

Review Article

Male reproductive health and infertility

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Phytocannabinoids, the Endocannabinoid System and Male Reproduction

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The endocannabinoid system (ECS) is comprised of a set of lipid-derived messengers (the endocannabinoids, ECBs), proteins that control their production and degradation, and cell-surface cannabinoid (CB) receptors that transduce their actions. ECB molecules such as 2-arachidonoyl-*sn*-glycerol (2-AG) and anandamide (arachidonoyl ethanolamide) are produced on demand and deactivated through enzymatic actions tightly regulated both temporally and spatially, serving homeostatic roles in order to respond to various challenges to the body. Key components of the ECS are present in the hypothalamus-pituitary-gonadal (HPG) axis, which plays critical roles in the development and regulation of the reproductive system in both males and females. ECB signaling controls the action at each stage of the HPG axis through CB receptors expressed in the hypothalamus, pituitary, and reproductive organs such as the testis and ovary. It regulates the secretion of hypothalamic gonadotropin-releasing hormone (GnRH), pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH), estrogen, testosterone, and affects spermatogenesis in males. Δ^9 -tetrahydrocannabinol (THC) and other phytocannabinoids from *Cannabis sativa* affect a variety of physiological processes by altering, or under certain conditions hijacking, the ECB system. Therefore, phytocannabinoids, in particular THC, may modify the homeostasis of the HPG axis by altering CB receptor signaling and cause deficits in reproductive function. While the ability of phytocannabinoids, THC and/or cannabidiol (CBD), to reduce pain and inflammation provides promising opportunities for therapeutic intervention for genitourinary and degenerative disorders, important questions remain regarding their unwanted long-term effects. It is nevertheless clear that the therapeutic potential of modulating the ECS calls for further scientific and clinical investigation.

Keywords: Cannabinoids; Endocannabinoids; Male; Reproduction; Spermatogenesis; Therapeutics

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INTRODUCTION

The prevalence of cannabis use in the United States increases every year [1,2], and it is likely to keep growing as the availability of cannabis-based products continues to increase and risk perception by the general public of their adverse effects is lowered [3-5]. In particular, recent approval of the cannabidiol (CBD) medi-

cine, Epidiolex[®] (GW Pharmaceuticals, Cambridge, UK), by the U.S. Food and Drug Administration (FDA) for the treatment of seizures associated with severe forms of epilepsy further accelerated the use of this non-psychoactive cannabinoid [5-9]. Furthermore, off-label uses of CBD from both physician's recommendation and self-treatment are increasing [6-9].

Initial research on the plant *Cannabis sativa* (mari-

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juana) focused on understanding its toxicity to humans because it was considered an addictive, illegal, and only harmful psychotropic drug without medical benefits; however, thanks to recent research we now have a much better understanding of the plant [10-13]. Δ^9 -tetrahydrocannabinol (THC), the main psychoactive and intoxicating substance of cannabis, was first described in the 1940s [14,15], and fully characterized in 1964 by precisely defining the structure to be (-)-*trans*- Δ^9 -tetrahydrocannabinol [16]. Two isoforms of cannabinoid (CB) receptors were discovered, cloned, and determined to be the direct bodily target proteins for THC in the 1990s [17-19]. THC activates and under certain conditions hijacks/over-stimulates CB receptors to exert its various pharmacological effects [10]. Interestingly, research later revealed the existence of unique lipid neurotransmitters that are produced by the body that serve as agonists for CB receptors: the endocannabinoids (ECBs) [12]. In addition, the biosynthetic and metabolizing pathways regulating local levels of ECBs have been characterized [10,13]. Research suggests that the endocannabinoid system (ECS) is a complex but essential signaling system found in most body organs where it serves regulatory roles for many biological functions including control of emotions, learning and memory, regulation of food intake and energy metabolism, regulation of body temperature, pain reception, immune response and inflammation, and maintaining body homeostasis.

Research during the last two decades also proposed that the ECS is a key modulator for reproductive functions in males and females. In this mini-review, we provide a brief overview of current knowledge about the ECS in the male reproductive system. Most, if not all, molecular components of the ECS are present in the male reproductive system, where ECB signals are thought to control the homeostasis of the hypothalamus-pituitary-gonadal (HPG) axis and critical testicular physiology, including spermatogenesis and the functions of Leydig and Sertoli cells [20-23]. Then, we discuss the potential therapeutic utility of CBs in treating male genitourinary disorders, but also their possible side effects on male reproduction.

THE ENDOCANNABINOID SYSTEM

The ECS is comprised of two G protein-coupled cell-surface receptors, CB₁ and CB₂, two lipid-derived ECB

molecules — arachidonoyl ethanolamide (anandamide, AEA) and 2-arachidonoyl-*sn*-glycerol (2-AG) — and proteins involved in the formation, transport, and deactivation of ECB molecules [10] (Fig. 1). The activation of the ECS is regulated through the expression of CB₁ and CB₂ receptors and their coupling with intracellular signaling pathways, as well as temporal and local changes in the concentration of ECB molecules, which are produced on demand through cleavage of distinct phospholipid precursors.

