Spatial distribution of cannabinoid receptor 1 and fatty acid amide hydrolase in the cat ovary and oviduct

Andrea Pirone, Carla Lenzi, Angela Briganti, Francesco Abbate, Maria Levanti, Francesca Abramo, Vincenzo Miragliotta*

Department of Veterinary Sciences, University of Pisa, Pisa, Italy

Keywords:

CatOvaryOviductCB1RFAAHEndocannabinoid system

ABSTRACT

Involvement of the endocannabinoid system in female reproduction has been extensively described in humans with the cognate receptors and ligands being found in the ovaries and genital tract. In human, an imbalance of the endocannabinoid system is linked with both ectopic pregnancy and infertility. In bovine species anandamide levels regulate aspects of sperm-oviduct interaction. Here we report the immunohistochemical distribution of cannabinoid receptor 1 (CB1R) and fatty acid amide hydrolase (FAAH) in cat ovary and oviduct, using paraffin- embedded tissue samples and commercially available antibodies. We found a differential expression of both CB1R and FAAH during different stages of ovarian function and in the oviduct. CB1R was detected only in tertiary follicle granulosa cells while more immature follicles were negative. FAAH was instead found in ovarian pre-antral follicles, the oocyte cytoplasm, and in granulosa cells of primary, secondary and tertiary follicles. Secondary and tertiary follicles were also FAAH immunoreactive. Luteal cells were immunopositive for both CB1R and FAAH. Because CBR1 in oviduct was found only in ciliated cells, it might represent a specific marker at least in cats. In contrast, FAAH immunoreactivity was observed in both ciliated and non-ciliated cells. The present study may thus serve as the starting point for further investigations on the role of the endocannabinoid system in cat reproduction. Additional work will be needed to assess whether the morphological distribution of CB1R and FAAH changes in different conditions such as pre-pubertal age, follicular phase of the sexual cycle and pregnancy.

1. Introduction

Cannabinoid receptors together with a group of endogenous lipid ligands and the enzymes for their biosynthesis and degradation constitute the endocannabinoid system (ECS) (Iannotti et al., 2016). The ECS mediates the effects of cannabinoids, including Δ9-tetrahy- drocannabinol (THC) the chief psychoactive component in marijuana (Skaper and Di Marzo, 2012). At least two cannabinoid receptors are known, cannabinoid receptor 1 (CB1R) and cannabinoid receptor 2 (CB2R). CB1R is a G-protein-coupled receptor abundantly expressed in the brain (Covey et al., 2015) that mediates the effects of the endogenous cannabinoids anandamide (AEA) (Devane et al., 1992) and 2-arachidonoyl-glycerol (2-AG) (Mechoulam et al., 1995). Although CB1R was first described as a central nervous system receptor, CB1R-mediated actions were proposed in the liver, pancreas, gastro- intestinal system, skeletal muscle, adipose tissue, skin and embryo development (Campora et al., 2012; Kunos and Tam, 2011; Pirone et al., 2015; Stander et al., 2005). Fatty acid amide hydrolase (FAAH) is

the principal catabolic enzyme for AEA and other fatty acid amides, and in brain is found in soma and dendrites of postsynaptic elements (where AEA is also synthetized). CB1R, in contrast, is located on presynaptic terminals (Pirone et al., 2015). This anatomical distribution underlies the so-called retrograde inhibitory mechanism which suppresses pre- synaptic neurotransmitter release (Kreitzer and Regehr, 2001).

The ECS also plays a complex role in the female reproductive system. Its components are located throughout the reproductive tract, including the rat and human ovary (Bagavandoss and Grimshaw, 2010; El-Talatini et al., 2009), oviduct (Horne et al., 2008), myometrium (Brighton et al., 2011), and endometrium (Taylor et al., 2010). Studies performed in a human granulosa cell line indicated that the intrinsic ovarian ECS plays a role in estradiol synthesis (Ernst et al., 2016). AEA is found in human follicular fluid (Schuel et al., 2002), and alterations in its plasma levels

are related to fertility/infertility in healthy women (Maccarrone et al., 2002). Moreover, ECS signaling was shown to be involved in the oocyte maturation (Agirregoitia et al., 2015; Agirregoitia et al., 2016; Lopez-Cardona et al., 2016; Peralta et al., 2011) and the fertilization process as well, as the fertilizing ability of sperm and sperm–oocyte interaction depends on AEA binding to either CB1R or the transient receptor potential cation channel subfamily V member 1 (TRPV1) receptor. This occurs in a two-step process: first, AEA negatively affects human sperm motility in a CB1R-dependent manner while acting as a capacitating signal at physiological concen- trations (Rossato et al., 2005); second, once capacitation is completed, AEA effects are mediated by TRPV1 activation to prevent spontaneous acrosomal reaction that results only from sperm–egg interactions which maximize sperm-fertilizing potential (Francavilla et al., 2009). AEA also plays key roles post-fertilization, being involved in the implantation process, placentation and pregnancy (Battista et al., 2015). Aberrant plasma levels of AEA, whether too high or too low, are deleterious for pregnancy (Wang et al., 2004).

Domestic cats are well-known for their reproductive proclivity, and suppression of fertility in this species is often desirable. Although surgical contraception is highly effective, but its cost and irreversibility may desirable for professional cat breeders (Goericke-Pesch et al., 2014).

As far as we are aware, a role for the ECS in domestic animal reproduction has only been described in bovine species (Gervasi et al., 2013) in terms of the effects that ECS ligands (i.e AEA) might play in sperm-oviduct interaction. Morphological data on the distribution of CB1R and FAAH is limited to human (El-Talatini et al., 2009) and rats (Bagavandoss and Grimshaw, 2010).

