



Platinum Priority – From Lab to Clinic

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Expression of Fatty Acid Amide Hydrolase (FAAH) in Human, Mouse, and Rat Urinary Bladder and Effects of FAAH Inhibition on Bladder Function in Awake Rats

Frank Strittmatter^{a,b,†}, Giorgio Gandaglia^{c,†}, Fabio Benigni^{c,*}, Arianna Bettiga^c, Patrizio Rigatti^c, Francesco Montorsi^c, Christian Gratzke^a, Christian Stief^a, Giorgia Colciago^c, Petter Hedlund^{c,d}

^aDepartment of Urology, Munich University, Munich, Germany; ^bDepartment of Clinical and Experimental Pharmacology, Lund, Sweden; ^cUrological Research Institute, San Raffaele University, Milan, Italy; ^dDepartment of Clinical Pharmacology, Linköping, Sweden

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Abstract

Background: Cannabinoid receptor (CB)–mediated functions may be involved in the regulation of bladder function, but information on endocannabinoid signals during micturition is scarce.

Objective: Investigate the expression of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) in human, rat, and mouse bladders and study the effects of inhibition of FAAH during urodynamics in awake rats.

Design, setting, and participants: Bladder tissue from humans, mice, and rats was used for measurements. Female Sprague-Dawley rats were administered the FAAH inhibitor oleoyl ethyl amide (OEtA) or vehicle intravenously (IV) or intravesically (IVES) with or without rimonabant (CB1 antagonist) or SR144528 (CB2 antagonist).

Measurements: Real-time transcriptase-polymerase chain reaction, Western blot, immunohistochemistry, and cystometry in awake rats.

Results and limitations: Messenger RNA and protein for FAAH was expressed in the mucosa of human, mouse, and rat urinary bladders. Immunoreactivities for FAAH and CB2 were codistributed in rat and human urothelium. IV OEtA (0.3 mg/kg) to rats increased intercontraction intervals (ICIs), micturition volume (MV), bladder capacity (BC), and threshold pressure (TP) by $17 \pm 1\%$, $16 \pm 1\%$, $17 \pm 1\%$, and $19 \pm 5\%$, respectively (all $p < 0.05$ vs baseline). IVES OEtA (1 and 10 mg/l) in rats dose-dependently increased ($p < 0.05$ vs baseline) ICI ($19 \pm 2\%$ and $35 \pm 5\%$), MV ($15 \pm 3\%$ and $32 \pm 4\%$), BC ($16 \pm 2\%$ and $34 \pm 4\%$), and TP ($15 \pm 1\%$, $21 \pm 3\%$). SR144528 (IVES 5 mg/l) abolished all effects of OEtA, whereas rimonabant only counteracted effects of OEtA on TP.

Conclusions: Bladder mucosa of all species expressed FAAH. Rat and human urothelium coexpressed FAAH and CB2. The FAAH inhibitor OEtA altered urodynamic parameters that reflect sensory functions of micturition in rats. Suggesting a role for the endocannabinoid system in bladder mechanoafferent functions of rats, effects of IVES OEtA were abolished by an IVES CB2 antagonist and partly counteracted by an IVES CB1 antagonist.

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[†] These authors contributed equally to the manuscript.

* Corresponding author. Urological Research Institute, San Raffaele University, 20132 Milan, Italy. Tel. +39 3664247852; Fax: +39 0226435659.

E-mail address: benigni.fabio@hsr.it (F. Benigni).

1. Introduction

The effect of cannabis on urge incontinence in multiple sclerosis patients has brought attention to the endocannabinoid system as a target for lower urinary tract symptoms (LUTS) [1]. The endocannabinoid system consists of the cannabinoid receptors, their endogenous ligands, and enzymes that regulate the biosynthesis and degradation of the endocannabinoids [2,3]. Messenger RNA (mRNA) and protein for cannabinoid receptor (CB) are expressed in the urinary bladder of several species, including humans, and a role for CB-mediated functions during experimental urodynamics and in sensory mechanisms and inflammation of the urinary bladder is proposed [4–7]. However, central nervous system (CNS) side effects of exogenous cannabinoids that are associated with activation of CB1 have raised concerns about the suitability of such agents in the treatment of pain or LUTS [8,9]. Potential endocannabinoid targets where analgesic effects may be separated from psychotropic effects are peripheral CB2 receptors [8]. The CB2 subtype is expressed mainly outside the CNS, and a role for peripheral CB2 functions in normal micturition and experimental detrusor overactivity is presented [8–10].

Pharmacologic targeting of the homeostasis of endogenous cannabinoids may also offer opportunities to avoid undesirable CNS effects of exogenous cannabinoids [3]. Fatty acid amide hydrolase (FAAH) is the key enzyme for the degradation of the endogenous cannabinoid anandamide (AEA) but also other fatty acid amides [11]. Mice that lack FAAH have increased levels of endocannabinoids, including AEA in the CNS, and they display analgesia, anti-inflammation, and normal motility and cognition [12,13]. In a transgenic mice model, central and peripheral fatty acid amide transmitter systems can be functionally uncoupled to separately modify the concentrations of these signaling lipids in peripheral tissues but not in CNS structures [14].

