GASTROENTEROLOGY 2005;129:941–951

Fatty Acid Amide Hydrolase Controls Mouse Intestinal Motility In Vivo

RAFFAELE CAPASSO,* ISABEL MATIAS,† BEAT LUTZ,§,|| FRANCESCA BORRELLI,* FRANCESCO CAPASSO,* GIOVANNI MARSICANO,§,|| NICOLA MASCOLO,* STEFANIA PETROSINO,† KRISZTINA MONORY,§,|| MARTA VALENTI,† VINCENZO DI MARZO,† and ANGELO A. IZZO*

*Department of Experimental Pharmacology, University of Naples "Federico II," Naples, Italy; †Endocannabinoid Research Group, Institute of Biomolecular Chemistry, National Research Council, Pozzuoli (NA), Italy; §Molecular Genetics of Behaviour, Max Planck Institute of Psychiatry, Munich, Germany; and ||Department of Physiological Chemistry, Johannes Gutenberg-University Mainz, Mainz, Germany

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.

© 2005 by the American Gastroenterological Association
0016-5085/05/$30.00
doi:10.1053/j.gastro.2005.06.018

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 $\pm$ 5 nm and emission 590 $\pm$ 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

bromide. RNA was treated with RNAse-free DNAse I (Ambion DNA-free kit) according to the manufacturer's recommendations, to digest contaminating genomic DNA. Subsequently DNAse and divalent cations were removed.

The expression of mRNA for GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and FAAH was examined by reverse transcription (RT) coupled to the polymerase chain reaction (PCR). Total RNA was reverse transcribed using oligo dT primers. DNA amplifications were carried out in PCR buffer (Q-Biogen) containing 2 μL cDNA, 500 μmol/L dNTP, 2 mmol/L $MgCl_2$, 0.8 μmol/L each primer, and 0.5 U Taq polymerase (Q-Biogen). The thermal reaction profile consisted of a denaturation step at 94°C for 1 minute, annealing at 60°C for 1 minute, and an extension step at 72°C for 1 minute. A final extension step of 10 minutes was carried out at 72°C. Thirty PCR cycles were observed to be optimal and in the linear portion of the amplification curve (data not shown). The reaction was performed in a PE Gene Amp PCR System 9600 (Perkin Elmer). After reaction, the PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide for UV visualization.

The specific oligonucleotides were synthesized on the basis of cloned cDNA sequences of GAPDH, FAAH, and CB_1 common to the rat and mouse. For GAPDH, the primers sequences were 5′-CCCTTCATTGACCTCAACTACATGGT-3′ (nt 208–233; sense) and 5′-GAGGGCCATCCACAGTCTTCTG-3′ (nt 655–677; antisense, accession No. AH007340). The FAAH sense and antisense primers were 5′-GTGGTGCT(G/A)ACCCCCATGCTGG-3′ (nt 1407–1428) and 5′-TCCACCTCCCGCATGAACCGCAGACA-3′ (nt 1683–1708, accession No. AF098010). The CB_1 sense and antisense primers were 5′-GATGTCTTTGGGAAGATGAACAA GC-3′ (nt 1095–1119) and 5′-AGACGTGTCTGTGGACACAGACATGG-3′ (nt 1380–1405). The expected sizes of the amplicons were 470 bp for GAPDH, 300 bp for FAAH, and 309 bp for CB_1. The expression of the housekeeping gene GAPDH was used as an internal standard. No PCR products were detected when the reverse transcriptase step was omitted (data not shown).

Drugs

N-Arachidonoylserotonin (AA-5-HT) was synthesized as described previously.[19] PIP, oleamide, oleoylethanolamide, PEA, anandamide, and I-RTX were purchased from Tocris Cookson (Bristol, United Kingdom), loperamide hydrochloride from Sigma (Milan, Italy). Rimonabant (SR141716A; [(*N*-piperidin-1-yl)-5-(4-chlorophenyl)-1-2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride]) and SR144528 (*N*-[-1S-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide) were a kind gift from Drs. Madaleine Mossè and Francis Barth (SANOFI-Recherche, Montpellier, France).

AA-5-HT and palmitoylisopropylamide were dissolved in DMSO/Tween 80 (1:4), oleamide and palmitoylethanolamide in ethanol (4 μL/mouse), oleoylethanolamide and iodoresiniferatoxin in DMSO, anandamide in Tocrisolve (soya oil/water [1:4 emulsion]), and loperamide in 2% DMSO. The drug vehicles (20 μL/mouse of DMSO/Tween 80, 4 μL/mouse DMSO, 4 μL/mouse ethanol, 40 μL/mouse Tocrisolve, or 50 μL/mouse 2% DMSO) had no effect on intestinal motility.

Results

Motility

Intraperitoneal administration of AA-5-HT (1–20 mg/kg; Figure 1*A*) and PIP (1–20 mg/kg; Figure 1*B*) produced a dose-dependent inhibition of transit. Both com-

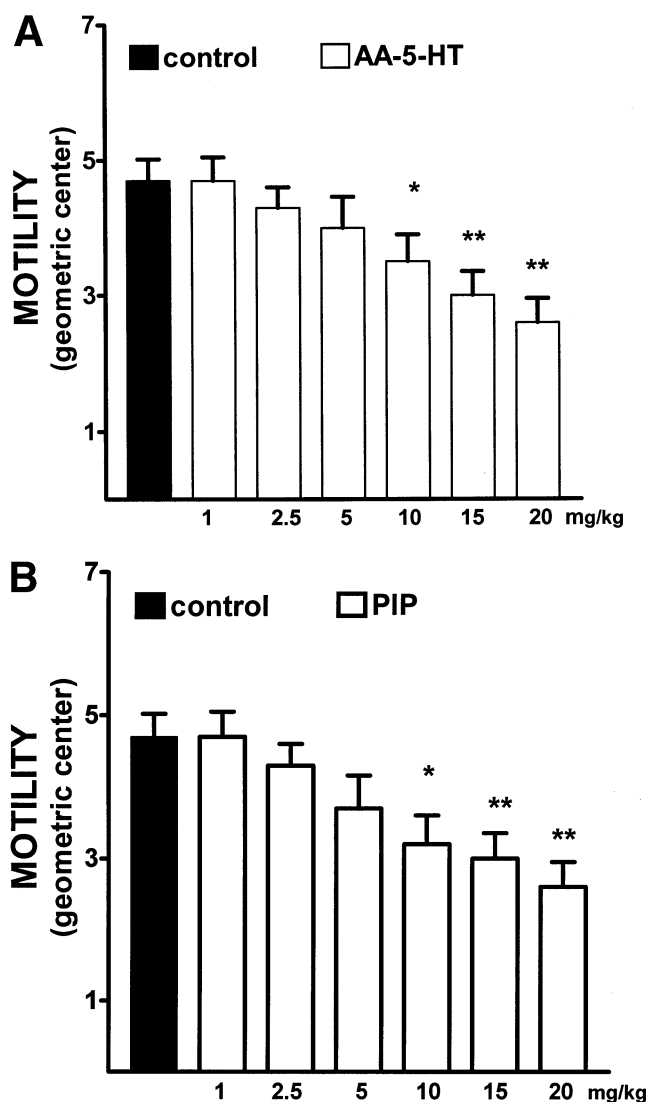

Figure 1. Inhibitory effect of IP-injected arachidonoylserotonin (*AA-5-HT*) and palmitoylisopropylamide (*PIP*) (both at doses ranging from 1 to 20 mg/kg) on intestinal transit in mice. Transit was expressed as the geometric center of the distribution of a fluorescent marker along the small intestine (see Materials and Methods section). *Bars* represent the mean ± SEM of 8–11 animals. *$P < .05$ and **$P < .01$ vs corresponding control.