Targeting the ECS with cannabinoid receptor agonists is under investigation for a number of therapeutic indications (Abramo et al., 2014; Campora et al., 2012; Pertwee, 2012). The potential use of cannabimimetic compounds in companion animals as analgesic/anti- inflammatory drugs was reviewed recently (Re et al., 2007). In a pilot study in cats, palmidrol [palmitoylethanolamide (PEA)], an AEA congener, was used to treat eosinophilic granuloma with full resolution of clinical signs (Scarampella et al., 2001). Immunophenotypical characterization of the ECS components might be of utility in predicting wanted and unwanted effects when a new cannabimimetic compound is used in a new target species. The present study was designed to begin addressing this issue by assessing the morphological distribution of CB1R and FAAH in cat ovary and oviduct.

2. Materials and methods

2.1. Animals and samples

Five non-pregnant female domestic cats in the luteal phase of the sexual cycle (diestrus), referred for spaying to our local veterinary teaching hospital, were used in the study. A written informed consent was obtained from each cat's owner prior to surgery. After premedica- tion with dexmedetomidine and methadone IM and intravenous induc- tion with propofol, hair was clipped and the surgical site aseptically prepared. Anaesthesia was then maintained with isoflurane in oxygen and fentanyl infusion for the nociception control. Gonads and oviducts were collected and promptly immersed in modified Davidson's solution (Latendresse et al., 2002) for 24 h. Tissues were then processed for routine paraffin embedding and 5µm thick sections prepared for morphological evaluation (hematoxylin and eosin staining) and im- munohistochemistry.

This research was carried out according to the international regulation on the use of animals for scientific purposes (Directive 2010/63/EU).

2.2. Immunohistochemistry

Immunohistochemistry was performed on three different section per animal in a single session in order to expose tissues to analogous experimental conditions. A rabbit polyclonal anti-CB1R antibody (1:50, abcam, ab23703) and a goat polyclonal anti-FAAH antibody (1:100,

abcam, ab110840) were used. Epitope retrieval was carried out at 120 °C in a pressure cooker for 5 min with a Tris/EDTA buffer, pH 9.0. Sections were pretreated in 1% H_2O_2 (in 0.1 M phosphate buffered saline (PBS), pH 7.4, 10min) to quench endogenous peroxidase activity, then rinsed with 0.05% tween-20 detergent (in 0.1 M PBS, 3 × 10 min), and blocked with 5% normal goat serum (NGS) (s-1000, Vector Labs, Burlingame, CA) for CB1R and 2.5% normal horse serum (NHS) (PK-7200, Vector Labs) for FAAH (in 0.1 M PBS, 1 h). To enable comparison of FAAH and CB1R expression, adjacent sections were incubated overnight at 4 °C in a solution containing anti-CB1R antibody with 2% NGS or anti-FAAH antibody with 2% NHS, 0.05% triton X-100 (in 0.1 M PBS). Sections were then rinsed in 0.1 M PBS, (3 × 10 min), followed by incubation with a biotinylated anti-rabbit IgG (BA-1000, Vector Labs) for CB1R or biotinylated anti-goat IgG (BA-9500, Vector Labs) for FAAH diluted 1:300 in PBS. Sections were again rinsed in 0.1 M PBS, for 3 × 10 min. Staining was visualized by incubating the sections in diaminobenzidine (sk-4105, Vector Labs) solution. Photomicrographs were obtained using a Nikon Ni-e microscope equipped with the Nis Elements Br Microscope Imaging Software (Nikon Instruments, Calenzano, Italy). Immunostaining was graded by two independent investigators as minimal, moderate or strong.

Specificity of immunohistochemical staining was also confirmed as follows: blocking with the corresponding peptide (abcam, ab50542), substitution of primary antibody, anti-rabbit/goat IgG, or ABC complex with PBS or non-immune serum. Under these conditions staining was abolished. Moreover, specificity of the anti-CB1R antibody used has been demonstrated previously in dogs (Campora et al., 2012); felis catus CB1R is 91% identical to canine CB1R at the gene level. Further, the FAAH antibody used was previously tested in dogs in our laboratory (Pirone et al., 2016); in analogy to CB1R, the felis catus FAAH nucleotide sequence is predicted to be 91% identical to the canine one.

To further verify the specificity of the used antibodies, a second set of cat ovaries were collected during spaying and promptly frozen in liquid nitrogen for subsequent western blot analyses. Briefly, 30 µg of proteins were resolved by 12% SDS-PAGE gels and transferred onto nitrocellulose membranes (0.2 µm) using a voltage of 25 V for 7 min (Trans-Blot®TurboTM Transfer System; Bio-Rad). After electrophoresis, the membranes were blocked and then incubated with appropriately diluted primary antibodies. HRP-conjugated goat anti-rabbit (1:10,000, Enzo life science, ADI-SAB-300J) and HRP-conjugated donkey anti-goat SantaCruz (1:10,000, Santa Cruz Biotechnology, Inc, sc 2020) were used as secondary antibodies. The chemiluminescent images were acquired by LAS 4010 (GE Health Care).

3. Results

3.1. CB1R immunoreactivity

Western blot analyses showed the presence of two immunoreactive bands (of 66 and 81kDa); either primary antibody omission and blocking with the corresponding peptide completely abolished immu- nostaining (Fig. 1). Cat ovaries were characterized by the presence of primordial, primary, secondary, tertiary and pre-ovulatory follicles as well as active corpora lutea. Primordial follicles did not show CB1R immunoreactivity either in their squamous epithelium or oocyte structures (Fig. 2a). The same was the case for primary and secondary follicles (Fig. 2c and Fig. 3a, respectively) while tertiary follicles showed minimal cytoplasmic immunoreactivity for both granulosa and corona radiata (Fig. 3c). The same minimal staining was observed in the secondary oocyte cytoplasm while both theca interna and externa cells where devoid of staining, as were stromal structures and ovarian surface epithelium (OSE).

