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Review article

The role of the endocannabinoid system in aetiopathogenesis of endometriosis: A potential therapeutic target

Keisuke Tanaka[a,b], Leah Mayne[a], Akram Khalil[a], David Baartz[a], Lars Eriksson[c], Sally-Anne Mortlock[d], Grant Montgomery[d], Brett McKinnon[d,e], Akwasi A. Amoako[a,b,*]

[a] Department of Obstetrics and Gynaecology, The Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia
[b] Faculty of Medicine, University of Queensland, Brisbane, Queensland, Australia
[c] The University of Queensland, UQ Library, Brisbane, Queensland, Australia
[d] The Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia
[e] Department of Obstetrics and Gynaecology, University Hospital of Berne, Berne, Switzerland

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ABSTRACT

Endometriosis affects a large proportion of women during their reproductive years and is associated with pain and infertility, also affecting psychological wellbeing and quality of life. The pathogenesis of the disease remains unclear, although it is believed to be multifactorial. The endocannabinoid system (ECS) consists of a number of ligands, receptors and enzymes, and has gained interests in endometriosis research. This review aims to summarise all available evidence reporting the roles of the ECS in endometriosis. A literature search of the PubMed, EMBASE, and Web of Science electronic medical databases was performed. Original and review articles published in peer-reviewed journals were included. No publication date or publication status restrictions were imposed. Significant differences in the concentrations and expressions of the components of the ECS were reported in the eutopic and ectopic endometrium, and the systemic circulation of women with endometriosis compared to controls. Endometriosis appears to be associated with downregulation of CB1 receptors and upregulation of TRPV1 receptors. The role of CB1 and progesterone in anti-inflammatory action and the role of TRPV1 in inflammation and pain are of particular interests. Furthermore, the ECS has been reported to be involved in processes relevant to endometriosis, including cell migration, cell proliferation, apoptosis, inflammation, and interacts with sex steroid hormones. The ECS may play a role in disease establishment, progression, and pain in endometriosis. However, reports are based on studies of limited size and there are inconsistencies among the definition of their control groups. There are also conflicting reports regarding precise involvement of the ECS in endometriosis. Future research with larger numbers, strict inclusion and exclusion criteria and detailed clinical information is imperative.

Contents

* Corresponding author at: Teaching & Research, Level 6, Ned Hanlon Building, Royal Brisbane and Women's Hospital, Butterfield St, Herston, QLD 4029, Australia.
E-mail address: a.amoako@uq.edu.au (A.A. Amoako).

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Introduction

Endometriosis is defined as the presence of endometrial-like tissue outside the uterus and it is accompanied by a chronic inflammatory reaction. The prevalence of endometriosis is 10–15 % among women of reproductive age, and it often causes symptoms such as dysmenorrhoea, pelvic pain, dyspareunia, dyschezia and infertility [1]. It significantly impacts the families, partners and carers of those with endometriosis, as well as the social and economic participation, physiological, mental and psychological health of those affected [2,3].

The pathogenesis of endometriosis remains unclear. Several theories including the Sampson's theory of retrograde menstruation, coelomic metaplasia, differentiation of extrauterine/progenitor cells originating from bone marrow, and lymphatic or haematogenous dissemination have been proposed [4]. The importance of hormones on endometriosis is well established, as oestrogen and progesterone regulate the growth of endometrial tissue by stimulating and inhibiting cell proliferation respectively [4–6]. However it is likely that aetiopathogenesis of endometriosis is multifactorial involving hormonal, genetic, biological and environmental factors [7].

Currently, available treatments are not always effective and recurrence following treatments is common [8]. The first-line medical treatment such as combined oral contraceptives or progestins is generally safe, effective and well tolerated, but approximately 25 % of women will require intervention because of inadequate treatment response or intolerance to adverse effects [9]. Other medications including gonadotropin-releasing hormone (GnRH) analogues, danazol and gestrinone may cause major adverse effects substantially impacting on the quality of life [9]. An ideal medical treatment would eliminate endometriotic lesions, prevent recurrence and not impede ovulation with minimum side effect profile. Consequently, novel therapies capable of accomplishing these are required.