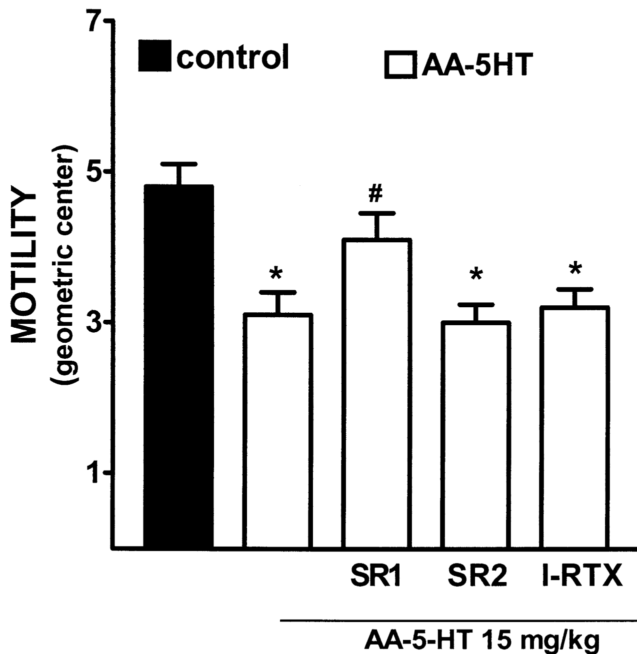

Figure 2. Effect of IP-injected *N*-arachidonoylserotonin (*AA-5-HT,* 15 mg/kg) on intestinal transit in mice pretreated (IP) with the CB_1 receptor antagonist rimonabant (*SR1,* 0.1 mg/kg, IP) or the CB_2 receptor antagonist SR144528 (SR2, 1 mg/kg, IP) or the vanilloid receptor antagonist 5′iodoresiniferatoxin (*I-RTX,* 0.75 mg/kg IP). Transit was expressed as the geometric center of the distribution of a fluorescent marker along the small intestine (see Materials and Methods section). *Bars* represent the mean ± SEM of 8–11 animals. **P* < .05 vs control and #*P* < .05 vs AA-5-HT alone.

pounds gave rise to significant inhibitory effects for the 10-mg/kg dose. A per se noneffective dose of the CB_1 receptor antagonist rimonabant (0.1 mg/kg), but not the CB_2 receptor antagonist SR144528 (1 mg/kg, IP) nor the vanilloid receptor antagonist I-RTX (0.75 mg/kg, IP), significantly reduced the inhibitory effect of AA-5-HT (15 mg/kg, IP) on motility (Figure 2). In absence of AA-5-HT, I-RTX or SR144528 did not affect motility (geometric center: control: 4.5 ± 0.6, I-RTX: 4.7 ± 1.1, SR144528: 4.8 ± 0.7, n = 6 for each experimental group, $P > .2$).

The results concerning the experiments carried out on FAAH- and CB_1 receptor-deficient mice are shown in Figure 3. AA-5-HT (15 mg/kg) significantly ($P < .05$) reduced motility in both $FAAH^{+/+}$ (Figure 3*A*) and in CB_1 $receptor^{+/+}$ (Figure 3*B*) mice. However, AA-5-HT produced no significant effect both in FAAH- and in CB_1 receptor-deficient mice. Compared with wild-type mice, FAAH- and CB_1 receptor-deficient mice showed a slight trend toward decreased or increased motility, respectively (Figure 3).

Figure 4 shows the effect of IP-injected oleamide (1–20 mg/kg), oleoylethanolamide (1–20 mg/kg), and PEA (1–20 mg/kg) on motility. These amides significantly reduced intestinal motility, the effect being significant starting from the 5 mg/kg (oleamide) or 10 mg/kg (PEA and oleoylethanolamide) doses. In the presence of rimonabant (0.1 mg/kg), a significant inhibitory effect was observed only for PEA, and a significant reversion was achieved only for oleamide (Figure 5). Moreover, a dose of AA-5-HT (5 mg/kg, IP) which per se did not affect significantly intes-

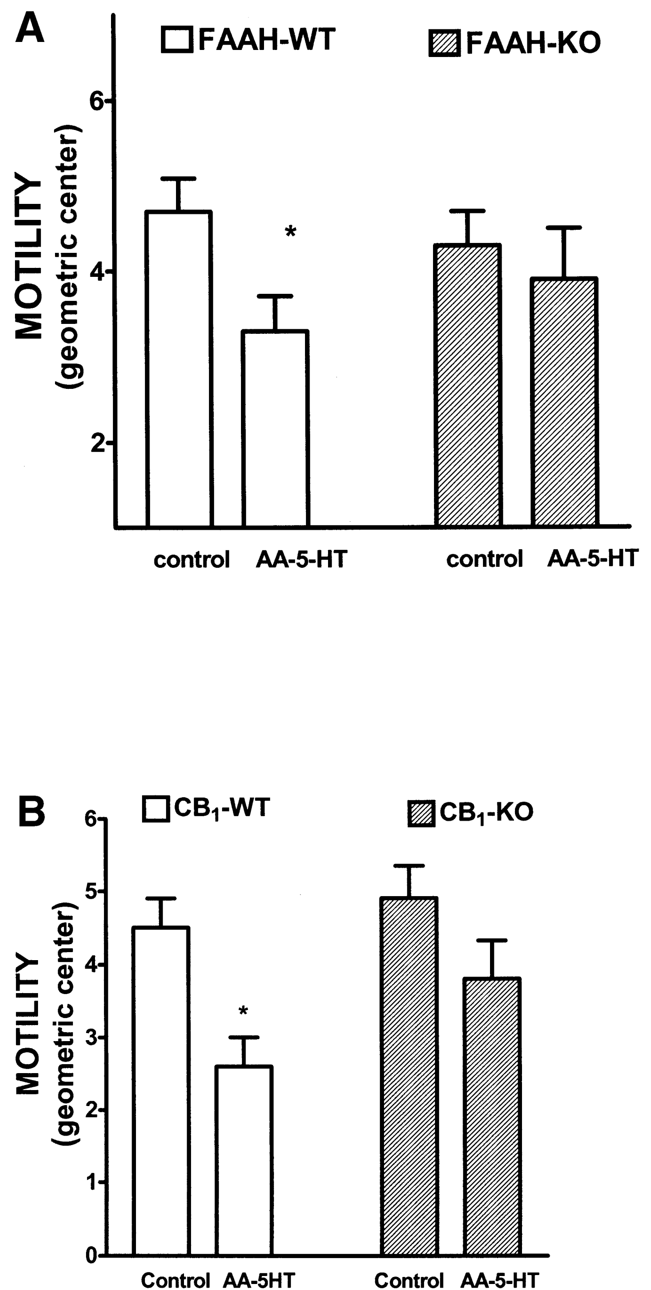

Figure 3. Effect of the FAAH inhibitor *N*-arachidonoylserotonin (*AA-5-HT,* 15 mg/kg, IP) on intestinal transit in FAAH-deficient (*FAAH-KO*) (*A*) or in CB_1-deficient (*CB_1-KO*) (*B*) mice (as compared with the corresponding wild-type (WT) littermates). Transit was expressed as the geometric center of the distribution of a fluorescent marker along the small intestine (see Materials and Methods section). Each *bar* represents the mean ± SEM of 6–8 animals. **P* < .05 vs corresponding control (WT mice).