Corpora lutea showed moderate immunostaining of the secretory cell cytoplasm (Fig. 4a) while strong immunoreactivity was observed for oviduct ciliated cells (Fig. 5a). Non-ciliated epithelial cells of the oviduct and stroma were consistently devoid of staining.

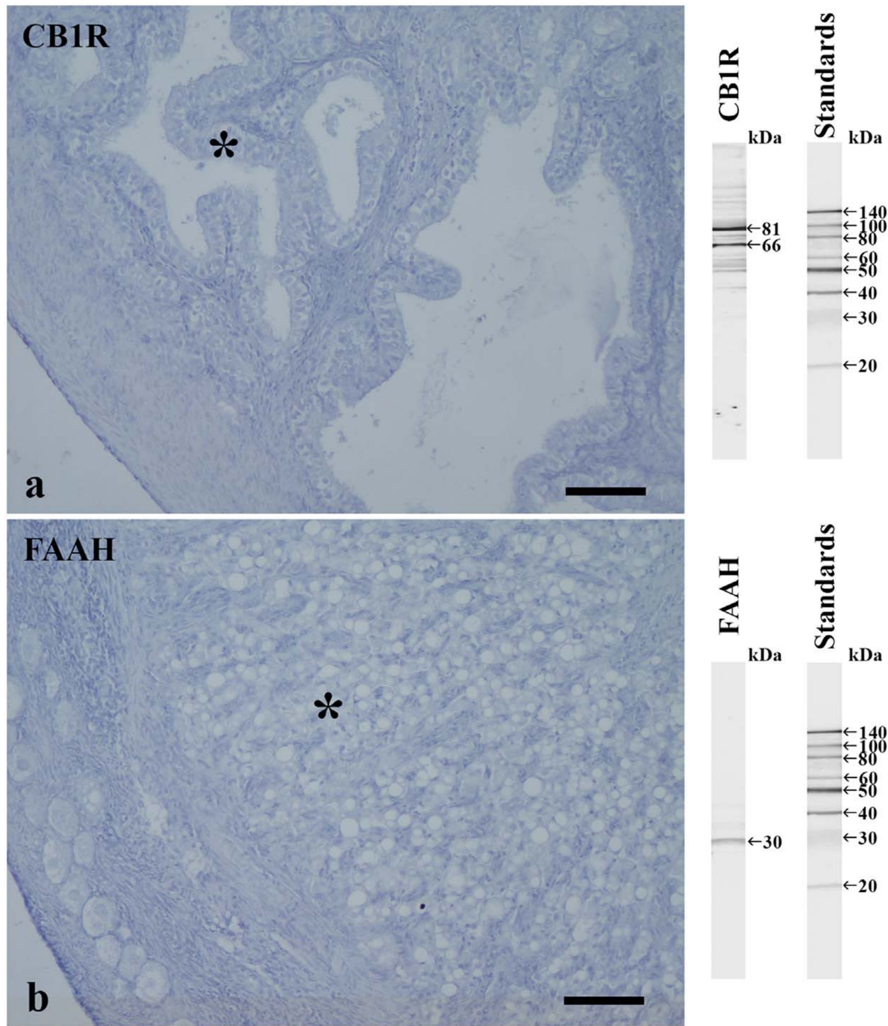
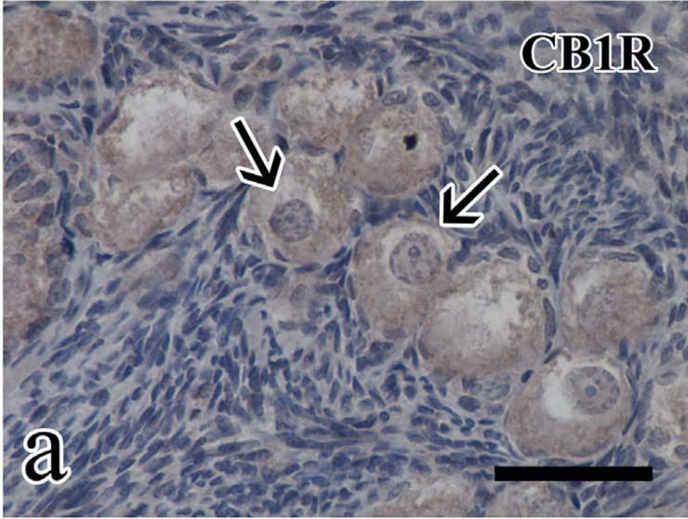
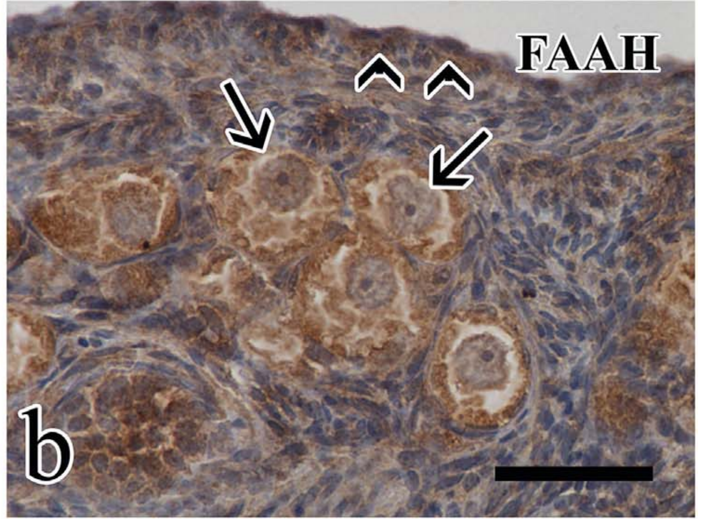
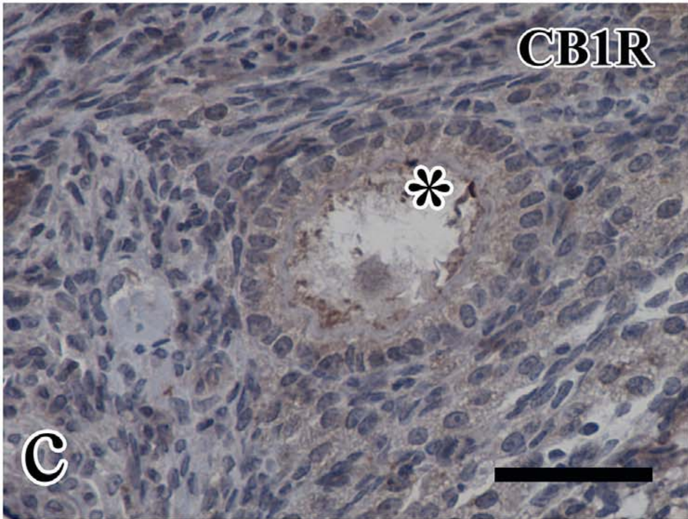