In recent years, the endocannabinoid system (ECS) has gained interests in endometriosis research. Endocannabinoids were first discovered in 1992 [10]. Endocannabinoids and their receptors are found through the body: in the brain, lungs, digestive system, connective tissues, hormone releasing glands, skin/hair, bone, the immune system, and the reproductive organs and influence multiple metabolic pathways [11]. In the female genital system, they are located in the endometrium, the myometrium, the ovarian cortex and the medulla and the uterine tubes, and have important roles in menstrual cycle, ovarian maturation, embryo transplantation and implantation [12–19]. Furthermore, the ECS has been shown to affect specific mechanisms critical to endometriosis establishment and maintenance including cell migration, cell proliferation, cell survival and inflammation in other systems [20–22].

Given the potential of the ECS to influence endometriosis pathogenesis, this manuscript aims to summarise previous studies to evaluate if pharmacological therapies targeting the ECS might be considered for the management of endometriosis.

Materials and methods

A systematic search of the PubMed, EMBASE, and Web of Science electronic medical databases was performed. Search terms include those corresponding to endometriosis (endometriosis and endometrioma) combined with terms describing ECS or its components (endocannabinoid, THC, CB1, CB2, AEA, PEA, OEA, MAGL, DAGL, NAPE and FAAH). Original and review articles published in peer-reviewed journals in the English language were identified. No publication date or publication status restrictions were imposed. A manual cross-reference search of the eligible papers was performed to identify additional relevant articles.

Results

The endocannabinoid system

The ECS is made up of endogenous phospholipid-based ligands, their molecular targets (two well-characterized G-protein–coupled cannabinoid receptors, CB1 and CB2), synthetic and degradation enzymes, and protein transporters [23]. Endocannabinoids bind to the same receptors as the principal biologically active component of Cannabis sativa, Δ9-tetrahydrocannabinol (Δ9-THC). The most well characterised endocannabinoids are anandamide (AEA) and 2-arachdonoylglycerol (2-AG), and they act primarily at G-protein-coupled cannabinoid receptors CB1 and CB2 (Table 1) [24,25]. The biological effects of AEA and 2-AG are terminated by cellular uptake via a putative endocannabinoid membrane transporter, followed by enzymatic degradation. Endocannabinoids are synthesised on demand from phospholipid precursors and are not stored [23]. AEA is produced via at least four separate pathways with the most prominent via cleavage of its precursor N-acyl-phosphatidylethanolamine (NAPE) into AEA and phosphatidic acid by N-acyl-phosphatidyl

Table 1
The components of the endocannabinoid system and related molecules.

Endocannabinoids	Receptors	Synthesising enzymes	Degrading enzymes
AEA	CB1, CB2, TRPV1, GPR55	NAPE-PLD	FAAH
2-AG	CB1, CB2	DAGL	MAGL, FAAH
Structural analogues of endocananbinoids			
OEA	PPAR, GPR119	NAPE-PLD	MAGL, FAAH
PEA	PPAR, GPR55	NAPE-PLD	MAGL, FAAH

AEA: anandamide; TRPV1: transient receptor potential vanilloid type 1; NAPE-PLD: N-acyl-phosphatidyl ethanolamine-specific phospholipase D; FAAH: fatty acid amide hydrolase; 2-AG: 2-arachdonoylglycerol; DAGL: sn-1-diacylglycerol lipase; MAGL: monoacylglycerol lipase; OEA: N-oleoylethanolamine; PPAR: Peroxisome proliferator activator receptor; PEA: N-palmitoylethanolamine.

ethanolamine-specific phospholipase D (NAPE-PLD) (Fig. 1) [26,27]. 2-AG is also released on demand after conversion of diacylglycerol by a sn-1-diacylglycerol lipase (DAGL) [28]. Both AEA and 2-AG are degraded through the action of specific enzymes. AEA is predominantly metabolised to arachnidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH) [29]. 2-AG is predominantly degraded by monoacylglycerol lipase (MAGL) and to a lesser extent by FAAH [30,31].

Structural analogues of endocannabinoids with low affinities for cannabinoid receptors such as N-oleoylethanolamine (OEA) and N-palmitoylethanolamine (PEA) have been identified. These compounds produce an "entourage effect" through being alternative substrates for FAAH and MAGL and thereby increasing the potency of endocannabinoids [32,33].

In addition to the established cannabinoid receptors CB1 and CB2, two putative CB receptors (GPR55 and GPR119) have been identified [34]. Certain cannabinoid ligands interact with GPR55 with high affinity [35–37] while GPR119 can recognise OEA and PEA [35].