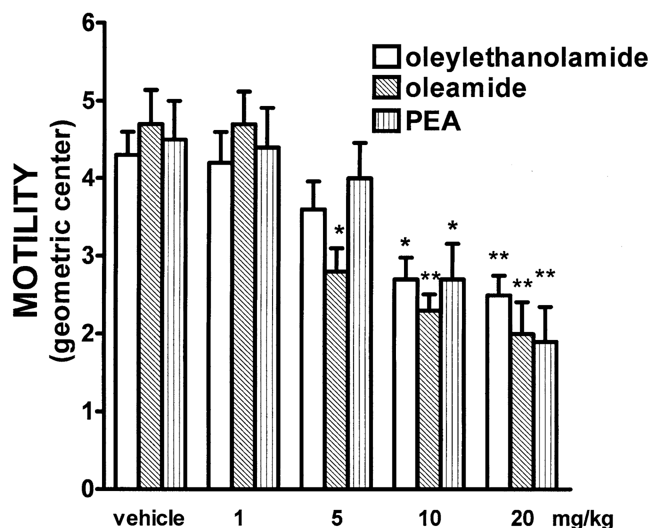

Figure 4. Inhibitory effect of IP-injected *oleamide*, *oleoylethanolamide*, and *palmitoylethanolamide* (*PEA*) on intestinal transit in mice. Transit was expressed as the geometric center of the distribution of a fluorescent marker along the small intestine (see Materials and Methods section). Each *bar* represents the mean ± SEM of 7–10 animals. $*P < .05$ and $**P < .01$ vs vehicle control.

tinal motility increased the inhibitory effect of both anandamide and PEA on motility (Figure 6*A* and 6*B*). However, AA-5-HT did not affect significantly the dose response curve to the opioid drug loperamide (percentage inhibition of motility: loperamide, 0.03 mg/kg 20% ± 5%; loperamide 0.03 + AA-5-HT, 30% ± 7%; loperamide 0.1 mg/kg, 34% ± 6%; loperamide 0.1 + AA-5-HT, 42% ± 6%; loperamide 0.3 mg/kg, 42% ± 6%; loperamide 0.3 + AA-5-HT, 48% ± 6%; loperamide 1 mg/kg, 55% ± 5%; loperamide 1 mg/kg + AA-5-HT, 59% ± 6%; loperamide 3 mg/kg, 69% ± 7%; loperamide 3 mg/kg + AA-5-HT, 66% ± 7%; n = 6–8 for each experimental group).

Endocannabinoid and Palmitoylethanolamide Content in the Small Intestine

Table 1 shows that anandamide, 2-AG, and PEA were increased in the small intestine of animals treated with AA-5-HT (1–15 mg/kg, IP). AA-5-HT significantly increased anandamide and 2-AG levels starting from the 10 mg/kg dose, whereas PEA levels were increased only at the highest dose of AA-5-HT tested (15 mg/kg). By contrast, small intestines from FAAH-deficient mice revealed significantly increased levels of anandamide (but not 2-AG or PEA) as compared with intestines from $FAAH^{+/+}$ mice (Table 2). The effect of oleoylethanolamide and oleamide on the intestinal levels of endocannabinoids and PEA is reported in Table 3. Both amides significantly increased the intestinal level of anandamide and reduced the level of 2-AG. PEA levels did not change after administration of either oleoylethanolamide or oleamide.

Study of FAAH mRNA Levels Determined by the Semiquantitative RT-PCR

Agarose gel analysis of RT-PCR from total RNA from mouse intestine showed intense bands of the sizes expected for a FAAH mRNA transcript (300 bp) as well as a GAPDH mRNA transcript (470 bp). The absence of FAAH amplicons when omitting the reverse-transcription reaction confirmed the absence of genomic DNA contamination in the RNA sample.

When analyzed by densitometry scanning, and normalized to the respective GAPDH transcript bands, FAAH transcript bands were found to be expressed at similar levels in all the regions of the small intestine as well as in the proximal and in the distal colon (Figure 7).

In line with previous studies,[27] abundant CB_1 transcript levels were found in the small intestine of both wild-type and FAAH-deficient mice. However, no significant difference between the 2 genotypes was observed (data not shown).

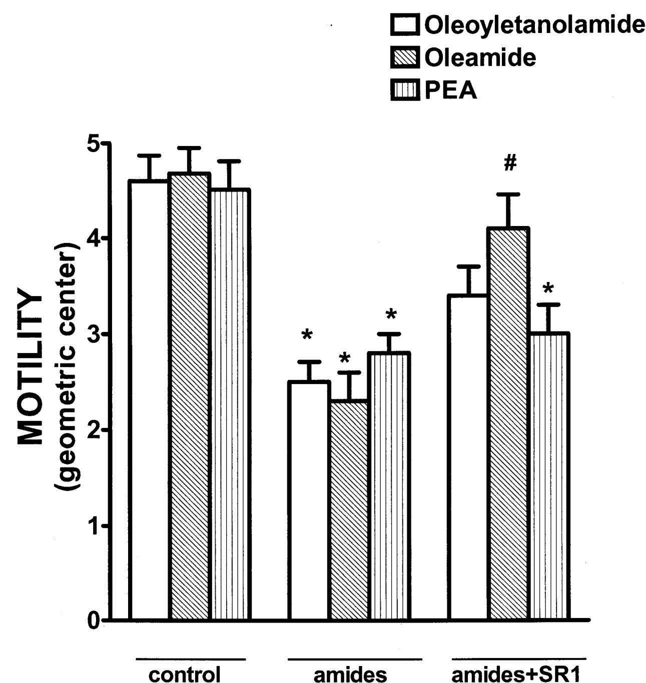

Figure 5. Effect of IP-injected *oleamide*, *oleoylethanolamide*, and *palmitoylethanolamide* (*PEA*) (all these biologic amides were used at the dose of 10 mg/kg) alone or in the presence of the CB_1 receptor antagonist rimonabant (SR1, 0.1 mg/kg, IP). Transit was expressed as the geometric center of the distribution of a fluorescent marker along the small intestine (see Materials and Methods section). *Bars* represent the mean ± SEM of 7–11 animals. $*P < .05$ vs control and $\#P < .05$ vs AA-5-HT alone.