FAAH is indicated to be present in the rat bladder, but it is not known if FAAH is expressed in the human bladder or if pharmacologic modification of FAAH has effects on micturition [15].

The aim of the current study was to compare FAAH expression at genetic and protein levels in human, rat, and mouse bladders and to study effects on micturition of the inhibition of FAAH during awake urodynamics in rats.

2. Material and methods

2.1. Ethical approval

All procedures were approved by the ethics committees of San Raffaele University, Milan, Italy, and Lund University, Lund, Sweden.

2.2. Human tissues

As assessed by a uropathologist, normal urinary bladder tissue was harvested peroperatively from six patients (mean age: 67 yr; four men and two women) undergoing cystectomy due to bladder cancer (without chemotherapy).

2.3. Animals

Normal female Sprague-Dawley rats ($n = 49$; 225–300 mg) and normal Balb C mice ($n = 6$; 30–35 mg) were used. Rodents were killed by carbon dioxide asphyxia.

2.4. Real-time polymerase chain reaction

Detrusor and bladder mucosa was processed for real-time polymerase chain reaction (RT-PCR) [16]. Briefly, cDNA product was amplified by 35 PCR cycles (30 s, 95 °C, annealing 1 min 58 °C, and extension 30 s, 72 °C) and final elongation at 72 °C for 5 min for FAAH and actin. Each PCR product was amplified by 35 PCR cycles and validated by size determination after separation on 2% agarose gel.

2.5. Western blot

Detrusor and bladder mucosa was processed for Western blot [17]. Primary rabbit antibodies for FAAH (1:200; Cayman Chemicals, Ann Arbor, MI, USA) or glyceraldehyde phosphate dehydrogenase (1:1000, Santa Cruz Biotech, Santa Cruz, CA, USA) and species-directed secondary horseradish peroxidase antibodies (1:1000, Santa Cruz) were used. Control experiments without FAAH primary antibodies or in the presence of FAAH blocking peptide (Cayman) were performed.

2.6. Immunohistochemistry

Bladder specimens were processed for immunohistochemistry [16]. A rabbit FAAH (1:200; Cayman) primary antibody and species-directed secondary Alexa Green/Red antibodies (1:600, Santa Cruz) were used. Control experiments without FAAH primary antibodies or in the presence of FAAH blocking peptide (Cayman) were performed. In separate experiments, double stainings using a mouse FAAH antibody (1:100; Sigma Aldrich, Stockholm, Sweden) and a rabbit CB2 antibody (1:200, Cayman) were performed. Hematoxylin and eosin stainings of the bladder specimens were used for histologic identification of structures of the bladder wall.

2.7. Cystometry

Under anesthesia, a polyethylene catheter (PE-50, Clay-Adams, USA) was implanted in the bladder dome. For intravenous (IV) administration of drugs, a PE-50 catheter was implanted into the femoral vein [9,16]. Conscious rats underwent cystometry 3 d after catheter implantation. Pressures, micturition volumes (MVs), and compliance were analyzed [18].

2.8. Drugs and solutions

A saline solution of OEtA (1 mg/ml; Cayman) or vehicle (saline) was given IV in weight-controlled doses of 0.3 mg/kg. For intravesical (IVES) administrations, saline solutions of OEtA (1 or 10 mg/l), SR144528 (5 mg/l; Cayman), or

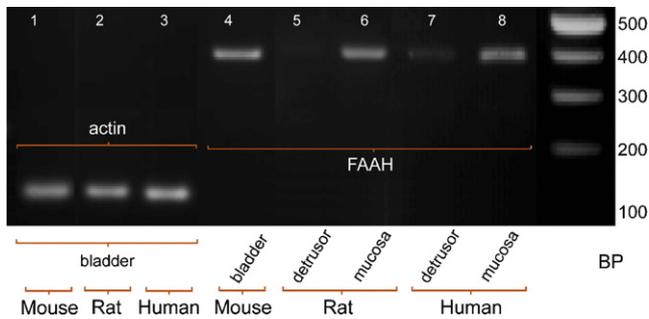


Fig. 1 – Real-time polymerase chain reaction. Similar results were obtained in tissue specimens ($n = 4$ for each species). Representative bands for mRNA encoding for fatty acid amide hydrolase (FAAH) in bladders from mice (lanes 1 and 4), rat bladder (lane 2), rat detrusor (lane 5), rat bladder mucosa (lane 6), human bladder (lane 3), human detrusor (lane 7), and human bladder mucosa (lane 8). Forward FAAH primers used were CTTGCCCTCAGAGAGGA (mouse), CTTGCCCTCAGAGAGGAG (rat), and GCACACGCTGGTCCCTTCTTG (human). Reverse FAAH primers used were CTTTTCAGCTGACCGAGGAC (mouse), TCAGTGACGGTGCCCGAGTT (rat), and GTTTTCAGCCGAACGAGACTTCATGTG (human). Primers for actin were used as housekeeping genes. Right margin indicates size of base pairs (BP).

rimonabant (5 mg/l; Cayman) were used. The doses used for SR144528 and rimonabant were selected based on previous uses in rats [19]. The number of experiments was based on power calculations in SigmaPlot (SYSTAT software, USA). Rats were randomly assigned to treatments.