The transient receptor potential vanilloid type 1 (TRPV1) and transient receptor potential ankyrin-type1 (TRPA1) receptors are structurally related ligand-gated Ca^{2+} permeable ion channels that are considered as an integral part of the ECS [38]. They are molecular integrators of a broad range of inflammatory stimuli including prostaglandins and AEA and play crucial roles in pain and inflammation [39]. Cannabinoid and TRPV1 receptors are often found in the same organs, tissues and cells, where they can have opposing or similar functions [40,41].

The endocannabinoid system in the endometrium

The ECS components are widely distributed in the human endometrium throughout the menstrual cycle [19] and the uterus is known to contain the highest concentrations of AEA within the reproductive tract [42]. CB1 receptor immunoreactivity has been reported to be more intense in the glandular epithelium compared with the stroma and its expression in the glands was not regulated throughout the menstrual cycle [19]. However, these findings are in contrast with other studies which have reported an upregulation of CB1 receptor mRNA and protein during the secretory phase in endometrial samples obtained from women without endometriosis [43,44].

CB2 receptor expression in the glandular cells is significantly higher in the proliferative phase compared to the secretory phase, while its expression is similar in the stromal cells between the two phases in women without endometriosis or adenomyosis [45].

The enzyme NAPE-PLD is upregulated in the menstrual, early-proliferative and late secretory glands with its lowest levels in the early secretory phase, and this enzyme is also found in the stroma [19]. Glandular expression of the enzyme FAAH is upregulated during the menstrual phase, downregulated in the early proliferative to the mid-secretory phase and then increased during the late-secretory phase, with similar expression in the stroma [19]. The expressions of these two enzymes in the endometrium suggest their critical role in controlling AEA concentration during the menstrual cycle.

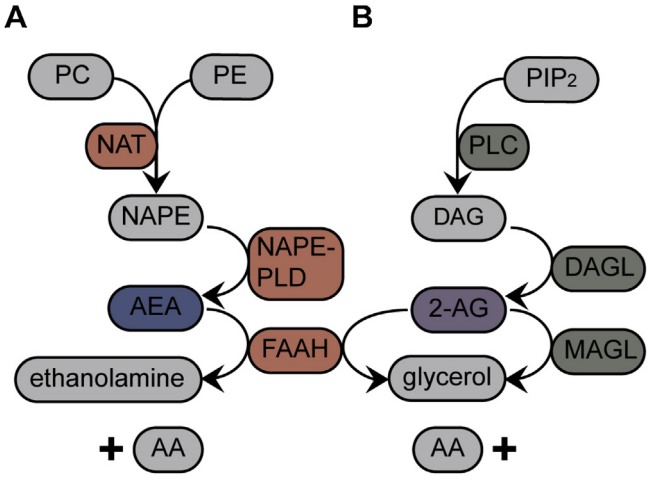

Fig. 1. Primary biosynthetic and degradation pathways of AEA and 2-AG. A) *N*-acyltransferase (NAT) transfers a fatty acyl group derived from the Sn-1 position of phospholipids, such as 1,2-sn-di-arachidonyolphosphatidylcholine (PC), to the primary amino group of phosphatidylethanolamine (PE) to form *N*-acyl-phosphatidylethanolamine (NAPE). NAPE is catalysed by NAPE-hydrolysing phospholipase D (NAPE-PLD) to form *N*-arachidonoylethanolamine (AEA). AEA degradation to ethanolamine and arachidonic acid (AA) occurs primarily through hydrolysis of by membrane-bound fatty acid amide hydrolase (FAAH). B) Phosphatidylinositol-4,5-bisphosphate (PIP2) is hydrolysed to diacylglycerol (DAG) by phospholipase C (PLC). DAG in turn is converted to 2-arachidonoylglycerol (2-AG) through the action of one of two diacylglycerol lipase (DAGL) isozymes, DAGLα and DAGLβ. Degradation of 2-AG to glycerol and AA occurs primarily through monoacylglycerol lipase (MAGL) and to a lesser extent FAAH.

The endocannabinoid system in eutopic endometrium in women with endometriosis

CB1 mRNA and protein are lower in the endometrial tissue from women with endometriosis compared to controls, regardless of the cycle phase [43]. This is in contrast with another study reporting no difference in CB1 receptor expression during the proliferative phase between patients with and without endometriosis [44].

There are no reports of significant difference in the expression of NAPE-PLD and FAAH in the endometrium of patients with and without endometriosis throughout the menstrual cycle [44]. TRPV1 receptor expression in the endometrium of women with and without endometriosis are similar throughout the menstrual cycle [44]. However, eutopic endometrium of subset of patients with deep infiltrating endometriosis shows high levels of TRPV1 receptor mRNA compared to those without endometriosis [39].