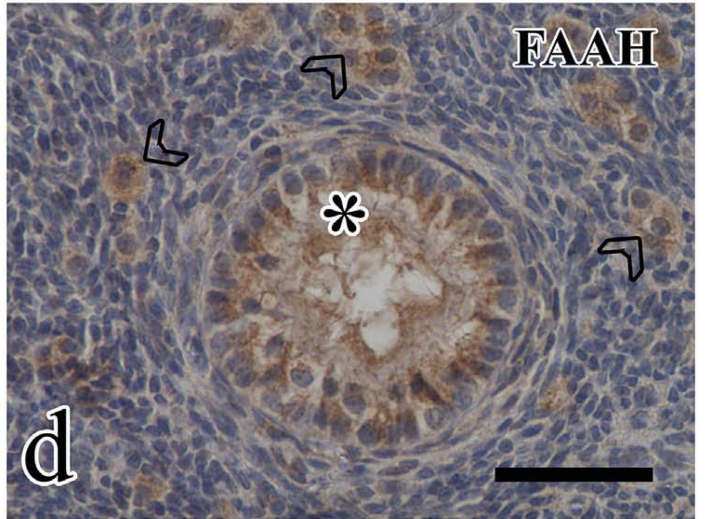
Fig. 1. Photomicrographs of primary antibody controls and western blot results obtained on proteins extracted from cat ovary. a) section of cat oviduct where the anti-CB1R antibody was blocked with the corresponding peptide; asterisk indicates the epithelium; scale bar is equal to 20 µm. b) section of cat ovary where the anti FAAH antibody was omitted; asterisk indicates the corpus luteum; scale bar is equal to 20 µm.

Fig. 2. Photomicrographs of cat ovary immunostained for CB1R (a, c) and FAAH (b, d) showing primordial (arrows) and primary (asterisks) ovarian follicles. Arrowheads indicate the OSE while empty arrowheads indicate scattered stromal cells immunoreactive for FAAH. Scale bar: 50 µm.