2.9. Calculations

Values are given as mean plus or minus standard error of the mean. For multiple comparisons, Student-Newman-Keuls analysis of variance was used. Paired comparisons were made with the Student t test. All calculations are based on the number of individual animals.

3. Results

3.1. Real-time polymerase chain reaction

Agarose gel electrophoresis of the amplified FAAH cDNA products from full-wall bladders (mice) and mucosa (rat and humans) verified the presence of mRNA encoding FAAH as single bands of the expected base-pair size, whereas

weak FAAH signals were observed in detrusor from rats and humans (Fig. 1).

3.2. Western blot

Full-wall bladder (mouse) and mucosa (rat and human) exhibited bands at the expected weights for FAAH (Fig. 2). Control experiments did not yield any bands (data not shown).

3.3. Immunohistochemistry

The urothelium from mice, rats, and humans exhibited intense immunoreactivity for FAAH (Fig. 3). No FAAH immunoreactivity was detected in other structures of the bladder. Control stainings did not exhibit immunoreactive signals (data not shown). In double-stained sections of rat bladder, FAAH immunoreactivity was located in the upper urothelial cell layers, whereas immunoreactivity for CB2 was distributed in immediate apposition in deeper urothelial cells (Fig. 4). In sections of the human bladder, FAAH immunoreactivity was also expressed in the upper urothelial layers (Fig. 4). In these cells, FAAH and CB2 immunoreactivities were co-localized, whereas deeper urothelial cells only expressed immunoreactivity for CB2 (Fig. 4).

3.4. Cystometry

After IV administration of OEtA (Table 1), intercontraction intervals (ICIs), MV, bladder capacity (BC), and threshold pressure (TP) were increased by $17 \pm 1\%$, $16 \pm 1\%$, $17 \pm 1\%$, and $19 \pm 5\%$ (all $p < 0.05$ vs vehicle) in awake rats. OEtA (IV) did not change residual volume (RV), basal pressure (BP), maximum pressure (MP), or compliance (C). Vehicle (IV) did not have any effect on urodynamic parameters.

After IVES administration of OEtA (Figs. 5 and 6; Table 2), increases of $19 \pm 2\%$ and $35 \pm 5\%$ for ICI, $15 \pm 3\%$, $32 \pm 4\%$, for MV, $16 \pm 2\%$, $34 \pm 4\%$ for BC, and $15 \pm 1\%$, $21 \pm 3\%$, for TP were recorded for OEtA at 1 and 10 mg/l, respectively (all $p < 0.05$ vs vehicle). IVES OEtA did not change RV, BP, MP, or C.

SR144528 (IVES 5 mg/l; Fig. 6; Table 3a) abolished all effects of IVES OEtA (1 mg/l), whereas rimonabant (Fig. 6; Table 3b) only counteracted effects of OEtA on TP. Vehicles (IVES) did not have any effects on urodynamic parameters.

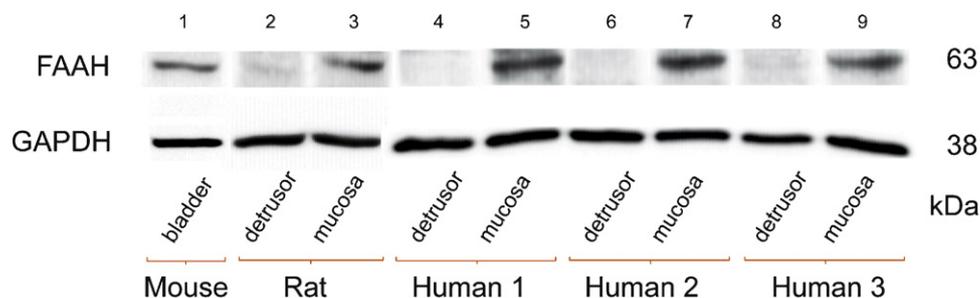


Fig. 2 – Western blot. Similar results were obtained in tissue specimens ($n = 4$ for each species). Representative bands for fatty acid amide hydrolase (FAAH) protein at the expected size of 63 kDa in bladders from mice (lane 1), rat detrusor (lane 2), rat bladder mucosa (lane 3), detrusor from three different humans (lanes 4, 6, and 8), and bladder mucosa from three different humans (lanes 5, 7, and 9). Glyceraldehyde phosphate dehydrogenase (GAPDH) (32 kDa) was used as a control.