The endocannabinoid system in ectopic endometrium (endometriotic tissue)

It has been reported that the CB1 and CB2 receptors are equally present in the epithelial and stromal cell lines derived from eutopic endometrium and deep infiltrating endometriotic nodules [46]. This is in contrast with a more recent study reporting that CB1 and CB2 receptor levels were significantly lower in endometriotic or adenomyotic tissue compared to eutopic endometrium obtained from those without endometriosis or adenomyosis [45]. Furthermore FAAH and NAPE-PLD levels decreased in endometriotic and adenomyotic tissues compared to the control, suggesting that synthesis and degradation of AEA become concomitantly slower in both epithelial and stromal cells during disease pathogenesis, and authors argued that FAAH enzyme rather than the CB1 or the NAPE-PLD enzyme regulates endocannabinoid activity [45]. MAGL and DAGL RNA and protein expression is decreased in glandular and stromal cells in endometriosis and adenomyosis group compared to that of the control group [45].

There was a remarkable elevation of TRPA1 mRNA expression in the ectopic endometrium of rectosigmoid deep infiltrating endometriosis lesions [39]. Furthermore significantly elevated TRPV1 receptor mRNA level was detected in both ectopic and eutopic endometrium of women with endometriosis [39]. Local inflammation and sensory neural sprouting play a key role in the pathogenesis of endometriosis-related pain, which is mediated by a broad range of pro-inflammatory molecules [47,48]. These stimulate TRPV1 and TRPA1 activity both on sensory nerve

terminals and non-neural structures, which in turn further trigger the pain. TRPA1 and TRPV1 expressions were shown to have correlations with the severity of pain symptoms, including dysmenorrhoea, dyspareunia and dyschezia [39].

The endocannabinoid expression at the systemic level in women with endometriosis

Plasma levels of endocannabinoids AEA, 2-AG and OEA are significantly higher in the secretory phase compared to the proliferative phase in patients with endometriosis. In the secretory phase, plasma AEA and OEA levels were also significantly higher in patients with endometriosis compared with controls. In contrast, in patients without endometriosis, there is no difference in the plasma levels of AEA, 2-AG, OEA and PEA between the proliferative and secretory phase [44]. The increased systemic levels of AEA detected in women with endometriosis during secretory phase could be due to higher NAPE-PLD, lower FAAH levels or reduced degradation of AEA, however there was no significant difference in the transcript levels of NAPE-PLD and FAAH in women with endometriosis compared to controls across the menstrual cycle [44].

Endocannabinoid receptor signalling actions and consequences in endometrial tissue: cell migration, cell proliferation, and apoptosis

The precise pathogenesis of endometriosis is unknown, but mechanisms that allow endometrial cells to exit the uterine cavity, implant and proliferate have been hypothesized. Therefore, cell motility, adhesion, proliferation and apoptosis are cellular behaviours especially relevant to endometiosis.

Synthetic endocannabinoid, methanandamide, stimulates endometrial stromal cell migration in a dose-dependent manner via CB1 and not via CB2 as indicated by the use of CB1 selective antagonist AM251 (Table 2) [49]. This effect was mediated through ERK1/2 and PI3K/Akt pathways as selective inhibitors of both pathways can prevent the stimulatory effect of methanandamide (Fig. 2) [49]. An effect of endocannabinoids on endometrial cell migration has through a CB1-independent mechanism [50]. Using the CB1 antagonist (SR141716A) and CB2 antagonist (SR144528) and the GPR18 antagonist (CBD) it was shown that SR144528 and CBD significantly attenuated the response to AEA, suggesting a signalling mechanism mediated through CB2 and GPR18 [50].

in vitro treatment of stromal endometriotic cells with cannabinoid agonist (WIN 55212-2) decreased cell proliferation associated with the inhibition of Akt suggesting cannabinoid agonists exert anti-proliferative effects on endometriotic stromal cells through the Akt pathway [46]. The *in vivo* effects of cannabinoid agonist WIN 55212-2 were evaluated on nude mice implanted with human deep infiltrating endometriotic nodules. WIN 55212-2 abrogated the growth of endometriotic tissue implanted in nude mice, suggesting the beneficial effects of cannabinoid agonists on deep infiltrating endometriosis have been confirmed *in vivo* [46].