3

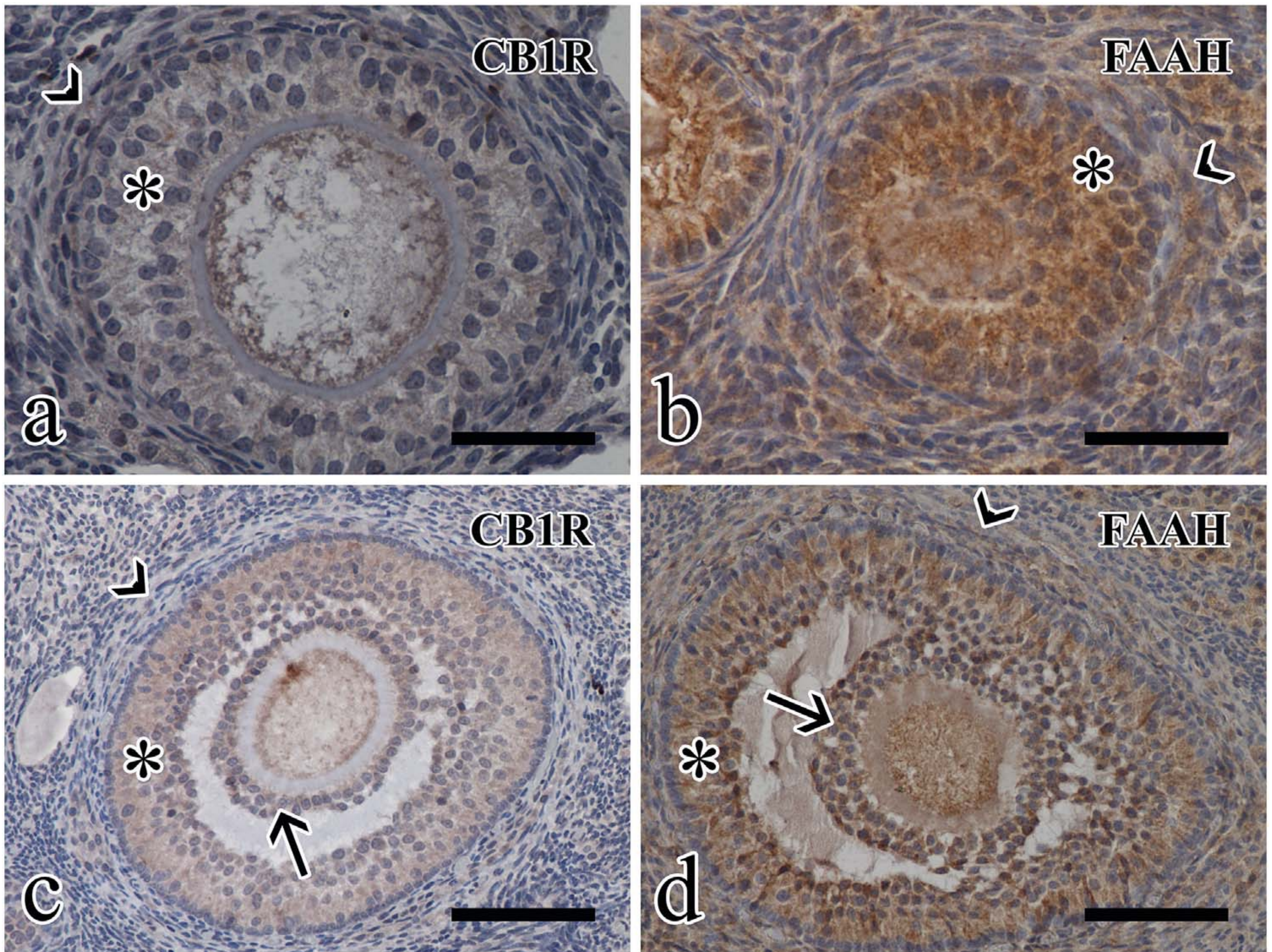


Fig. 3. Photomicrographs of cat ovary immunostained for CB1R (a, c) and FAAH (b, d) showing secondary (a, b) and tertiary (c, d) ovarian follicles. Arrowheads indicate thecal cells while asterisks indicate granulosa cells and arrows point to corona radiata. Scale bar: 50 μm in a and b, and 100 μm in c and d.

3.2. FAAH immunoreactivity

Western blot analyses showed the presence of one immunoreactive band of 30 kDa; the primary antibody omission completely abolished immunostaining (Fig. 1). Primordial follicles showed moderate immu- noreactivity in oocyte cytoplasm (Fig. 2b), as well as granulosa cell cytoplasm of primary follicles (Fig. 2d). FAAH was expressed also in the OSE (Fig. 2b) along with scattered immunoreactive cells throughout the ovarian cortex (Fig. 2d). While moderate immunostaining was observed in granulosa cells of the secondary follicles (Fig. 3b) labeling was minimal in tertiary follicles (Fig. 3d). The oocyte cytoplasm displayed a moderate level of FAAH immunoreactivity in both secondary and tertiary follicles. Theca cells showed minimal immunoreactivity in both secondary and tertiary follicles. Corpora lutea evidenced strong im- munostaining of the secretory cell cytoplasm while the surrounding stroma was consistently devoid of staining (Fig. 4b). Moderate FAAH immunoreactivity was noted in all oviduct epithelial cells, being predominantly located in the apical domain. Oviduct lamina propria displayed scattered FAAH immunoreactive cells (Fig. 5b).

4. Discussion

The present study describes a differential expression of CB1R and FAAH in the cat in the oviduct and during different

stages of ovarian cycle. CB1R was detected in tertiary follicle granulosa cells but not in more immature follicles. Analogous findings have been reported for rat (Bagavandoss and Grimshaw, 2010) where only pre-antral (tertiary) follicles showed CB1R immunostaining in granulosa cells. Likewise, in human CB1R was primarily located in the large antral follicles, with more modest expression in small follicles (El-Talatini et al., 2009). In humans, CB1R immunoreactivity was shown to increase from benign to malignant tumors, it can be thus hypothesized that proliferative activity (i.e. more mature follicles and malignant neoplastic lesions) is some- what related to CB1R expression (Messalli et al., 2014). The latter study also reported CB1R immunoreactivity in the cytoplasm of human secondary oocytes, consistent with our data in cats (Messalli et al., 2014). In contrast, these regions were CB1R negative in the rat (Bagavandoss and Grimshaw, 2010).

FAAH, the principal AEA-catabolizing enzyme, displayed an expres- sion pattern distinct from that of CB1R. It was indeed found in ovarian pre-antral follicles, in oocyte cytoplasm, and in granulosa cells of primary, secondary and tertiary follicles. Secondary and tertiary

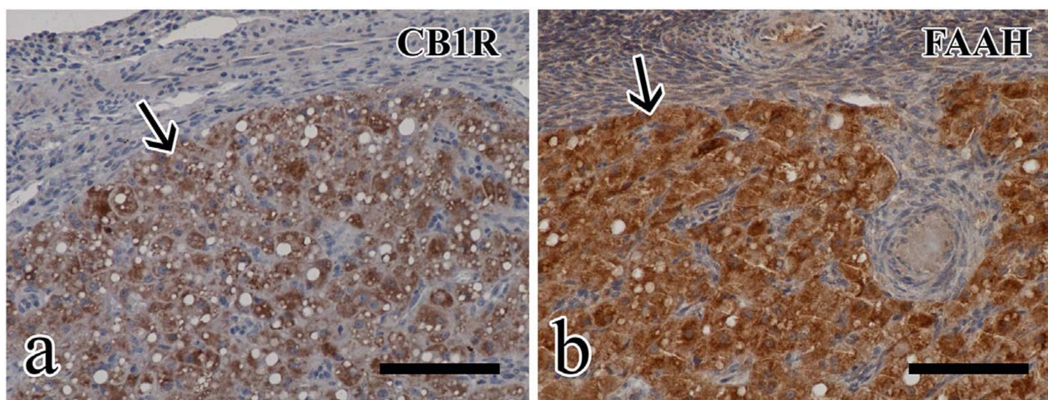


Fig. 4. Photomicrographs of cat ovary immunostained for CB1R (a) and FAAH (b) showing corpora lutea. Arrows indicate luteal cells immunostained with CB1R and FAAH in a and b, respectively. Scale bar: 100 μm.