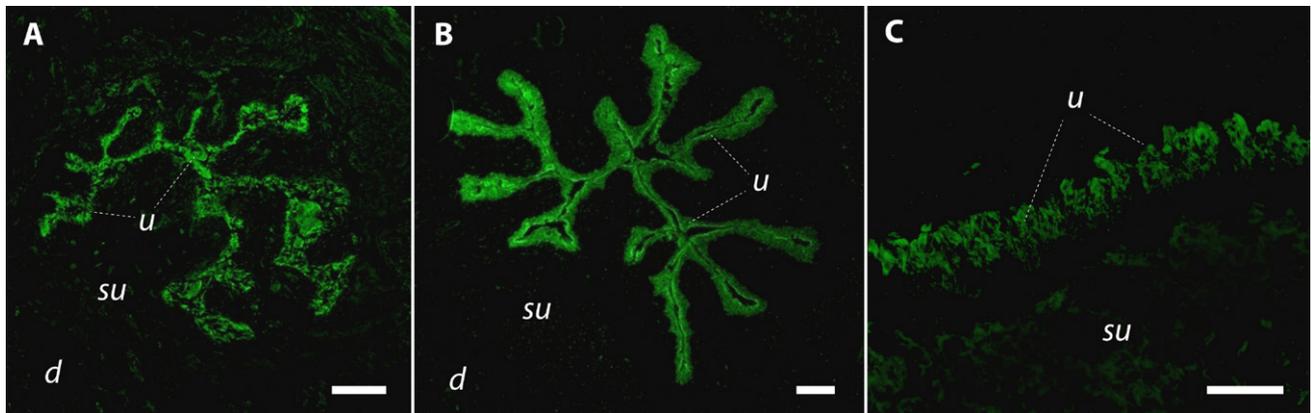


Fig. 3 – Immunohistochemistry. Similar results were obtained in tissue specimens ($n = 4-5$ for each species). The distribution of fatty acid amide hydrolase (FAAH) immunoreactivity in the urothelium of urinary bladders from (A) mouse (Alexa Green), (B) rat (Alexa Green), and (C) human (Alexa Green). *u* = urothelium; *su* = suburothelium; *d* = detrusor. Scale bars = 100 μm .

4. Discussion

In the current study we demonstrate for the first time that FAAH is expressed at genetic and protein levels in the human bladder mucosa and that this expression is similar to that of mouse and rat urinary bladders. We also show that FAAH and CB2 immunoreactivities are codistributed in the urothelium of rat and human bladders. In addition, we report that systemic or IVES administration of a FAAH inhibitor in rats during awake cystometry alters urodynamic parameters that

reflect sensory functions of the micturition reflex and that effects of IVES FAAH inhibition appear mainly to be mediated by CB2.

Several endocannabinoid amide and fatty acid derivatives are ligands for one or both CB subtypes [2,8]. FAAH, the principal enzyme that hydrolyzes AEA, is encoded for by highly conserved genes in humans, mice, and rats [11]. Suggesting the presence and transcription of these genes in the urinary bladder, we located mRNA for FAAH mainly in the bladder mucosa of human and rat specimens, and in full-wall