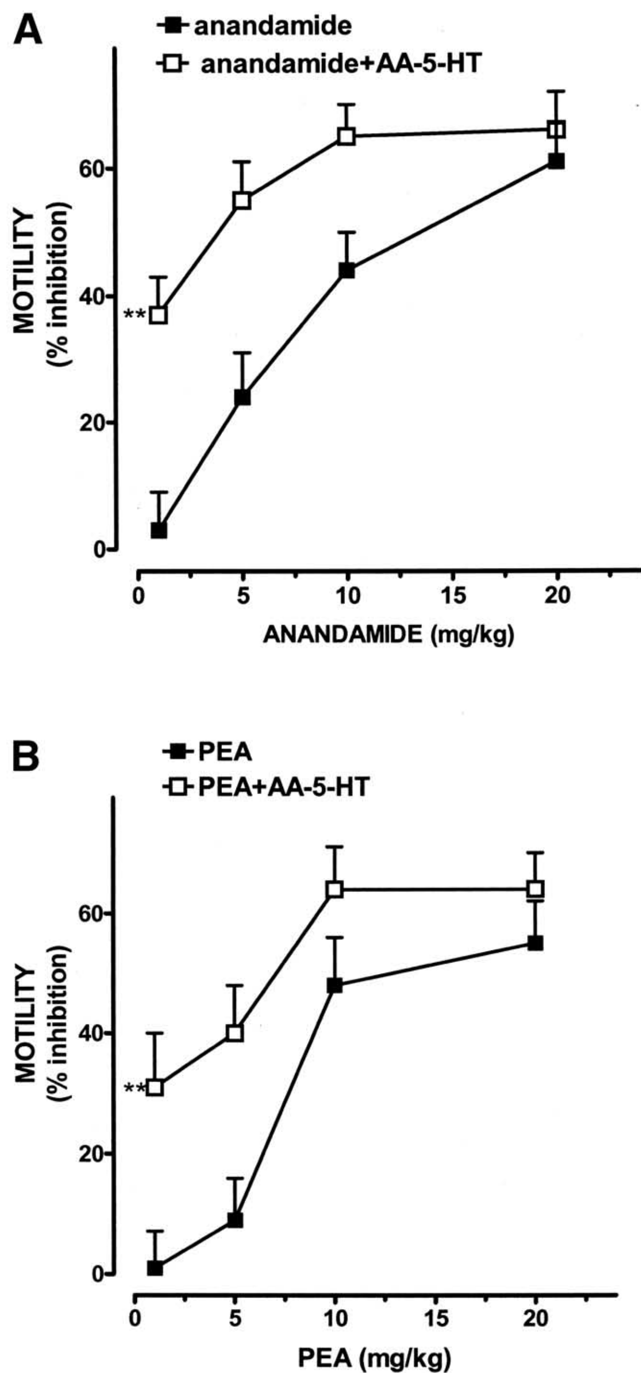

Figure 6. Dose-related inhibition of intestinal motility by *anandamide* (*A*) or *palmitoylethanolamide* (*PEA*) (*B*) alone or in animals treated with the FAAH inhibitor AA-5-HT (5 mg/kg, IP). *Bars* represent the mean ± SEM of 6–8 animals. $**P < .01$ vs anandamide (or PEA) dose-response curve (statistical significance between 2 dose-effect curves).

Discussion

Inhibitors of FAAH are considered to constitute a potential therapeutic approach in the treatment of several disorders, including pain and anxiety and some symptoms of multiple sclerosis.[2] However, the physiologic importance and the therapeutic potential of FAAH in the gastrointestinal tract have been largely unexplored so far. We have previously shown that endocannabinoids significantly contribute to intestinal motility in mice under both physiologic and pathologic conditions by activating enteric CB_1 receptors.[3] In particular, we not only found that agonists of CB_1 receptors inhibit motility and that a CB_1 antagonist, rimonabant, causes the opposite effect, but also that a selective inhibitor of endocannabinoid cellular uptake via the putative endocannabinoid membrane transporter, VDM11, can also inhibit motility in mouse colon[12] and small intestine.[30] In the latter case, however, the endocannabinoid uptake inhibitor was effective only under pathologic conditions, such as paralytic ileus, and not in control mice.[30] Our present data indicate that, also under physiologic conditions, inhibition of the inactivating mechanism subsequent to endocannabinoid uptake, ie, enzymatic hydrolysis via FAAH, leads to inhibition of small intestine motility. These results are supported by biochemical data showing an intense mRNA band of FAAH in different regions of both the small and the large intestine and suggest that this enzyme, rather than a putative endocannabinoid transporter, controls endocannabinoid levels in the small intestine under physiologic conditions. Previous investigators have shown that FAAH activity was by far the highest in the liver, followed by the intestine, brain, testis, and many other organs in the rat.[31]

We have shown that AA-5-HT and PIP, 2 selective FAAH inhibitors that display little or no affinity for cannabinoid receptors,[19,20] significantly inhibit intestinal motility. It is noteworthy that AA-5-HT significantly reduced motility at doses (10 and 15 mg/kg) previously shown to be inactive in the "open field," "hot plate," and rectal hypothermia tests, which are predictive of CB_1 activation in rodents.[19] AA-5-HT was also previously found to be ineffective against anandamide cellular uptake, a mechanism that, however, does not seem to be involved in the control of intestinal motility in the mouse small intestine under physiologic conditions (see above).[30,32] AA-5-HT significantly reduced motility in wild-type, but not in FAAH-deficient mice, thus conclusively confirming that the inhibitory effect of the inhibitor was due to FAAH inhibition and not to nonspecific effects or to interaction with CB_1 receptors (see also fourth paragraph of the Discussion section). Accordingly, AA-5-HT significantly increased the intestinal levels of anandamide, 2-AG, and PEA. Although the effect of PEA was observed only at the highest dose tested (15 mg/kg) of the FAAH inhibitor, anandamide and 2-AG were significantly elevated also by the 10 mg/kg dose of AA-5-HT, which, like the 15 mg/kg dose,

Table 1. Levels of Anandamide, 2-Arachidonylglycerol, and Palmitoylethanolamide in Mouse Small Intestine in Control Mice and in Mice Treated With the FAAH Inhibitor Arachidonoylserotonin

		AA-5-HT (IP)			
	Control	1 mg/kg	5 mg/kg	10 mg/kg	15 mg/kg
Anandamide (pmol/mg lipid)	1.08 ± 0.0	1.02 ± 0.15	1.00 ± 0.05	1.85 ± 0.20[a]	1.55 ± 0.17[a]
2-AG (nmol/mg lipid)	1.23 ± 0.20	1.30 ± 0.30	1.70 ± 0.20	2.30 ± 0.10[a]	2.15 ± 0.24[a]
PEA (pmol/mg lipid)	6.60 ± 0.73	6.09 ± 0.70	6.11 ± 0.74	5.70 ± 0.45	9.36 ± 0.62[a]