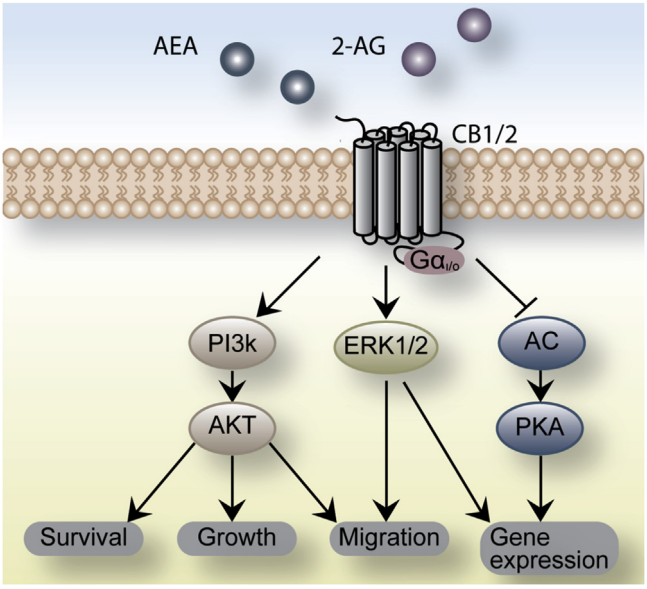

Fig. 2. Major signalling pathways of the cannabinoid receptors. 2-arachidonoylglycerol (2-AG) or *N*-arachidonoylethanolamine (AEA) signalling at the cannabinoid receptors (CB1/2) results in activation of the associated Gαi/o subunit. This is associated with downstream activation of phosphatidyl inositol 3 kinase (PI3k) / protein kinase B (AKT) and extracellular signal-regulated kinase1/2 (ERK1/2) pathways and decreased protein kinase A (PKA) activity via inhibition of adenyl cyclase (AC). These pathways affect cell survival and apoptosis, growth, migration and gene expression.

The apoptotic cell index between endometriotic and adenomyotic patients and age-matched controls decreased significantly compared to the control group [45]. CB1 and CB2 agonist mediated dose-dependent, fast anti-proliferative and pro-apoptotic effects, suggesting endocannabinoids increase apoptosis in endometriosis and adenomyosis, and CB1 and CB2 antagonists can be considered as potential therapeutic agents for these conditions.

The endocannabinoid system and inflammation in endometriosis

Local inflammation plays a key role in the pathogenesis of endometriosis and endometriosis-related pain, which is mediated by a broad range of pro-inflammatory molecules. Mast cells produce and release a variety of degranulation products, such as nerve growth factor, which may interact with nociceptive

Table 2
Endocannabinoid receptor signalling actions and consequences in endometrial tissue.

Cell action	Authors Publication year	Number of samples	ECS / pathways involved	Comments
Migration	Gentilini et al 2010	40 women undergoing gynaecological laparoscopy	CB1 ERK1/2 pathway	Synthetic endocannabinoid stimulated endometrial cell migration in a dose-dependent manner
	McHugh et al 2010	Human endometrial cell line	CB2 GPR18	CB2 antagonist and GPR18 antagonist significantly attenuated the response to AEA
Proliferation	Leconte et al 2010	14 women with DIE	Cannabinoid agonist Akt pathway	Cannabinoid agonist decreased endometrial cell proliferation
Apoptosis	Bilgic et al 2017	20 women with endometriosis	CB1&2	CB1 and CB2 agonist mediated dose dependent pro-apoptotic effects
Inflammation	Iuvone et al 2008	15 women with endometriosis	CB2	Activation of CB2 is associated with the nitric oxide release process in endometrial inflammation

ECS: Endocannabinoid system, DIE: Deeply infiltrating endometriosis.

neurons causing their activation or sensitisation [51,52]. The presence of increased activated and degranulating mast cells in deeply infiltrating endometriosis, and the close histological relationship between mast cells and nerves strongly suggest that mast cells could contribute to the development of pain and hyperalgesia in endometriosis [53].

In a study analysing CB1 mRNA and protein expression in human endometrial tissues and mRNA expression in isolated stromal cells after exposure to a substance triggering inflammation or a progesterone receptor antagonist, the loss of CB1 was associated with inflammation suggesting an anti-inflammatory action of progesterone via CB1 [43]. It was shown that cannabinoid receptors were present in inflammatory endometrial tissue, and selective activation of CB2 was associated with the nitric oxide release process existing in endometrial inflammation [54].

PEA may have an effect on inflammation by inhibiting the activity of the pro-inflammatory enzymes such as COX, eNOS, and iNOS [55] and by reducing mast cell activation in mice studies [56].