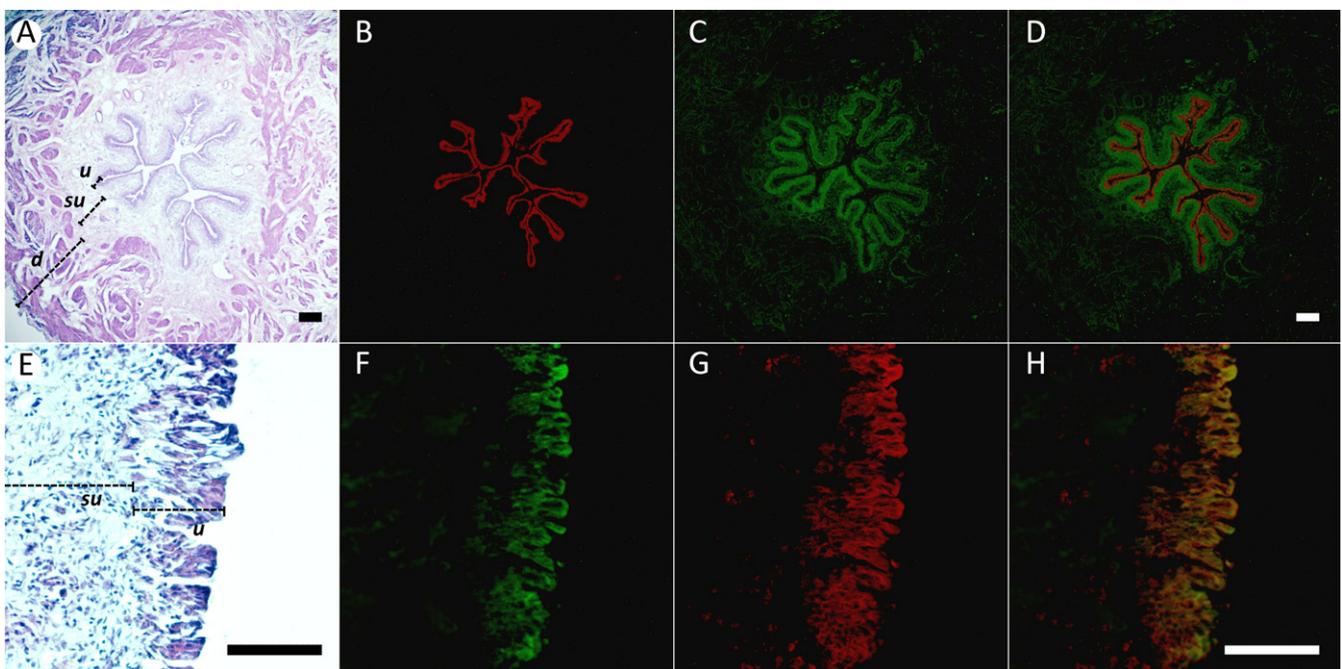


Fig. 4 – Histology and immunohistochemistry. Similar results were obtained in rat ($n = 3$) or human tissue specimens ($n = 4$). (A) Hematoxylin and eosin (HtxE) staining of the rat bladder. (B) Fatty acid amide hydrolase (FAAH) immunoreactivity (Alexa Red) in upper layers of the rat urothelium (the next adjacent section to [A]). (C) Cannabinoid receptor (CB) 2 immunoreactivity (Alexa Green) in deeper layers of the rat urothelium (same section as in [B]). (D) Merged section showing FAAH immunoreactivity (Red) and CB2 immunoreactivity (Green) in the rat urothelium. (E) HtxE staining of the human bladder. (F) FAAH immunoreactivity (Alexa Green) in upper layers of the human urothelium (the next adjacent section to [E]). (G) CB2 immunoreactivity (Alexa Red) in the human urothelium (same section as in [F]). (H) Merged section showing co-localization of FAAH and CB2 immunoreactivities in the human urothelium (yellow) and also CB2 immunoreactivity (red) in deeper urothelial cells. Dotted bars in HtxE-stained sections indicate *u* = urothelium; *su* = suburothelium; *d* = detrusor. Scale bars = 100 μm . A total of three to four experiments for each species were performed with similar results.

specimens from mice. For all species, we also showed that mRNA for FAAH was likely translated in protein biosynthesis because Western blot and/or immunohistochemistry revealed FAAH protein signals in the mucosa. Hence FAAH-related signals may have a function in the urothelium and/or suburothelium. In double-stained sections, we demonstrated that FAAH mainly was found in the outer urothelial layers, and as previously reported [9], we confirmed the presence of CB2 in the urothelium. Whereas FAAH was co-localized with CB2 in human urothelial cells, FAAH and CB2 immunoreactivities were closely located in apposition of the rat urothelium. This codistribution may suggest that CB2 on urothelial cells or afferent nerve terminals in the urothelium could be targets for FAAH substrates. It has been shown that activation of urothelial CB receptors attenuates calcitonin gene-related peptide release in the mouse bladder [20].

TP and ICIs reflect sensory functions of the micturition reflex, and the latter parameter also subsequently controls volumes and BC [9]. After systemic administration of OEtA, all these parameters were increased compared with vehicle. This is in line with investigations showing that FAAH inhibition reduces sensation to non-noxious stimuli [3]. Inhibition of FAAH also attenuates sensation to thermal provocation and reduces neurogenic or inflammatory pain, and mice with depletion of FAAH exhibit reduced nociception and hypoalgesic behaviors in a model of visceral pain [3,12]. Small sensory neurons of the rat dorsal root ganglion (DRG) express FAAH, and functional data from cultured sensory DRG neurons may indicate a role for FAAH and AEA in modulating peripheral nociceptive transmission [21,22]. In addition, intraplantar injection of a FAAH inhibitor increased locally the levels of fatty acid amides including AEA and attenuated mechanically evoked responses of spinal neurons [23].

Increased levels of AEA in the brain or spinal cord are reported for mice without FAAH or after systemic administration of FAAH inhibitors, but with no signs of disruptions in motility, body temperature, and cognition [3,24]. In contrast, hypoactivity, hypothermia, and psychotropic activity, which are associated with activation of CB1 in the CNS, restrict dosing and limit the clinical usefulness of exogenous nonselective cannabinoids [3,8,24].

Transgenic mice without peripheral FAAH have increased levels of AEA in peripheral tissues but not in the CNS, and they still exhibit a preserved anti-inflammatory phenotype [14]. These findings suggest that central and peripheral FAAH may separately regulate endocannabinoid turnover and may be targeted for separate therapeutic advantages [14]. In this context, altered peripheral expression of FAAH is reported in a rat model for chronic inflammation [25]. It is not known if FAAH activity is altered in functional visceral disorders of humans, but a genotype variation of the FAAH gene was found to be associated with symptom phenotypes of the irritable bowel syndrome [26]. In humans, monkeys, and rabbits, but not in rats or mice, a second FAAH isoenzyme (denoted FAAH-2) that was sensitive to the main classes of FAAH inhibitors was identified in peripheral tissues but not in brain [27].

Table 1 – Urodynamic parameters at vehicle baseline and after intravenous oleoyl ethyl amide 0.3 mg/kg in female rats

Urodynamic parameters intravenous OEtA	Intervals, min, and volumes, ml			Pressures, cm H ₂ O			Compliance, ml/cm H ₂ O
	ICI	MV	RV	BC	BP	TP	
Vehicle baseline (n = 6)	4.0 ± 0.3	0.66 ± 0.05	0.003 ± 0.0001	0.67 ± 0.05	11.1 ± 0.4	25.2 ± 0.5	0.121 ± 0.002
OEtA 0.3 mg/kg (n = 6)	4.7 ± 0.4*	0.77 ± 0.06*	0.006 ± 0.0002	0.78 ± 0.06*	11.3 ± 0.7	30.0 ± 1.3*	0.132 ± 0.009

OEtA = oleoyl ethyl amide; ICI = intercontraction interval; MV = micturition volume; RV = residual volume; BC = bladder capacity; BP = basal pressure; TP = threshold pressure; MP = maximum pressure. Compliance for micturition is calculated as MV/TP – BP. Values are given as mean plus or minus standard error of the mean. For comparisons (paired t test) versus vehicle: * = $p < 0.05$.