NOTE. Results are expressed as the mean ± SEM from 4 animals.
2-AG, 2-arachidonylglycerol; PEA, palmitoylethanolamide.
[a]$P < .05$ vs control.

exerted inhibition of motility per se. These results suggest that (1) AA-5-HT effectively inhibits FAAH in vivo (being endocannabinoids preferentially metabolized by FAAH compared with PEA) and that (2) there is a correlation between the effects of AA-5-HT on the levels of endocannabinoids/PEA (which are known to inhibit intestinal motility in vivo)[3] and the effect of AA-5-HT on intestinal motility, suggesting that the former may be responsible for the latter. The increased intestinal levels of endocannabinoids are in line with the ability of nonselective FAAH inhibitors (ie, phenylmethanesulfonyl fluoride and methylarachidonyl fluorophosphates) to enhance the content of both anandamide and 2-AG in the ileum of rats treated with *Clostridium difficile* toxin A.[33] Moreover, in the present study, we have shown that a per se noneffective dose of AA-5-HT increased the inhibitory effect of both anandamide and PEA on intestinal motility, thus confirming that the FAAH enzyme may metabolize both amines. Others have found that the nonselective FAAH inhibitor phenylmethylsulphonyl fluoride markedly increased the potency of anandamide in the isolated guinea pig ileum.[34]

Monoacylglycerol lipase (MAGL) has been proposed to be the enzyme most responsible for 2-AG inactivation in the brain.[35] In addition, Kathuria et al[36] showed that the FAAH inhibitor URB597 significantly increased the brain levels of anandamide (as well as oleoylethanolamide and PEA) but not of 2-AG. However, it is very unlikely that the increase in intestinal 2-AG levels observed in the present study is due to inhibition of MAGL because AA-5-HT does not inhibit this enzyme in cell free homogenates up to a concentration of 50 μmol/L.[37] An acid hydrolase for PEA that has very low affinity for anandamide and oleoylethanolamide, and is relatively abundant also in the gastrointestinal tract, has been identified and cloned very recently.[38] The effect of AA-5-HT on this enzyme has not been tested yet, but other FAAH inhibitors have been shown to be inactive on this alternative hydrolase. At any rate, the enhancement by AA-5-HT of anandamide as well as 2-AG tissue concentrations is in line with the similar effect observed with the same inhibitor on both endocannabinoids in thyroid carcinomas following intratumor administration.[39] It is possible that, unlike other FAAH inhibitors, AA-5-HT, because of its metabolic stability and the capability to form a stable (although not covalent) complex with FAAH,[19] can enhance the levels also of those substrates of this enzyme, such as PEA and, particularly, 2-AG, which are also metabolized by other hydrolases.

In fact, FAAH catalyzes the hydrolysis not only of endocannabinoids but also of several biologic fatty acid amides, including PEA, oleamide, and oleoylethanolamide. Endocannabinoids[18] and PEA[40] have been detected in the rodent intestine. Anandamide (through activation of CB_1 receptors),[41] palmitoylethanolamide (whose ability to reduce motility[40] has been confirmed here by using a more sensitive method), oleamide, and oleoylethanolamide (whose effects on motility were shown here for the first time) all reduce intestinal transit. Moreover, endocannabinoids may affect intestinal contractility through activation of vanilloid receptors[42] or by nonreceptor mediated mechanisms.[42,43] Considering this scenario, we investigated the receptors and endogenous FAAH substrates that are involved in the FAAH-mediated inhibition of motility. We showed that the inhibitory effect of AA-5-HT on motility was reduced (but not abolished) by the selective CB_1 receptor antagonist rimonabant, but not by the CB_2 receptor antagonist SR144528 or the vanilloid receptor antagonist I-RTX,

Table 2. Levels of Anandamide, 2-Arachidonylglycerol, and Palmitoylethanolamide in Mouse Small Intestine of Wild-Type or FAAH-Deficient Mice

	Wild-type mice	FAAH-deficient mice
Anandamide (pmol/mg lipid)	1.30 ± 0.33	3.61 ± 0.66[a]
2-AG (nmol/mg lipid)	1.34 ± 0.37	2.15 ± 0.28
PEA (pmol/mg lipid)	6.60 ± 1.81	7.80 ± 0.92

NOTE. Results are expressed as the mean ± SEM from 4 animals.
2-AG, 2-arachidonylglycerol; PEA, palmitoylethanolamide.
[a]$P < .05$ vs wild-type mice.

Table 3. Levels of Anandamide, 2-Arachidonylglycerol, and Palmitoylethanolamide in Mouse Small Intestine of Mice Treated With Oleoylethanolamide or Oleamide (10 mg/kg, IP)

	Control	Oleoylethanolamide (10 mg/kg, IP)	Oleamide (10 mg/kg, IP)
Anandamide (pmol/mg lipid)	1.02 ± 0.21	4.50 ± 0.95[a]	2.58 ± 0.55[b]
2-AG (nmol/mg lipid)	1.21 ± 0.09	0.67 ± 0.08[a]	0.77 ± 0.07[a]
PEA (pmol/mg lipid)	7.64 ± 0.65	7.61 ± 2.31	6.42 ± 0.21

NOTE. Results are expressed as the mean ± SEM from 4 animals.
2-AG, 2-arachidonylglycerol; PEA, palmitoylethanolamide.
[a]$P < .01$ vs control.
[b]$P < .05$ vs control.

thus suggesting an involvement of CB_1, but not CB_2 or vanilloid receptors. Experiments performed with CB_1 receptor-deficient mice confirmed the partial involvement of CB_1 receptors. Indeed, AA-5-HT induced a significantly larger reduction in motility in CB_1 receptor wild-type (42% inhibition) than in CB_1 receptor-deficient (22% inhibition) mice. Overall, these experiments suggest that, in addition to endocannabinoids acting through a CB_1 receptor-mediated mechanism, FAAH substrates inactive or weakly active at cannabinoid receptors, including PEA[40] and possibly oleamide and oleoylethanolamide, may be involved in the FAAH-mediated inhibition of motility. The lack of involvement of vanilloid receptors is in line with the ability of exogenously administered anandamide to reduce small intestinal transit through activation of CB_1, but not vanilloid, receptors.[41] However, despite the important role of CB_1 in the AA-5-HT-mediated inhibition of intestinal motility, the lack of a clear phenotype in CB_1-deficient mice under basal conditions suggest that other mechanisms could also be involved and might compensate the lifelong absence of CB_1 receptors in mutant mice. Given the high degree of colocalization of CB_1 and vanilloid TRPV1 receptors in myenteric cholinergic neurons,[44] such compensative mechanisms may indeed involve the latter receptors, which might become engaged by anandamide only when CB_1 receptors are genetically deleted. However, we did not investigate this hypothesis because the primary aim of this work was to investigate the role of FAAH in the intestinal motility of wild-type mice.