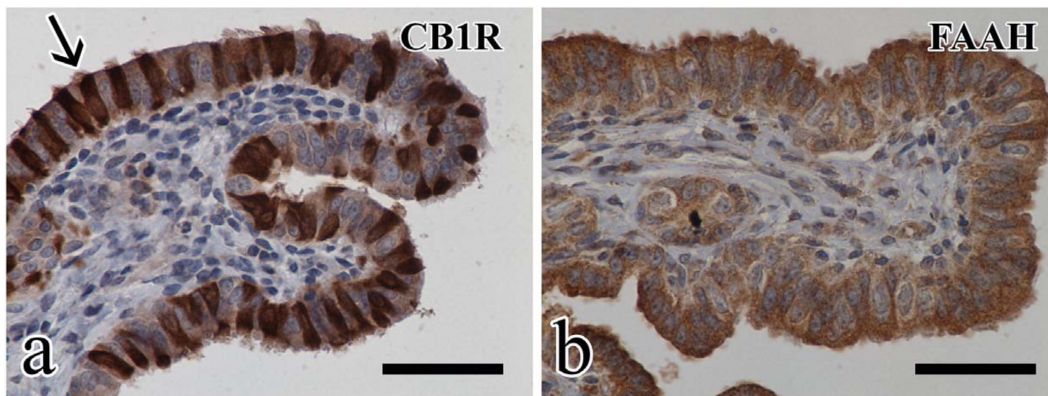


Fig. 5. Photomicrographs of cat oviduct immunostained for CB1R (a) and FAAH (b). Arrow indicates ciliated cells immunostained with CB1R (a). Both ciliated and non-ciliated cells are immunoreactive for FAAH (b). Scale bar: 50 μm.

follicles also showed FAAH immunoreactivity in thecal cells. These observations point to FAAH expression preceding that of CB1R during folliculogenesis. In human, FAAH was expressed only in thecal cells of secondary and tertiary follicles, as described here for cats, while granulosa cells were devoid of staining. These findings led El-Talatini et al. (2009) to suggest that AEA acts in an autocrine manner on thecal cells, while an alternate, FAAH-independent degradation pathway may be present in granulosa cells. Our findings contrast not only with these differences reported in human, but also those reported in rat, where FAAH was confined to the oocyte cytoplasm. On the other hand, our observation of FAAH immunoreactivity in the OSE and scattered cells in the ovarian cortex are congruous with what has been found in the rat (Bagavandoss and Grimshaw, 2010). In other species the OSE and subepithelial cords in the cortex are capable of differentiating into presumptive granulosa and germ cells (Bukovsky et

al., 2008), leading to the suggestion that FAAH might serve as a potential marker for tracking cells differentiating from the OSE (Bagavandoss and Grimshaw, 2010).

Luteal cells were immunoreactive for both CB1R and FAAH as already reported in rat and humans (Bagavandoss and Grimshaw, 2010; El-Talatini et al., 2009). In this context it is worth mentioning a recent study in ewes treated with CB1R/CB2R agonist, which negatively affected luteal progesterone secretion by decreasing luteal expression of luteinizing hormone receptor mRNA (Tsutahara et al., 2011). Since progesterone is required for implantation and pregnancy maintenance (Palomba et al., 2015), targeting the corpus luteum with endocanna- binoids may be a way to decrease fertility.

Collectively, the described findings on ovarian CB1R and FAAH distribution suggest that late stage follicles and corpora lutea – in contrast to early follicles – should respond to ECS ligands. We also examined oviduct sections, whose epithelium consists of both ciliated and non-ciliated cells (Okada et al., 2004). CB1R was found only in ciliated cells, suggesting its potential as a specific marker at least in cats. FAAH immunopositivity was instead observed in both ciliated and non-ciliated cells. The ECS is known to influence sperm-oviduct interaction, with AEA inhibiting bovine sperm binding and inducing sperm release from oviductal epithelial cells, either via CB1R activation (Gervasi et al., 2009) or the nitric oxide pathway (Osycka-Salut et al., 2012). After conception, in another phase of the reproductive process, high local (oviductal) and systemic AEA levels positively correlated with ectopic pregnancy (Gebeh et al., 2013; Gebeh et al., 2012) as well as aberrant expression of CB1R and FAAH in the oviduct epithelium (Gebeh et al., 2012). Due to the particular localization of CB1R and this latter report, along with our findings, we postulate that epithelial ciliated cell function might be impaired upon enhanced CB1R activa- tion. In fact, mice exposed to either a stable AEA analogue or delta9- tetrahydrocannabinol experienced pregnancy loss with embryo reten- tion in the oviduct (Wang et al., 2004). The same study also showed that CB1R and CB2R knockout mice, as well as wild-type mice treated with a CB1 selective antagonist are subject to an elevated frequency of pregnancy loss (Wang et al., 2004). These data point to an essential role

of maternal CB1R expression in the reproductive tract, and that either silenced or enhanced cannabinoid signaling may impair embryo trans- port from oviduct to uterus (Di Blasio et al., 2013).

The presented results may lead one to hypothesize that the local (to avoid CNS effects) application of CB1R agonists in cats may be a novel approach for decreasing fertility, via a reduction in progesterone levels (CB1R and FAAH were strongly expressed in corpora lutea cells) and impairment of oviduct ciliated cell function.

In conclusion, we found a differential expression of both CB1R and FAAH during different stages of ovarian function and in the oviduct, although this work may serve as a starting point for further research on the role of the ECS in cat reproduction, further investigations are needed to assess whether the morphological distribution of CB1R and FAAH undergo changes in different conditions such as pre-pubertal age, follicular phase of the sexual cycle and pregnancy.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

Authors are indebted to Dr. Stephen D. Skaper (Department of Pharmaceutical and Pharmacological Sciences, University of Padua) for fruitful discussions and English proofreading of the manuscript.

References

Abramo, F., Campora, L., Albanese, F., della Valle, M.F., Cristino, L., Petrosino, S., Di Marzo, V., Miragliotta, V., 2014. Increased levels of palmitoylethanolamide and other bioactive lipid mediators and enhanced local mast cell proliferation in canine atopic dermatitis. BMC Vet. Res. 10, 21.

