Fatty Acid Amide Hydrolase Controls Mouse Intestinal Motility In Vivo

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Background & Aims: **Fatty acid amide hydrolase (FAAH) catalyzes the hydrolysis both of the endocannabinoids (which are known to inhibit intestinal motility) and other bioactive amides (palmitoylethanolamide, oleamide, and oleoylethanolamide), which might affect intestinal motility. The physiologic role of FAAH in the gut is largely unexplored. In the present study, we evaluated the possible role of FAAH in regulating intestinal motility in mice in vivo.** ***Methods:*** **Motility was measured by evaluating the distribution of a fluorescent marker along the small intestine; FAAH messenger RNA (mRNA) levels were analyzed by reverse-transcription polymerase chain reaction (RT-PCR); endocannabinoid levels were measured by isotope-dilution, liquid chromatography, mass spectrometry.** ***Results:*** **Motility was inhibited by *N*-arachidonoylserotonin (AA-5-HT) and palmitoylisopropylamide, 2 selective FAAH inhibitors, as well as by the FAAH substrates palmitoylethanolamide, oleamide, and oleoylethanolamide. The effect of AA-5-HT was reduced by the CB_1 receptor antagonist rimonabant and by CB_1 deficiency in mice but not by the vanilloid receptor antagonist 5′-iodoresiniferatoxin. In FAAH-deficient mice, pharmacologic blockade of FAAH did not affect intestinal motility. FAAH mRNA was detected in different regions of the intestinal tract.** ***Conclusions:*** **We conclude that FAAH is a physiologic regulator of intestinal motility and a potential target for the development of drugs capable of reducing intestinal motility.**

The endogenous cannabinoid system includes cannabinoid (CB_1 and CB_2) receptors, their endogenous ligands (the endocannabinoids), and the enzymes for the synthesis and inactivation of these ligands.[1,2] The endocannabinoids anandamide and 2-arachidonylglycerol (2-AG) may reduce gastrointestinal motility through activation of enteric CB_1 receptors; potential therapeutic applications of this activity include the treatment of motility disorders such as gastroesophageal reflux disease, irritable bowel syndrome, diarrhea, and inflammatory bowel diseases.[3,4] Several experiments have demonstrated that the CB_1 receptor antagonist rimonabant (SR141716A), in the absence of any exogenous agonist, produces motility changes that are invariably opposite in direction to those caused by the cannabinoid receptor agonists. For example, rimonabant is known to increase (1) electrically induced contractions and peristalsis in isolated intestinal segments from rodents,[5–8] (2) occurrence of transient lower esophageal sphincter relaxation in dogs,[9] and (3) intestinal motility in mice in vivo, both in the small[10,11] and in the large[12] intestine. These effects cannot be attributed unequivocally to the displacement of endogenous cannabinoids because rimonabant may behave as an inverse agonist at CB_1 receptors in vitro.[13]

Inactivation of endocannabinoid signaling is dependent on cellular uptake, localization to appropriate intracellular compartments, and enzymatic hydrolysis. The latter reaction produces arachidonic acid and either ethanolamine (from anandamide) or glycerol (from 2-AG).[14] Although it is generally recognized that there is uptake, intracellular transport, and hydrolysis of anandamide, only the latter step has been conclusively assigned to a protein, the fatty acid amide hydrolase (FAAH).[2,14] FAAH is a membrane-associated protein that is localized to internal membranes, such as the endoplasmic reticulum, at which it is active. The broad substrate specificity of FAAH allows it to catalyze the hydrolysis not only of the endocannabinoids anandamide and 2-AG but also of palmitoylethanolamide (PEA), oleamide (a sleep-inducing factor),[15] and oleoylethanolamide, whose biologic effects may be independent of CB_1 receptors.[16,17] FAAH activity has been detected in the rodent

Abbreviations used in this paper: 2-AG, 2-arachidonylglycerol; AA-5-HT, *N*-arachidonoylserotonin; DMSO, dimethyl sulfoxide; FAAH, fatty acid amide hydrolase; I-RTX, 5′-iodoresiniferatoxin; PEA, palmitoylethanolamide; PIP, palmitoylisopropylamide; RT-PCR, reverse-transcription polymerase chain reaction.

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intestine and was found to be increased in the croton oil model of intestinal inflammation.[18] However, to date, selective FAAH inhibitors have not been evaluated in the gastrointestinal tract.

The present study investigates the possible role of FAAH in the control of intestinal motility in mice in vivo. To this end, we used the selective FAAH inhibitors *N*-arachidonoylserotonin (AA-5-HT)[19] and palmitoylisopropylamide (PIP)[20] as well as FAAH-deficient mice. In addition, we report the distribution of FAAH messenger RNA (mRNA) along the mouse intestinal tract.