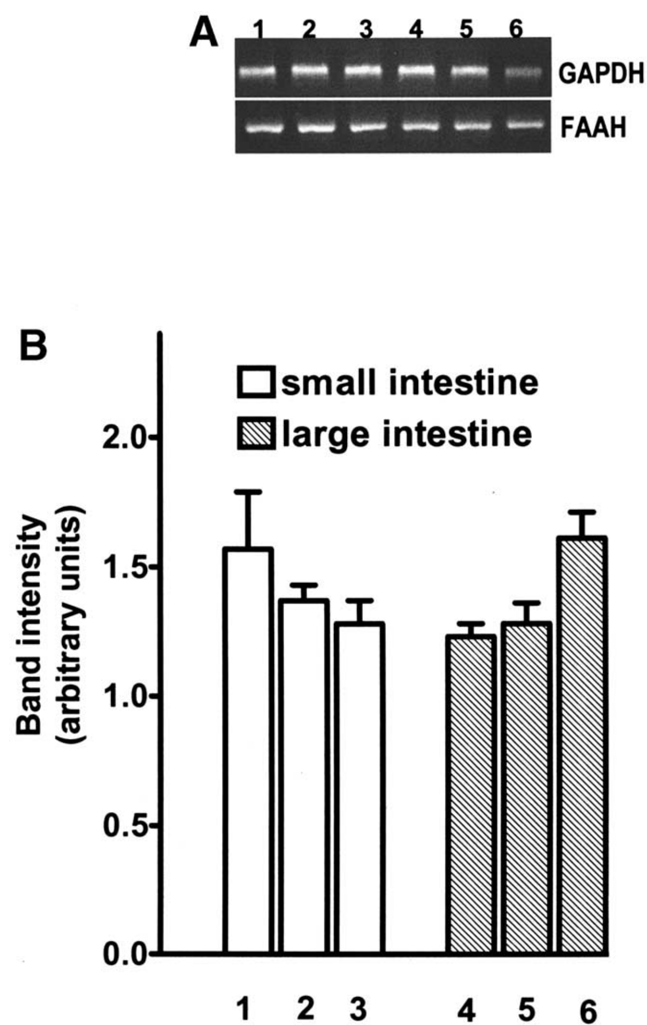

Figure 7. Distribution of the FAAH messenger RNA along the intestinal tract. (*A*) Agarose gel analysis of RT-PCR reaction from total RNA from different intestinal regions (*1*, duodenum; *2*, jejunum; *3*, ileum; *4*, cecum; *5*, proximal colon; *6*, distal colon). Bands sized as expected for FAAH (300 bp) and GAPDH (470 bp) mRNA transcripts when using primers selective for FAAH and GAPDH, respectively, are shown. (*B*) Band intensities were quantitatively evaluated (see Materials and Methods section). Each *bar* represents the mean ± SEM of 3 mice.

The inhibitory effect of oleamide, oleoylethanolamide, and PEA on small intestine motility might be due either to the interaction with specific targets[16,17] or to their inhibition of FAAH as competitive substrates and subsequent enhancement of the levels of other substrates for this enzyme ("entourage effects").[45,46] Interestingly, we observed a different inhibitory pattern when these biologic amides were evaluated in animals treated with the CB_1 receptor antagonist rimonabant. Indeed, only PEA still exerted the same inhibitory effect in the presence of rimonabant, whereas both oleoylethanolamide and oleamide became inactive, in agreement with their action being at least in part because of "direct" (as in the case of oleamide, which is a selective albeit not very potent endogenous agonist at CB_1 receptors)[47] or "indirect" (via

enhancement of endocannabinoid levels) activation of CB_1 receptors.

In view of the incomplete reversal of their actions following CB_1 blockade, we investigated further the mechanism of action of oleamide and oleoylethanolamide by looking at their effect on anandamide, PEA, and 2-AG levels in the mouse small intestine. We found that oleoylethanolamide and oleamide significantly elevate anandamide concentrations in the small intestine but do not exert the same effect on PEA, and they even slightly decrease 2-AG levels. These data suggest that oleoylethanolamide and oleamide may not elevate anandamide levels only by inhibiting FAAH because, in this case, they should have elevated also PEA and 2-AG levels as AA-5-HT did. However, it is also possible that, because AA-5-HT, unlike the 2 amides, is a FAAH inhibitor stable to wash up and degradation,[19] it may elevate the levels also of those FAAH substrates like PEA and 2-AG, which can be metabolized also by other enzymes apart from FAAH. The observation that, despite their opposing actions on the 2 endocannabinoids, oleamide and oleoylethanolamide still inhibit motility indicates that their stimulatory effect on anandamide levels prevails on their inhibitory action on 2-AG, or that, as suggested previously,[48] the 2 amides owe their pharmacologic actions to several mechanisms including, but not limited to, elevation of anandamide levels. It remains to be determined through which mechanism the 2 amides decrease 2-AG levels. Oleoylethanolamide activates peroxisomal proliferator-activated receptor-γ (PPAR-α),[17] and this nuclear receptor may regulate the expression of the lipase enzymes involved in 2-AG biosynthesis and degradation, much in the same way it does with other lipases. At any rate, the effects of oleamide and oleoylethanolamide on intestinal motility and, concomitantly, anandamide levels, both described here for the first time, still support the hypothesis that anandamide, and hence its specific degrading enzyme FAAH, are involved in the control of this important intestinal function.

FAAH genetic ablation in mice leads to increased sensitivity to exogenously administered anandamide in some assays and to a 15-fold increase in brain anandamide (but not 2-AG) levels.[22] We observed that, unlike the brain,[22] small intestines of FAAH-deficient mice possessed "only" approximately 2.8-fold higher levels of anandamide than small intestines from FAAH wild-type mice. The use of full-thickness intestinal segments, with eventually the presence of intestinal cells, which are not able to produce anandamide, might possibly explain why we did not observe a more dramatic increase in anandamide levels. In other experiments, it has been shown that FAAH-deficient mice showed significant protection against dinitrobenzene sulphonic acid-induced colonic inflammation.[49] Our experiments with FAAH-deficient mice further established that the inhibitory effect of AA-5-HT on intestinal motility is due to FAAH inhibition because the inhibitor did not reduce intestinal motility in these transgenic mice. Interestingly, in comparison with wild-type mice, FAAH-deficient mice showed only a trend toward decreased motility. However, this possible phenotypic character of FAAH-deficient mice did not achieve statistical significance, indicating that, as opposed to acute enzymatic inhibition, congenital FAAH inactivation may be largely compensated by other endogenous mechanisms. Nevertheless, experiments were performed to clarify the different effects on motility between acute pharmacologic and congenital FAAH blockade: We found that, in contrast to the acute treatment with AA-5-HT, FAAH-deficient mice showed significantly increased levels of anandamide, but not of 2-AG (nonsignificant 60.4% increase, compared with a significant 87.0% increase after AA-5-HT 10 mg/kg and 74.8% increase after AA-5-HT 15 mg/kg) or PEA (nonsignificant 18.2% increase, compared with a significant 41.8% increase after AA-5-HT 15 mg/kg), which is a relevant finding in the light of the observation that both 2-AG and PEA may inhibit intestinal motility.[3] Therefore, the lack of significant effect on 2-AG and PEA levels might possibly explain why genetic ablation of FAAH is not as efficacious as acute pharmacologic blockade of the enzyme in reducing small intestine motility and suggests that lifelong effects of FAAH deficiency might be partially compensated, leading to normal motility in basal conditions. The most likely mechanisms compensating for the lack of FAAH-catalyzed hydrolysis of 2-AG and PEA would involve the above-mentioned MAGL[35] and *N*-acylethanolamine acid amido hydrolase,[38] respectively. These 2 enzymes are abundant in the gut and have been found to catalyze selectively the hydrolysis of 2-AG and PEA, respectively. On the other hand, it is very unlikely that changes in CB_1 receptor expression could explain such different effects because we did not observe any significant variation in small intestine CB_1 mRNA receptor expression between wild-type and FAAH-deficient mice (data not shown). Similarly, we found that CB_1-deficient mice showed only a trend toward increased motility, which is not in agreement with the ability of acutely administered rimonabant to increase motility. It is very likely that compensatory mechanisms, such as the above-mentioned coexpression of TRPV1 receptors[44] in myenteric neurons, are involved also in this case. However, because this was not the primary aim of our study, this difference between