TRPV1 and TRPA1 receptors are important molecular integrators of a broad range of inflammatory stimuli (protons, bradykinin, prostaglandins, lipoxygenase products, anandamide, nitric oxide, hydrogen peroxide, formaldehyde, methylglyoxal, acrolein and reactive oxygen species (ROS)) and play crucial roles in pain and inflammation (Fig. 3) [39].

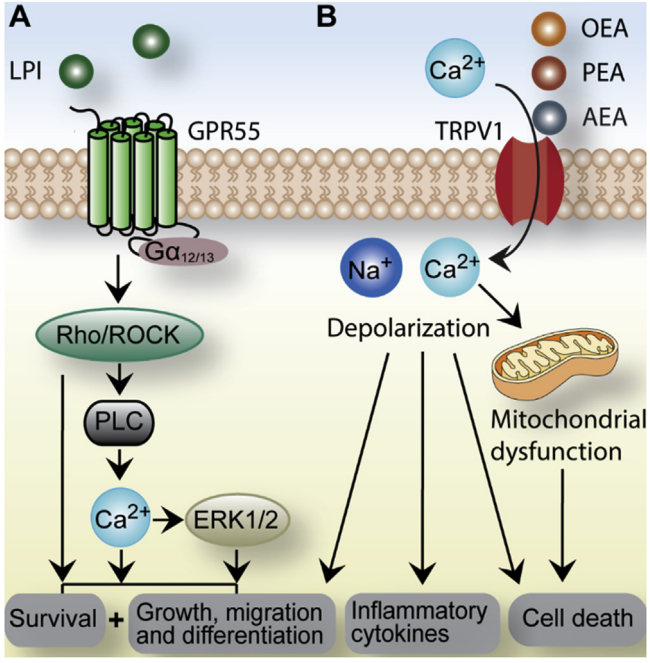

Fig. 3. Major signalling pathways of GPR55 and TRPV1. A) Lysophophatidylinositol (LPI), an intermediate of 2-AG biosynthesis, is the main endogenous ligand for GPR55 which couples to Gα12/13 and signals through ras homolog gene family member A (RhoA)/ Rho-associated protein kinase (ROCK)/phospholipase C (PLC) pathways. RhoA/ROCK signalling results in Ca^{2+} release from intracellular stores. Increased intracellular Ca2+ results in phosphorylation and activation of extracellular-regulated protein kinase (ERK). These pathways affect cell survival, growth, migration and differentiation. B) *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA) and *N*-arachidonoylethanolamine (AEA) can activate the transient receptor potential cation channel subfamily V member 1 (TRPV1). When activated with a classical agonist, such as capsaicin, TRPV1 causes an intracellular influx of Ca^{2+} and Na^{+} and consequently membrane depolarization. This directly affects cellular proliferation, migration and release of inflammatory cytokines and potentially cell death secondary to oxidative stress. Persistently increased intracellular Ca^{2+} can also induce mitochondrial dysfunction.

The endocannabinoid system and endometriosis-related pain, quality of life and sexual function

PEA is a shorter and fully saturated analogue of AEA, and is an endogenous agonist of CB1. PEA accumulates during inflammation and processes a number of anti-inflammatory actions and reduces mast cell degranulation [57]. It has been reported to exert anti-inflammatory and analgesic effects in both acute and neuropathic pain conditions [58–60]. The presence of increased activated and degranulating of mast cells in deeply infiltrating endometriosis, and the close histological relationship between mast cells and nerves strongly suggest that mast cells could contribute to the development of pain and hyperalgesia in endometriosis [53].

There have been a number of studies and a meta-analysis investigating the effects of PEA on endometriosis pain symptoms and quality of life (Table 3) [61–66]. PEA in combination with polydatin, a phytoalexin polyphenolic compound that down-regulates inflammation, significantly decreased endometriosis-related dysmenorrhoea, dyspareunia and pelvic pain compared to placebo after 3 months [62]. Furthermore PEA with polydatin was found to be as effective as hormonal therapies including leuprorelin acetate (synthetic analogue of GnRH) and ethinylestradiol + drospirenone in reducing endometriosis related pain symptom without the anti-ovulatory effects [64]. A recent meta-analysis evaluating clinical effectiveness of PEA with polydatin in reducing endometriotic chronic pelvic pain concluded that it resulted in clinically relevant improvement of chronic pelvic pain and dysmenorrhoea while improving deep dyspareunia to a limited degree [66].

