2J2. Although some of the metabolites had significantly different values for their kinetic parameters, similar deviations in these kinetic values have been seen for the metabolism of AEA by other P450s and for the different regioisomers formed during P450-mediated metabolism of AA (Daikh et al., 1994; Kiss et al., 2000; Snider et al., 2008; Pratt-Hyatt et al., 2010; Sridar et al., 2011). The metabolism of AEA by CYP2J2 is not as efficient, based on the $k_{cat}/K_M$ values, as that by other P450s such as CYP2D6, CYP2B6, and CYP3A4 (Snider et al., 2008; Pratt-Hyatt et al., 2010; Sridar et al., 2011); however, in the presence of 50 μM AEA, the total turnover rate for the formation of all the products by recombinant CYP2J2 was calculated to be about 7 nmol of total product formed/nmol of CYP2J2/min. For comparison, in the presence of 100 μM AA, two separate laboratories have determined that the turnover for AA was 0.065 and 0.165 nmol of total product formed/nmol of CYP2J2/min. This is a difference of more than 40-fold. In agreement with the studies by McDougle et al. (2014), this suggests that AEA may be a much better substrate for CYP2J2 in vivo than AA.

The pharmacologic relevance of CYP2J2 activity in the intestine has previously been suggested by reports of its contribution to the intestinal metabolism of certain drugs (Hashizume et al., 2002; Matsumoto et al., 2002). Although CYP2J2 is present in the intestine, it does not appear to be a significant contributor to AEA metabolism in the intestine in comparison with other P450s. In fact, when CYP2J2 was inhibited, more of the 8,9- and 11,12-EET-EA products were produced, possibly as a result of compensation from other, more efficient P450s for the loss of CYP2J2 activity (Kaspera et al., 2014). On the other hand, CYP3A4 and CYP2C9 are responsible for the formation of about 50% of the 20-HETE-EA and 11,12-EET-EA produced in the intestine, respectively. Other P450s, such as CYP2C19 and CYP2D6, both of which are known to be expressed at appreciable levels in the intestine, probably contribute significantly to AEA metabolism in the intestine as well (Paine et al., 2006; Snider et al., 2008). Since the intestinal absorption of AA was reported to be in the μM-mM range (Chow and Hollander, 1978), under physiologic conditions, CYP2J2-catalyzed activity will probably

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**Table 1**

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<th>Product formation by cytochrome P450s in the presence of specific inhibitors</th>
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<td>Control</td>
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<td>11,12-EET-EA</td>
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**Fig. 7.** Contributions of individual P450s to the metabolism AEA by HIMs. The HIMs (200 μg) were incubated with AEA (100 μM) for 30 minutes at 37°C in the presence of vehicle, the selective CYP3A inhibitor ketoconazole (1 μM), the selective CYP2C9 inhibitor sulfaphenazole (10 μM), or the selective CYP2J2 inhibitor danazol (100 nM). The reactions were quenched with ice-cold ethyl acetate, and the metabolites were extracted and analyzed as described in Materials and Methods. (A) Epoxide formation in pmol/min per milligram of protein, (B) hydroxylated products formed in pmol/min per milligram of protein. Statistical significance was determined by the GraphPad Prism 6 program using the Holm-Sidak method. $^*P < 0.05$. Each bar represents the mean ± S.E.M.
be inhibited by roughly 30% owing to the presence of AA in the intestine.

Previous studies from our laboratory have suggested a physiologic importance for the 5,6-EET-EA metabolite of AEA since it exhibits nanomolar affinity for the CB2 receptor and decreases intracellular cAMP accumulation in Chinese hamster ovary cells expressing the CB2 receptor (Snider et al., 2009). Moreover, this metabolite is more biologically stable than the parent AEA, with an estimated half-life of 32 minutes (Snider et al., 2009). This half-life is also four times longer than the half-life reported for the structurally similar 5,6-EET metabolite formed by the metabolism of AA (Fulton et al., 1998). Since the 5,6-EET-EA is one of the AEA metabolites formed in the intestine and its presence can be quantified in the presence of sEH unlike 14,15-EET-EA, which is formed at very low levels, it may play an important anti-inflammatory role in the intestine.

Intestinal motility is usually attributed to CB1 receptor activation; however, in an inflammatory state, the CB2 receptor may predominate (Mathison et al., 2004). It is widely known that CB2 receptors are expressed on immune cells and that the gut has an abundant supply of immune cells (Cabral and Staab, 2005; Klein, 2005; Lunn et al., 2006). Moreover, activation of the CB2 receptor has been shown to inhibit the release of inflammatory mediators that are known to encourage intestinal peristalsis (Izzo, 2004; Mathison et al., 2004).

The activation of CB2 receptors expressed on human colonic epithelial cells has been found to inhibit the tumor necrosis factor-α–induced release of the inflammatory cytokine interleukin-8 (Ihenetu et al., 2003). Since we have identified 5,6-EET-EA as the major AEA metabolite formed in intestinal mucusomes, and it can activate the CB2 receptor at nanomolar concentrations, these observations may indicate a physiologic function for 5,6-EET-EA in the inflamed gastrointestinal system; however, the physiologic function of the CB2 receptor in the gut has not been well studied, and more studies are needed to confirm this hypothesis.

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Authorship Contributions

Participated in research design: Walker, Hollenberg.
Conducted experiments: Walker, Griffin, Hammar.
Performed data analysis: Walker, Griffin, Hammar.
Wrote or contributed to the writing of the manuscript: Walker, Hollenberg.

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