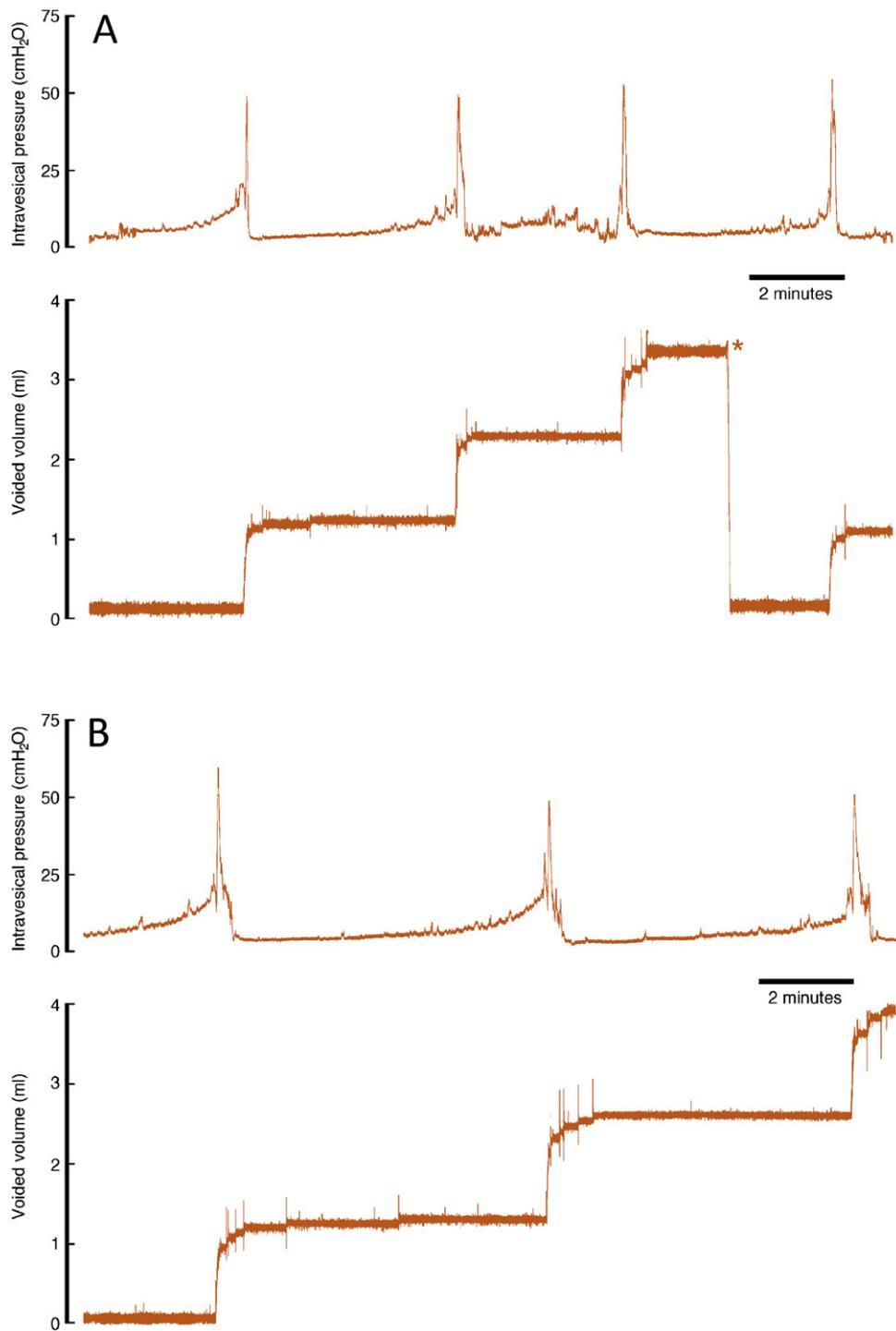


Fig. 5 – Rat cystometry. Original tracings of intravesical pressure (cm H₂O) and voided volume (ml) from one awake rat during continuous cystometry during intravesical infusion of (A) vehicle and during intravesical infusion of (B) oleoyl ethyl amide (1 mg/l). * Indicates emptying of cup for voided volume.

Development of more advanced pharmacologic tools may allow for selective inhibition of peripheral FAAH.