acute pharmacologic vs genetic CB_1 blockade should be investigated more in-depth in future studies.

In conclusion, the present study provides strong evidence for a role of FAAH in the physiologic regulation of intestinal motility. Degradation of endocannabinoids acting through CB_1 receptors appears to play a major role in the FAAH-dependent regulation of intestinal motility. However, other molecules, such as palmitoylethanolamide and possibly oleamide and oleoylethanolamide, whose biologic effects are likely to be partly or completely independent of CB_1 receptors and whose presence in the gastrointestinal tract[50] as well as in some foods[51] has been clearly demonstrated, are regulated by FAAH and could participate in the physiologic inhibition of intestinal motility. Pharmacologic targeting of FAAH, which is expected to be devoid of the unwanted psychotropic effects typical of "direct" CB_1 activation, might constitute a new mechanistic approach for treating disorders of intestinal hyperactivity.

References

1. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 2002;54:161–202.
2. Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Disc 2004;3:771–784.
3. Coutts AA, Izzo AA. The gastrointestinal pharmacology of cannabinoids. An update. Curr Opin Pharmacol 2004;4:572–579.
4. Hornby PJ, Prouty SM. Involvement of cannabinoid receptors in gut motility and visceral perception. Br J Pharmacol 2004;141:1335–1345.
5. Pertwee RG, Fernando SR, Nash JE, Coutts AA. Further evidence for the presence of cannabinoid CB_1 receptors in guinea-pig small intestine. Br J Pharmacol 1996;118:2199–2205.
6. Izzo AA, Mascolo N, Borrelli F, Capasso F. Excitatory transmission to the circular muscle of the guinea-pig ileum: evidence for the involvement of cannabinoid CB_1 receptor. Br J Pharmacol 1998;124:1363–1368.
7. Begg M, Dale N, Llaudet E, Molleman A, Parsons ME. Modulation of the release of endogenous adenosine by cannabinoids in the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum. Br J Pharmacol 2002;137:1298–1304.
8. Mancinelli R, Fabrizi A, Del Monaco S, Azzena GB, Vargiu R, Colombo GC, Gessa GL. Inhibition of peristaltic activity by cannabinoids in the isolated distal colon of mouse. Life Sci 2001;69:101–111.
9. Lehmann A, Blackshaw LA, Branden L, Carlsson A, Jensen J, Nygren E, Smid SD. Cannabinoid receptor agonism inhibits transient lower esophageal sphincter relaxations and reflux in dogs. Gastroenterology 2002;123:1129–1134.
10. Izzo AA, Mascolo N, Borrelli F, Capasso F. Defecation, intestinal fluid accumulation and motility in rodents: implications of cannabinoid CB_1 receptors. Naunyn Scmideberg's Arch Pharmacol 1999;359:65–70.
11. Casu MA, Porcella A, Ruiu S, Saba P, Marchese G, Carai MAM, Reali R, Gessa GL, Pani L. Differential distribution of functional cannabinoid CB1 receptors in the mouse gastroenteric tract. Eur J Pharmacol 2003;459:97–105.
12. Pinto L, Izzo AA, Cascio MG, Bisogno T, Hospodar-Scott K, Brown DR, Mascolo N, Di Marzo V, Capasso F. Endocannabinoids as physiological regulators of colonic propulsion in mice. Gastroenterology 2002;123:227–234.
13. MacLennan SJ, Reynen PH, Kwan J, Bonhaus DW. Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB1 and CB2 receptors. Br J Pharmacol 1998;124:619–622.
14. Deutsch DG, Ueda N, Yamamoto S. The fatty acid amide hydrolase (FAAH). Prostaglandins Leukot Essent Fatty Acids 2002;66:201–210.
15. Cravatt BF, Prospero-Garcia O, Siuzdak G, Gilula NB, Henriksen SJ, Boger DL, Lerner RA. Chemical characterization of a family of brain lipids that induce sleep. Science 1995;268:1506–1509.
16. Lambert DM, Di Marzo V. The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimimetic? Curr Med Chem 1999;6:757–773.
17. Fu J, Gaetani S, Oveisi F, Lo Verme J, Serrano A, Rodriguez De Fonseca F, Rosengarth A, Luecke H, Di Giacomo B, Tarzia G, Piomelli D. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. Nature 2003;425:90–93.
18. Izzo AA, Fezza F, Capasso R, Bisogno T, Pinto L, Iuvone T, Esposito G, Mascolo N, Di Marzo V, Capasso F. Cannabinoid CB_1-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation. Br J Pharmacol 2001;134:563–570.
19. Bisogno T, Melck D, De Petrocellis L, Bobrov MYU, Gretskaya NM, Bezuglov VV, Sitachitta N, Gerwick WH, Di Marzo V. Arachidonoylserotonin and other novel inhibitors of fatty acid amide hydrolase. Biochem Biophys Res Commun 1998;248:515–522.
20. Jonsson KO, Vandevoorde S, Lambert DM, Tiger G, Fowler CJ. Effects of homologues and analogues of palmitoylethanolamide upon the inactivation of the endocannabinoid anandamide. Br J Pharmacol 2001;133:1263–1275.
21. Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgansberger W, Di Marzo V, Lutz B. The endogenous cannabinoid system controls extinction of aversive memories. Nature 2002;418:530–534.
22. Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci U S A 2001;98:9371–9376.
23. Kalff JC, Buchholz BM, Eskandari MK, Hierholzer C, Schraut WH, Simmons RL, Bauer AJ. Biphasic response to gut manipulation and temporal correlation of cellular infiltrates and muscle dysfunction in rat. Surgery 1999;126:498–509.
24. Schwarz NT, Kalff JC, Turler A, Engel BM, Watkins SC, Billiar TR, Bauer AJ. Prostanoid production via COX-2 as a causative mechanism of rodent postoperative ileus. Gastroenterology 2001;121:1354–1371.
25. Shook JE, Burks TF. Psychoactive cannabinoids reduce gastrointestinal propulsion and motility in rodents. J Pharmacol Exp Ther 1989; 249:444–449.
26. Rigoni M, Trevisani M, Gazzieri D, Nadaletto R, Tognetto M, Creminon C, Davis JB, Campi B, Amadesi S, Geppetti P, Harrison S. Neurogenic responses mediated by vanilloid receptor-1 (TRPV1) are blocked by the high affinity antagonist, iodo-resiniferatoxin Br J Pharmacol 2003;138:977–985.
27. Izzo AA, Capasso F, Costagliola A, Bisogno T, Marsicano G, Ligresti A, Matias I, Capasso R, Pinto L, Borrelli F, Cecio A, Lutz B, Mascolo N, Di Marzo V. An endogenous cannabinoid tone attenuates cholera toxin-induced fluid accumulation in mice. Gastroenterology 2003;125:765–774.