Agirregoitia, E., Ibarra-Lecue, I., Totorikaguena, L., Mendoza, R., Exposito, A., Matorras, R., Uriguen, L., Agirregoitia, N., 2015. Dynamics of expression and localization of the cannabinoid system in granulosa cells during oocyte nuclear maturation. Fertil. Steril. 104 (3), 753–760.

Agirregoitia, E., Totorikaguena, L., Exposito, A., Mendoza, R., Matorras, R., Agirregoitia, N., 2016. Dynamic of expression and localization of cannabinoid-degrading enzymes FAAH and MGLL in relation to CB1 during meiotic maturation of human oocytes. Cell Tissue Res. 365 (2), 393–401.

Bagavandoss, P., Grimshaw, S., 2010. Temporal and spatial distribution of the cannabinoid receptors (CB1, CB2) and fatty acid amide hydroxylase in the rat ovary. Anat. Rec. 293 (8), 1425–1432.

Battista, N., Bari, M., Maccarrone, M., 2015. Endocannabinoids and reproductive events in health and disease. Handbook of Experimental Pharmacology, vol. 231. pp. 341–365.

Brighton, P.J., Marczylo, T.H., Rana, S., Konje, J.C., Willets, J.M., 2011. Characterization of the endocannabinoid system, CB(1) receptor signalling and desensitization in human myometrium. Br. J. Pharmacol. 164 (5), 1479–1494.

Bukovsky, A., Gupta, S.K., Virant-Klun, I., Upadhyaya, N.B., Copas, P., Van Meter, S.E., Svetlikova, M., Ayala, M.E., Dominguez, R., 2008. Study origin of germ cells and formation of new primary follicles in adult human and rat ovaries. Methods Mol. Biol. 450, 233–265.

Campora, L., Miragliotta, V., Ricci, E., Cristino, L., Di Marzo, V., Albanese, F., Federica Della Valle, M., Abramo, F., 2012. Cannabinoid receptor type 1 and 2 expression in the skin of healthy dogs and dogs with atopic dermatitis. Am. J. Vet. Res. 73 (7), 988–995.

Covey, D.P., Wenzel, J.M., Cheer, J.F., 2015. Cannabinoid modulation of drug reward and the implications of marijuana legalization. Brain Res. 1628 (Pt. A), 233–243.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258 (5090), 1946–1949.

Di Blasio, A.M., Vignali, M., Gentilini, D., 2013. The endocannabinoid pathway and the female reproductive organs. J. Mol. Endocrinol. 50 (1), R1–9.

El-Talatini, M.R., Taylor, A.H., Elson, J.C., Brown, L., Davidson, A.C., Konje, J.C., 2009. Localisation and function of the endocannabinoid system in the human ovary. PLoS One 4 (2), e4579.

Ernst, J., Grabiec, U., Greither, T., Fischer, B., Dehghani, F., 2016. The endocannabinoid system in the human granulosa cell line KGN. Mol. Cell. Endocrinol. 423, 67–76.

Francavilla, F., Battista, N., Barbonetti, A., Vassallo, M.R., Rapino, C., Antonangelo, C., Pasquariello, N., Catanzaro, G., Barboni, B., Maccarrone, M., 2009. Characterization of the endocannabinoid system in human spermatozoa and involvement of transient receptor potential vanilloid 1 receptor in their fertilizing ability. Endocrinology 150 (10), 4692–4700.

Gebeh, A.K., Willets, J.M., Marczylo, E.L., Taylor, A.H., Konje, J.C., 2012. Ectopic pregnancy is associated with high anandamide levels and aberrant expression of FAAH and CB1 in fallopian tubes. J. Clin. Endocrinol. Metab. 97 (8), 2827–2835.

Gebeh, A.K., Willets, J.M., Bari, M., Hirst, R.A., Marczylo, T.H., Taylor, A.H., Maccarrone, M., Konje, J.C., 2013. Elevated anandamide and related N-acylethanolamine levels occur in the peripheral blood of women with ectopic pregnancy and are mirrored by changes in peripheral fatty acid amide hydrolase activity. J. Clin. Endocrinol. Metab. 98 (3), 1226–1234.

Gervasi, M.G., Rapanelli, M., Ribeiro, M.L., Farina, M., Billi, S., Franchi, A.M., Perez Martinez, S., 2009. The endocannabinoid system in bull sperm and bovine oviductal epithelium: role of anandamide in sperm-oviduct interaction. Reproduction 137 (3), 403–414.

Gervasi, M.G., Marczylo, T.H., Lam, P.M., Rana, S., Franchi, A.M., Konje, J.C., Perez- Martinez, S., 2013. Anandamide levels fluctuate in the bovine oviduct during the oestrous cycle. PLoS One 8 (8), e72521.

Goericke-Pesch, S., Wehrend, A., Georgiev, P., 2014. Suppression of fertility in adult cats. Reproduction in domestic animals = Zuchthygiene 49 (Suppl. 2), 33–40.

Horne, A.W., Phillips, J.A., Kane, N., Lourenco, P.C., McDonald, S.E., Williams, A.R.W., Simon, C., Dey, S.K., Critchley, H.O.D., 2008. CB1 expression is attenuated in fallopian tube and decidua of women with ectopic pregnancy. PLoS One 3 (12).

Iannotti, F.A., Di Marzo, V., Petrosino, S., 2016. Endocannabinoids and endocannabinoid- related mediators: targets: metabolism and role in neurological disorders. Prog. Lipid Res. 62, 107–128.

Kreitzer, A.C., Regehr, W.G., 2001. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron 29 (3), 717–727.

Kunos, G., Tam, J., 2011. The case for peripheral CB(1) receptor blockade in the treatment of visceral obesity and its cardiometabolic complications. Br. J.

Pharmacol. 163 (7), 1423–1431.