Because our molecular biologic data suggest that FAAH is located in the bladder mucosa of all species studied and to avoid possible effects of the drug in the CNS after IV administration, we infused OEtA into the bladder of awake rats. In these experiments we recorded similar effects of the drug as during systemic administration, and we also

demonstrated dose-dependent effects of the drug on ICIs, MVs, and TPs. These findings that propose a role for FAAH and FAAH-related signals in sensory functions locally in the urinary bladder during the filling phase of micturition (ie, when an increased volume activates mechanoafferent bladder functions) are consistent with the reported effects of cannabior, a peripherally active CB₂ agonist [9]. Interestingly, in other models, CB₂ activation appears to

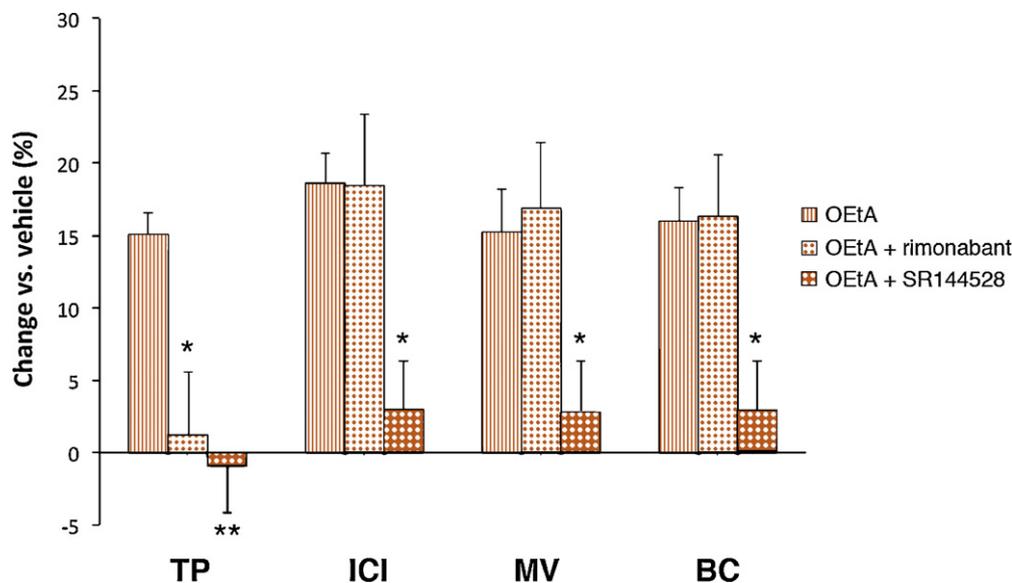


Fig. 6 – Rat cystometry. Comparison of percentage change of threshold pressure (TP), intercontraction interval (ICI), micturition volume (MV), and bladder capacity (BC) versus vehicle by intravesical administration of oleoyl ethyl amide (OEtA; 1 mg/l; $n = 6$), intravesical administration of OEtA (1 mg/l) plus rimonabant (5 mg/l; $n = 6$), and intravesical administration of OEtA (1 mg/l) plus SR144528 (5 mg/l; $n = 7$). Values are given as mean plus or minus standard error of the mean. For comparisons between groups (analysis of variance): * $p < 0.05$, ** $p < 0.01$.

exhibit functional selectivity and decrease the nociceptive signals of neurons that are sensitized or activated [8].

In the current investigation we could also show that IVES coadministration of OEtA and SR144528 abolished all effects of OEtA on ICIs, MVs, and TPs, suggesting that FAAH

substrates in the urinary bladder act via local CB2-mediated signals.

Similarly, palmitoylethanolamide, a non-CB1 non-CB2 cannabinoid-like compound that is proposed to interfere with the breakdown of endogenous cannabinoids, reduced

Table 2 – Urodynamic parameters at vehicle baseline and after intravesical infusion of oleoyl ethyl amide 1 or 10 mg/l in female rats

Urodynamic parameters intravesical OEtA	Intervals, min, and volumes, ml				Pressures, cm H ₂ O			Compliance, ml/cm H ₂ O
	ICI	MV	RV	BC	BP	TP	MP	
Vehicle baseline ($n = 6$)	4.4 ± 0.3	0.74 ± 0.04	0.005 ± 0.01	0.75 ± 0.04	6.1 ± 0.5	24.9 ± 1.8	59.3 ± 1.8	0.089 ± 0.005
OEtA 1 mg/l ($n = 6$)	5.2 ± 0.3*	0.86 ± 0.06	0.005 ± 0.01	0.87 ± 0.05*	6.4 ± 0.5	28.8 ± 2.0*	56.7 ± 1.8	0.092 ± 0.004
		$p = 0.07$						
Vehicle baseline ($n = 6$)	4.0 ± 0.3	0.68 ± 0.04	0.0 ± 0.0	0.68 ± 0.04	6.9 ± 0.6	22.8 ± 0.8	66.9 ± 3.0	0.109 ± 0.005
OEtA 10 mg/l ($n = 6$)	5.3 ± 0.2**	0.87 ± 0.04**	0.01 ± 0.003	0.88 ± 0.04**	7.4 ± 0.6	27.4 ± 1.0**	67.3 ± 2.6	0.128 ± 0.005

OEtA = oleoyl ethyl amide; ICI = intercontraction interval; MV = micturition volume; RV = residual volume; BC = bladder capacity; BP = basal pressure; TP = threshold pressure; MP = maximum pressure.

Compliance for micturition is calculated as $MV/TP - BP$. OEtA was infused (10 ml/h) continuously into the bladder for 45 min, resulting in total intravesical doses of 7.5 μg (1 mg/l) and 75 μg (10 mg/l). Values are given as mean plus or minus standard error of the mean.

For comparisons (paired t test) versus vehicle: * $p < 0.05$, ** $p < 0.01$.