28. Buckley MJ, Surowy C, Meyer M, Curzon P. Mechanism of action of A-85380 in an animal model of depression. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:723–730.
29. Bisogno T, Berrendero F, Ambrosino G, Cebeira M, Ramos JA, Fernandez-Ruiz JJ, Di Marzo V. Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. Biochem Biophys Res Commun 1999;256:377–380.
30. Mascolo N, Izzo AA, Ligresti A, Costagliola A, Pinto L, Cascio MG, Maffia P, Cecio A, Capasso F, Di Marzo V. The endocannabinoid system and the molecular basis of paralytic ileus in mice. FASEB J 2002;16:1973–1975.
31. Katayama K, Ueda N, Kurahashi Y, Suzuki H, Yamamoto S, Kato I. Distribution of anandamide amidohydrolase in rat tissues with a special reference to small intestine. Biochim Biophys Acta 1997;1347:212–218.
32. Calignano A, La Rana G, Makriyannis A, Lin SY, Beltramo M, Piomelli D. Inhibition of intestinal motility by anandamide, an endogenous cannabinoid. Eur J Pharmacol 1997;340:R7–R8.
33. McVey DC, Schmid PC, Schmid HH, Vigna SR. Endocannabinoids induce ileitis in rats via the capsaicin receptor (VR1). J Pharmacol Exp Ther 2003;304:713–722.
34. Pertwee RG, Fernando SR, Griffin G, Abadji V, Makriyannis A. Effect of phenylmethylsulphonyl fluoride on the potency of anandamide as an inhibitor of electrically evoked contractions in two isolated tissue preparations. Eur J Pharmacol 1995;272:73–78.
35. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci U S A 2002;99:10819–10824.
36. Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D. Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med 2003;9:76–81.
37. Cascio MG, Bisogno T, Matias I, De Petrocellis L, Orlando P, Di Marzo V. Enzymes for 2-AG biosynthesis and metabolism in cell lines, and their pharmacological inhibition. 2004 Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society, 2004:110.
38. Tsuboi K, Sun YX, Okamoto Y, Araki N, Tonai T, Ueda N. Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. J Biol Chem 2005;280:11082–11092.
39. Bifulco M, Laezza C, Valenti M, Ligresti A, Portella G, DI Marzo V. A new strategy to block tumor growth by inhibiting endocannabinoid inactivation. FASEB J 2004;18:1606–1608.
40. Capasso R, Izzo AA, Fezza F, Pinto A, Capasso F, Mascolo N, Di Marzo V. Inhibitory effect of palmitoylethanolamide on gastrointestinal motility in mice. Br J Pharmacol 2001;134:945–950.
41. Izzo AA, Capasso R, Pinto L, Di Carlo G, Mascolo N, Capasso F. Effect of vanilloid drugs on gastrointestinal transit in mice. Br J Pharmacol 2001;132:1411–1416.
42. Mang CF, Erbelding D, Kilbinger H. Differential effects of anandamide on acetylcholine release in the guinea-pig ileum mediated via vanilloid and non-CB_1 cannabinoid receptor. Br J Pharmacol 2001;134:161–167.
43. Kojima S, Sugiura T, Waku K, Kamikawa Y. Contractile response to a cannabimimetic eicosanoid, 2-arachidonoylglycerol, of longitudinal smooth muscle from the guinea-pig distal colon in vitro. Eur J Pharmacol 2002;444:203–207.
44. Anavi-Goffer S, McKay NG, Ashford ML, Coutts AA. Vanilloid receptor type 1-immunoreactivity is expressed by intrinsic afferent neurones in the guinea-pig myenteric plexus. Neurosci Lett 2002;319:53–57.
45. Maurelli S, Bisogno T, De Petrocellis L, Di Luccia A, Marino G, Di Marzo V. Two novel classes of neuroactive fatty acid amides are substrates for mouse neuroblastoma "anandamide amidohydrolase." FEBS Lett 1995;377:82–86.
46. Mechoulam R, Fride E, Hanus L, Sheskin T, Bisogno T, Di Marzo V, Bayewitch M, Vogel Z. Anandamide may mediate sleep induction. Nature 1997;389:25–26.
47. Leggett JD, Aspley S, Beckett SR, D'Antona AM, Kendall DA, Kendall DA. Oleamide is a selective endogenous agonist of rat and human CB1 cannabinoid receptors. Br J Pharmacol 2004;141:253–262.
48. Lichtman AH, Hawkins EG, Griffin G, Cravatt BF. Pharmacological activity of fatty acid amides is regulated, but not mediated, by fatty acid amide hydrolase in vivo. J Pharmacol Exp Ther 2002;302:73–79.
49. Massa F, Marsicano G, Hermann H, Cannich A, Krisztina M, Cravatt BF, Ferri G-L, Sibaev A, Storr M, Lutz B. The endogenous cannabinoid system protects against colonic inflammation. J Clin Invest 2004;113:1202–1209.
50. Gomez R, Navarro M, Ferrer B, Trigo JM, Bilbao A, Del Arco I, Cippitelli A, Nava F, Piomelli D, Rodriguez de Fonseca F. A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. J Neurosci 2002;22:9612–9617.
51. Crozier Willi G, Berger A, Di Marzo V, Bisogno T, De Petrocellis L, Fride E, Mechoulam R. Lipids in neural function: modulation of behavior by oral administration of endocannabinoids found in foods. Nestle Nutr Workshop Ser Clin Perform Programme 2001;5:169–184.

Received November 26, 2004. Accepted May 26, 2005.

Address requests for reprints to: Angelo A. Izzo, PhD, University of Naples Federico II, Department of Experimental Pharmacology, Via D. Montesano 49, Naples 80131, Italy. e-mail: aaizzo@unina.it; fax: (39) 081 678403; or Vincenzo Di Marzo, PhD, National Research Council, Pozzuoli (NA), Italy. e-mail: vdimarzo@icmib.na.cnr.it; fax: (39) 081 8041770.

Supported by *Cofinanziamento Murst*, Enrico and Enrica Sovena Foundation (Roma), SESIRCA (Regione Campania), MURST, Fondi Strutturali (to V.D.M.), and Marie Curie fellowship Program (to K.M.).

R.C. and I.M. contributed equally to this work.

The authors thank Dr. Ben Cravatt for providing FAAH-deficient mice.