PEA with transdatin resulted in significant improvement in both mental and physical component of the SF-12 quality of life questionnaire, while two hormonal therapy groups resulted in significant improvement only in the physical component [64]. A prospective study with 56 women assessed the effects of PEA with transdatin on pain, quality of life and sexual function using the visual analogic scale (VAS), quality of life questionnaire (SF-36), Female Sexual Function Index (FSFI), and the Female Sexual Distress Scale (FSDS) and reported improvement in pain symptoms, all categories of the SF-36 quality of life questionnaire and FSFI and FSDS score by the 6th and 9th month [65].

The endocannabinoid system and sex steroid hormones

The role of progesterone

Progesterone is a steroid hormone produced predominantly after ovulation by the corpus luteum and exerts its primary action through the intracellular progesterone receptor. Exposure to progesterone is recognised as protective against the development of endometriosis [67], and studies suggest endometriosis results in reduced progesterone expression [67–69].

Effect of progesterone on NAPE-PLD expression in the uterus was examined using ovariectomised mice. It was reported that progesterone down-regulates uterine NAPE-PLD expression, possibly leading to a decrease in AEA levels [70] although no correlation has been established between AEA and progesterone in normal cycling women [14].

The relationship between progesterone and ECS in the endometrium of women with endometriosis has been explored. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) was shown to cause progesterone-resistant cellular phenotype as a consequence of reduced progesterone receptor expression in adult endometrial stromal cells acquired from disease-free women [68]. The expression of CB1 mRNA and protein is increased during the progesterone-dominant secretory phase in healthy human endometrium, but the expression was minimal in the endometrial tissue obtained from women with endometriosis regardless of the

Table 3
Effect of palmitoylethanolamide on symptoms of endometriosis.

Authors Publication year	Number of women treated with PEA	Treatment	Results / Conclusions
Indraccolo 2010	4 women	PEA 400 mg and transpolydatin 40 mg, BD, 90 days	Pain relief as early as 1 month Reduction in the analgesic drugs requirement
Cobellis et al 2011	21 women	PEA 400 mg and transpolydatin 40 mg, BD, 3 months	A marked decrease in dysmenorrhoea, dyspareunia and pelvic pain
Giugliano 2013	47 women	PEA 400 mg and transpolydatin 40 mg, BD, 90 days	Pain intensity decreased significantly
Di Francesco et al 2014	10 women	PEA 400 mg and transpolydatin 40 mg, BD, 6 months	Pain symptom intensity significantly decreased As effective as hormonal therapy in reducing pain Quality of life (both physical and mental component) scores improved significantly compared to baseline
Caruso et al 2015	56 women	PEA 300 mg and α-lipoic acid 300 mg, BD, 9 months	Pain symptoms and quality of life improved significantly by the 6th month
Indraccolo et al 2017	Meta-analysis	PEA 400 mg and transpolydatin 40 mg, BD, 3 months	Clinically relevant improvement of chronic pain and dysmenorrhoea Deep dyspareunia was improved to a limited degree

PEA: Palmitoylethanolamide, BD: Twice a day.

cycle phase, suggesting that endometriosis patients exhibit alterations in cannabinoid responsiveness [43].

Progesterone treatment of the proliferative endometrial stromal cells led to a significant increase in expression of CB1 mRNA, and progesterone receptor antagonist (onapristone) largely prevented this effect. The use of proliferative phase stromal cells without a prior exposure to progesterone *in vivo* coupled with the use of onapristone to block progesterone action *in vitro* confirm progesterone's involvement in regulating CB1 expression in human endometrial stromal cells (Fig. 4) [43].

The role of oestrogen

The effects of oestrogen on cell migration was evaluated using human endometrial cancer cell lines, and it was found that cell migration was enhanced by oestrogen, but not modified by progesterone [71,72]. This capability of human endometrial cells to migrate in response to oestrogen relates to the oestrogen-dependence of endometriosis [5].

Effect of oestrogen on NAPE-PLD expression in the uterus was examined using ovariectomised mice. It was reported that oestrogen down-regulates uterine NAPE-PLD expression, suggesting that it induces a decrease in AEA levels [70]. A separate study also involving ovariectomised mice reported that oestrogen significantly decreased activity of FAAH compared with untreated controls [73].

The highest plasma AEA levels were measured at ovulation and the lowest level was measured in the late luteal phase, and there was a statistically significant positive correlation between AEA and oestradiol level, suggesting it may be involved in the regulation of AEA levels [14].