Table 3a – Urodynamic parameters at vehicle baseline and after intravesical infusion of SR144528 5 mg/l and oleoyl ethyl amide 1 mg/l plus SR144528 5 mg/l

Urodynamic parameters intravesical OEtA, SR144528	Intervals, min, and volumes, ml				Pressures, cm H ₂ O			Compliance, ml/cm H ₂ O
	ICI	MV	RV	BC	BP	TP	MP	
Vehicle baseline ($n = 7$)	4.6 ± 0.2	0.74 ± 0.03	0.013 ± 0.006	0.75 ± 0.03	7.0 ± 0.3	23.6 ± 0.6	62.6 ± 1.6	0.150 ± 0.007
SR144528 5 mg/l ($n = 7$)	4.9 ± 0.3	0.78 ± 0.05	0.040 ± 0.017	0.82 ± 0.04	5.8 ± 0.3	23.3 ± 0.5	63.0 ± 2.3	0.127 ± 0.009
OEtA 1 mg/l plus SR144528 5 mg/l ($n = 7$)	4.7 ± 0.2	0.77 ± 0.03	0.0 ± 0.0	0.78 ± 0.03	6.0 ± 0.3	23.0 ± 0.6	59.6 ± 2.4	0.139 ± 0.004

OEtA = oleoyl ethyl amide; ICI = intercontraction interval; MV = micturition volume; RV = residual volume; BC = bladder capacity; BP = basal pressure; TP = threshold pressure; MP = maximum pressure.

Compliance for micturition is calculated as $MV/TP - BP$. SR144528 was infused (10 ml/h) continuously into the bladder for 45 min alone and then simultaneously for 45 min with OEtA resulting in total intravesical doses of 7.5 μg for OEtA and 75 μg for SR144528. Values are given as mean plus or minus standard error of the mean. No differences on comparisons between groups (analysis of variance).

Table 3b – Urodynamic parameters at vehicle baseline and after intravesical infusion of rimonabant 5 mg/l and oleoyl ethyl amide 1 mg/l plus rimonabant 5 mg/l in female rats

Urodynamic parameters intravesical OEtA, rimonabant	Intervals, min, and volumes, ml				Pressures, cm H ₂ O			Compliance, ml/cm H ₂ O
	ICI	MV	RV	BC	BP	TP	MP	
Vehicle baseline (n = 6)	4.9 ± 0.4	0.81 ± 0.07	0.0 ± 0.0	0.81 ± 0.07	6.2 ± 0.6	22.1 ± 0.5	59.6 ± 4.3	0.144 ± 0.011
Rimonabant 5 mg/l (n = 6)	5.1 ± 0.5	0.85 ± 0.08	0.0 ± 0.0	0.85 ± 0.08	5.9 ± 0.7	21.9 ± 0.3	56.9 ± 4.9	0.132 ± 0.011
OEtA 1 mg/l plus rimonabant 5 mg/l (n = 6)	5.7 ± 0.5§	0.95 ± 0.07§	0.0 ± 0.0	0.95 ± 0.07§	6.0 ± 0.5	22.2 ± 0.3	56.2 ± 3.1	0.148 ± 0.005

Rimonabant was infused (10 ml/h) continuously into the bladder for 45 min alone and then simultaneously for 45 min with OEtA, resulting in total intravesical doses of 7.5 µg for OEtA and 75 µg for rimonabant. Values are given as mean plus or minus standard error of the mean. For comparisons between groups (analysis of variance): § *p* < 0.05.

referred hyperalgesia to inflammation of the urinary bladder via CB₂-mediated mechanisms [28]. Another FAAH inhibitor, URB597, has also been described as reducing referred hyperalgesia to bladder inflammation, but a downstream mechanistic basis was not investigated [15].

Local CB₁ antagonism in our study did not modify micturition intervals or volumes but had effects on TP. Hence a partial function for CB₁ on afferent signals in the rat bladder may be considered. Currently diverging information on the expression and/or activity of CB₁ in the urinary bladder of humans and other mammals calls for additional investigations to clarify the function for this receptor in the urinary bladder [5,6,29,30].

5. Conclusions

Human, mouse, and rat bladder mucosa expressed FAAH, and FAAH and CB₂ immunoreactivities were codistributed in the urothelium of rats and human bladders. IV or IVES administration of the FAAH inhibitor OEtA significantly altered urodynamic parameters that reflect sensory functions of micturition in rats. Effects of IVES OEtA were abolished by an IVES CB₂ antagonist and partly counteracted by an IVES CB₁ antagonist. A role for the endocannabinoid system in mechanoafferent bladder function of rats may be considered.

Author contributions: Fabio Benigni had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Hedlund, Benigni, Gratzke.

Acquisition of data: Strittmatter, Gandaglia, Bettiga, Colciago.

Analysis and interpretation of data: Strittmatter, Gandaglia, Bettiga, Benigni, Hedlund.

Drafting of the manuscript: Strittmatter, Gandaglia, Hedlund.

Critical revision of the manuscript for important intellectual content: Hedlund, Benigni, Gratzke.

Statistical analysis: Strittmatter, Gandaglia, Hedlund.

Obtaining funding: Montorsi, Rigatti, Stief, Hedlund.

Administrative, technical, or material support: Bettiga, Colciago, Benigni.

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