Latendresse, J.R., Warbrittion, A.R., Jonassen, H., Creasy, D.M., 2002. Fixation of testes and eyes using a modified Davidson’s fluid: comparison with Bouin's fluid and conventional Davidson’s fluid. Toxicol. Pathol. 30 (4), 524–533.

Lopez-Cardona, A.P., Sanchez-Calabuig, M.J., Beltran-Brena, P., Agirregoitia, N., Rizos, D., Agirregoitia, E., Gutierrez-Adan, A., 2016. Exocannabinoids effect on in vitro bovine oocyte maturation via activation of AKT and ERK1/2. Reproduction 152 (6), 603–612.

Maccarrone, M., Bisogno, T., Valensise, H., Lazzarin, N., Fezza, F., Manna, C., Di Marzo, V., Finazzi-Agro, A., 2002. Low fatty acid amide hydrolase and high anandamide levels are associated with failure to achieve an ongoing pregnancy after IVF and embryo transfer. Mol. Hum. Reprod. 8 (2), 188–195.

Mechoulam, R., Benshabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G.,

Bayewitch, M., Barg, J., Vogel, Z., 1995. Identification of an endogenous 2- Monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol. 50 (1), 83–90.

Messalli, E.M., Grauso, F., Luise, R., Angelini, A., Rossiello, R., 2014. Cannabinoid receptor type 1 immunoreactivity and disease severity in human epithelial ovarian tumors. Am. J. Obstet. Gynecol. 211 (3), 234.e1-6.

Okada, A., Ohta, Y., Brody, S.L., Watanabe, H., Krust, A., Chambon, P., Iguchi, T., 2004. Role of foxj1 and estrogen receptor alpha in ciliated epithelial cell differentiation of the neonatal oviduct. J. Mol. Endocrinol. 32 (3), 615–625.

Osycka-Salut, C., Gervasi, M.G., Pereyra, E., Cella, M., Ribeiro, M.L., Franchi, A.M., Perez- Martinez, S., 2012. Anandamide induces sperm release from oviductal epithelia through nitric oxide pathway in bovines. PLoS One 7 (2), e30671.

Palomba, S., Santagni, S., La Sala, G.B., 2015. Progesterone administration for luteal phase deficiency in human reproduction: an old or new issue? J. Ovarian Res. 8, 77.

Peralta, L., Agirregoitia, E., Mendoza, R., Exposito, A., Casis, L., Matorras, R., Agirregoitia, N., 2011. Expression and localization of cannabinoid receptors in human immature oocytes and unfertilized metaphase-II oocytes. Reprod. Biomed. Online 23 (3), 372–379.

Pertwee, R.G., 2012. Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 367 (1607), 3353–3363.

Pirone, A., Lenzi, C., Coli, A., Giannessi, E., Stornelli, M.R., Miragliotta, V., 2015. Preferential epithelial expression of type-1 cannabinoid receptor (CB1R) in the developing canine embryo. SpringerPlus 4, 804.

Pirone, A., Cantile, C., Miragliotta, V., Lenzi, C., Giannessi, E., Cozzi, B., 2016. Immunohistochemical distribution of the cannabinoid receptor 1 and fatty acid amide hydrolase in the dog claustrum. J. Chem. Neuroanat. 74, 21–27.

Re, G., Barbero, R., Miolo, A., Di Marzo, V., 2007. Palmitoylethanolamide, endocannabinoids and related cannabimimetic compounds in protection against tissue inflammation and pain: potential use in companion animals. Vet. J. 173 (1), 21–30.

Rossato, M., Ion Popa, F., Ferigo, M., Clari, G., Foresta, C., 2005. Human sperm express cannabinoid receptor Cb1, the activation of which inhibits motility, acrosome reaction, and mitochondrial function. J. Clin. Endocrinol. Metab. 90 (2), 984–991.

Scarampella, F., Abramo, F., Noli, C., 2001. Clinical and histological evaluation of an analogue of palmitoylethanolamide, PLR 120 (comicronized Palmidrol INN) in cats with eosinophilic granuloma and eosinophilic plaque: a pilot study. Vet. Dermatol. 12 (1), 29–39.

Schuel, H., Burkman, L.J., Lippes, J., Crickard, K., Forester, E., Piomelli, D., Giuffrida, A., 2002. N-Acylethanolamines in human reproductive fluids. Chem. Phys. Lipids 121 (1–2), 211–227.

Skaper, S.D., Di Marzo, V., 2012. Endocannabinoids in nervous system health and disease: the big picture in a nutshell. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 367 (1607), 3193–3200.

Stander, S., Schmelz, M., Metze, D., Luger, T., Rukwied, R., 2005. Distribution of cannabinoid receptor 1 (CB1) and 2 (CB2) on sensory nerve fibers and adnexal structures in human skin. J. Dermatol. Sci. 38 (3), 177–188.

Taylor, A.H., Abbas, M.S., Habiba, M.A., Konje, J.C., 2010. Histomorphometric evaluation of cannabinoid receptor and anandamide modulating enzyme expression in the human endometrium through the menstrual cycle. Histochem. Cell Biol. 133 (5), 557–565.

Tsutahara, N.M., Weems, Y.S., Arreguin-Arevalo, J.A., Nett, T.M., LaPorte, M.E., Uchida, J., Pang, J., McBride, T., Randel, R.D., Weems, C.W., 2011. Effects of endocannabinoid 1 and 2 (CB1; CB2) receptor agonists on luteal weight, circulating progesterone, luteal mRNA for luteinizing hormone (LH) receptors, and luteal unoccupied and occupied receptors for LH in vivo in ewes. Prostaglandins Other Lipid Mediators 94 (1–2), 17–24.

Wang, H., Guo, Y., Wang, D., Kingsley, P.J., Marnett, L.J., Das, S.K., DuBois, R.N., Dey, S.K., 2004. Aberrant cannabinoid signaling impairs oviductal transport of embryos. Nat. Med. 10 (10), 1074–1080.