Comment

Significant differences in the concentrations and expressions of the components of the ECS were reported in the eutopic and ectopic endometrium, and the systemic circulation of women with

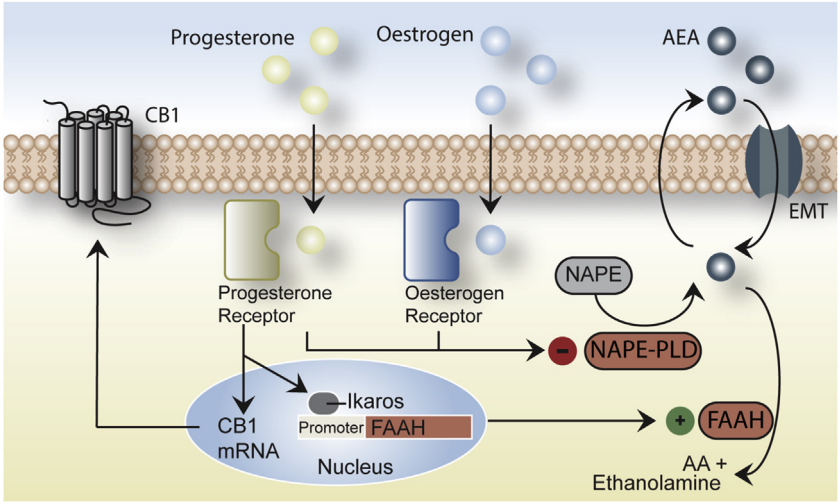

Fig. 4. Modulation of ECS by sex steroid hormones. Termination of AEA signalling is thought to occur through cellular uptake of *N*-arachidonoylethanolamine (AEA) via the putative endocannabinoid membrane transporter (EMT) and subsequent degradation to AA (arachidonic acid) and ethanolamine by fatty acid amide hydrolase (FAAH). Progesterone and signalling increases FAAH activity through up-regulation of the FAAH promoter via the transcription factor Ikaros. Progesterone and oestrogen signalling downregulates *N*-acyl-phosphatidylethanolamine- hydrolysing phospholipase D (NAPE-PLD) expression and consequently AEA production. Progesterone signalling increases cannabinoid receptor 1 (CB1) mRNA expression.

endometriosis compared to controls. Although there are conflicting reports regarding the role of the ECS in endometriosis, endometriosis appears to be associated with downregulation of CB1 receptors and upregulation of TRPV1 receptors.

The importance of downregulation of CB1 receptors in endometriosis is highlighted by the study reporting the association between the loss of CB1 and inflammation suggesting an anti-inflammatory action of progesterone via CB1. Endometriotic lesions are surrounded by inflammation. The association between a reduction in CB1 and inflammation may also suggest that endometriotic lesions are likely to have reduced ECS expression. On the other hand, if progesterone response is down regulated in ectopic tissue it may result in decrease in ECS activity. A developed progesterone resistance in endometriotic lesions could influence the endocannabinoid responsiveness. This would mean that treatment might be relevant for certain lesions/people and the potential for personalised medicine.

The finding of upregulation of TRPV1 receptors in endometriosis seems particularly relevant, as endometriosis is characterised by its inflammation and associated pain symptoms and TRPV1 receptors are known to play important roles in pain and inflammation. Poor association between a wide range of phenotypes or pain symptoms and the severity of the disease on laparoscopy may be due to the TRPV1 activity.

It is worthwhile noting that the ECS is significantly influenced by genetic variants [74] with a suspected genetic influence on the ECS contribution to neurological development [75]. Analysis of data from the Gtex portal indicates that expression quantitative trait loci (eQTL), genetic variants that occur in the population across the whole genome, exist for crucial genes in the ECS in a number of different tissue. Moreover, studies have shown variation in the occurrence of side effects for exogenous compounds may occur depending on the genetic variants in FAAH enzyme [76].

Further research with pre-defined strict inclusion and exclusion criteria ensuring the details of patients' characteristics and clinical information is required to investigate the exact roles of the ECS in endometriosis. There is a reasonable body of evidence supporting the efficacy of PEA in managing endometriosis-related symptoms. More *in vivo* studies need to be conducted to have a clinical value, and CB1 and TRPV1 may be a potential target for future molecular therapy. Finally, as the ECS is implicated throughout the human body, the development of biomarkers to identify suitable patients predicting the maximum benefit and the low side effect profile may be imperative.

Declaration of Competing Interest

We declare we have no conflict of interest.

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