Materials and Methods

Animals

Male ICR mice (Harlan Italy, Corezzana, MI) (20–22 g) were normally used, but, in our preliminary experiments, some female ICR mice were studied as well. No difference in sensitivity to FAAH inhibitors was found between males and females. Mice lacking CB_1 receptor and FAAH genes were generated and genotyped as previously described.[21,22] Female homozygous wild-type and homozygous mutant littermates (19–22 g) were used in the experiments. Mutant mice were in a mixed genetic background with a predominance of C57BL/6N contribution (5 backcrosses for both mutant lines). Mice were fed ad libitum with standard mouse food, except for the 12-hour period immediately preceding the experiments.

Functional Studies

Transit was measured by evaluating the intestinal location of rhodamine-B–labeled dextran.[23,24] Animals were given fluorescent-labeled dextran (100 μL of 25 mg/mL stock solution) via a gastric tube into the stomach. Twenty minutes after administration, the entire small intestine with its content was divided into 10 equal parts. The intestinal contents of each bowel segment were vigorously mixed with 2 mL saline solution to obtain a supernatant containing the rhodamine. The supernatant was centrifuged at 500 rpm to force the intestinal chime to a pellet. The fluorescence in duplicate aliquots of the cleared supernatant was read in a multiwell fluorescence plate reader (LS55 Luminescence spectrometer; Perkin Elmer Instruments; excitation 530 ± 5 nm and emission 590 ± 10 nm) for quantification of the fluorescent signal in each intestinal segment. From the distribution of the fluorescent marker along the intestine, we calculated the geometric center (GC) of small intestinal transit as follows:

$$GC = \sum(\text{fraction of fluorescence per segment} \times \text{segment number})$$

GC ranged from 1 (minimal motility) to 10 (maximal motility).[25] This procedure yielded an accurate, nonradioactive measurement of intestinal transit.[24]

Drug Administration

N-arachidonoylserotonin (AA-5-HT, 1–20 mg/kg), palmitoylisopropylamide (PIP, 1–20 mg/kg), oleamide (1–20 mg/kg), oleoylethanolamide (1–20 mg/kg), palmitoylethanolamide (PEA; 1–20 mg/kg), or vehicle were given intraperitoneally (IP) 30 minutes before the administration of the fluorescent marker. In some experiments, rimonabant (0.1 mg/kg), 5′-iodoresiniferatoxin (I-RTX; 0.75 mg/kg), or SR144528 (1 mg/kg) were given IP 10 minutes before AA-5-HT (15 mg/kg). Rimonabant (0.1 mg/kg) was also given 10 minutes before the administration of PEA, oleamide, or oleoylethanolamide (all at the dose of 10 mg/kg). I-RTX and SR144528 doses were selected on the basis of previous work.[26–28] In some experiments, the effect of IP-injected anandamide (1–20 mg/kg), PEA (1–20 mg/kg), or loperamide (0.03–3 mg/kg) was evaluated 30 minutes after the administration of AA-5-HT (5 mg/kg, IP)

Identification and Quantification of Endocannabinoids and Palmitoylethanolamide

Full-thickness small intestines from mice given (IP) vehicle, AA-5-HT (1–15 mg/kg), oleamide (15 mg/kg), or oleoylethanolamide (15 mg/kg), as well as from FAAH and wild-type deficient mice, were removed, and tissue specimens were immediately weighed, immersed into liquid nitrogen, and stored at −70°C until chromatographic separation of endocannabinoids. Tissues were extracted with chloroform/methanol (2:1, by volume) containing each of 200 pmol d_8-anandamide, d_4-palmitoylethanolamide, and d_5-2-AG, synthesized as described previously (for the former compounds),[29] or provided by Cayman Chemicals (for d_5-2-AG, Ann Arbor, MI). The lipid extracts were purified by silica column chromatography, carried out as described previously,[29] and the fractions containing anandamide, palmitoylethanolamide, and 2-AG were analyzed by isotope-dilution, liquid chromatography, atmospheric pressure, chemical ionization mass spectrometry (LC-APCI-MS) carried out in the selected monitoring mode as described in detail elsewhere.[21] Results were expressed as pmol or nmol per g of wet tissue. Because, during tissue extraction/purification, both d_8- and native 2-AG are partly transformed into the 1(3)-isomers and only a limited amount of arachidonic acid is present on the *sn*-1(3) position of (phospho)glycerides, the amounts of 2-AG reported here represent the combined mono-arachidonyl-glycerol peaks.

Semiquantitative RT-PCR for FAAH mRNA

Total RNA from both the small (duodenum, jejunum, and ileum) and the large (proximal and distal colon) intestine of each animal was extracted using Trizol reagent according to the manufacturer's recommendations (GibcoBRL). Following extraction, RNA was precipitated using ice-cold isopropanol, resuspended in diethyl pyrocarbonate-treated water (Sigma). The integrity of RNA was verified following separation by electrophoresis into a 1% agarose gel containing ethidium