1. Cannabinoid receptors

CB receptors are found in the central nervous system (CNS) and various peripheral organs where they serve important regulatory functions in synaptic plasticity, signal transduction, and inflammation [24]. In humans, the CB₁ receptor is encoded by the *CNR1* gene whereas the *CNR2* gene encodes CB₂ receptors. CB₁ and CB₂ receptors are highly homologous, sharing 48% identity in amino acid sequence. Both receptors signal through the transducing G proteins, G_i and G_o [25,26]. The CB₁ receptor is one of the most abundantly expressed receptors in the brain and mainly localized to the presynaptic axon terminals of both excitatory glutamatergic and inhibitory γ -amino-butyric acid (GABA)-ergic neurons [26]. As expected from their subcellular localization, CB₁ receptors regulate neural activities by controlling synaptic transmission mediated by well-known classical neurotransmitters, such as glutamate and GABA. In the neuronal presynaptic axons, activation of CB₁ receptors inhibits Ca²⁺ channel activity to reduce neurotransmitter release, and elevates K⁺ channel activity to suppress membrane excitability [10,26,27]. In addition, neuronal activity, such as elevation of intracellular calcium concentration in the postsynaptic spines, may trigger temporal biosynthesis of ECB molecules, which acts retrogradely on presynaptic CB₁ receptors across the synaptic cleft to modulate neurotransmitter release. Therefore, it was proposed that ECB signaling serves as a prompt negative feedback mechanism for synaptic activities, *i.e.*, a synaptic circuit breaker [27]. Other brain cells, such as astrocytes and microglia, also express CB₁ receptors [28-30], but their precise functions need to be elucidated further. Outside the CNS, CB₁ receptors are expressed in the peripheral nervous system, including liver, pancreas, small intestine, and skeletal muscle [24,31] and have been linked to diverse influences exerted by ECB messengers to maintain bodily

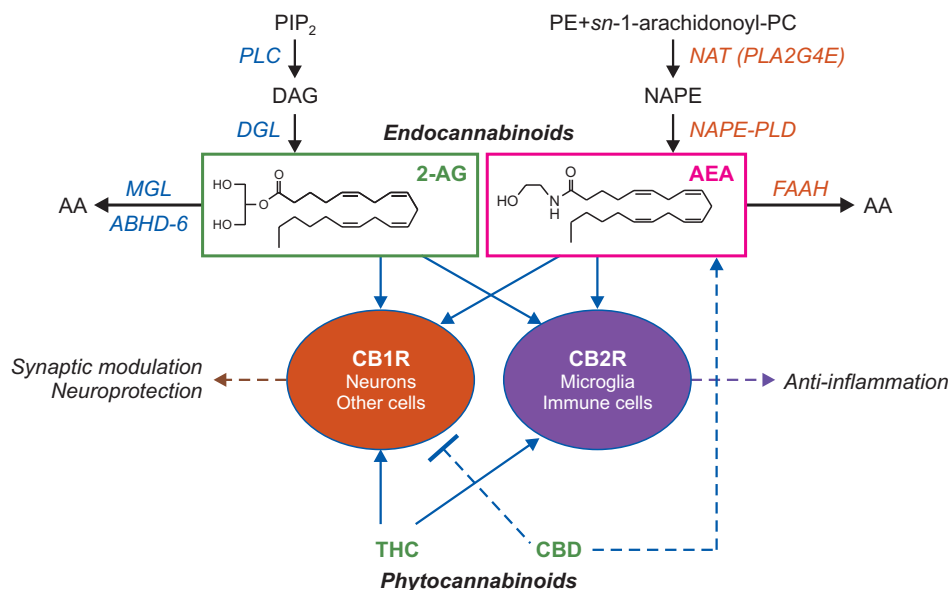


Fig. 1. Simplified overview of the endocannabinoid (ECB) system. The ECB system is comprised of the CB₁ and CB₂ cannabinoid receptors (CB1R and CB2R), the endogenous ligands for CB receptors, anandamide (arachidonoyl ethanolamide, AEA) and 2-arachidonoyl-*sn*-glycerol (2-AG), and proteins involved in the biosynthesis and inactivation of ECBs. Receptor-operated phospholipase C (PLC) converts phosphatidylinositol-4,5-bisphosphate (PIP₂) into 1,2-diacylglycerol (DAG). DAG is hydrolyzed by diacylglycerol lipase (DGL) forming 2-AG. 2-AG is subjected to hydrolytic cleavage catalyzed by monoacylglycerol lipase (MGL) or, to a lesser extent, α,β -hydrolase domain-containing protein 6 (ABHD-6). The biosynthesis of AEA starts from the production of *N*-arachidonoyl-phosphatidylethanolamine (NAPE), through the transfer of an arachidonate group from the *sn*-1 position of 1,2-diarachidonoyl-phosphatidylcholine (PC) to the free amino group of phosphatidylethanolamine (PE). NAPE is converted to AEA, catalyzed by a unique phospholipase D (PLD). AEA is degraded by the intracellular serine amidase, fatty acid amide hydrolase (FAAH). These ECB molecules bind and activate both CB1R and CB2R, which are also targeted by exogenously administered phytocannabinoids.

homeostasis [32], such as control of lipogenesis in the liver [33].

The CB₂ receptor is mainly found in cellular constituents of the immune systems—including monocyte-derived cells and lymphocytes [34]. CB₂ receptor signaling mainly plays a role in regulating inflammation, cytokine release, cell migration, and apoptosis. It is of note that the psychoactive properties of cannabis, an unwanted side effect when used for medical purposes, are mediated by the CB₁ receptor in the CNS, but not by the CB₂ receptor, making it a particularly appealing target for drug development.

Although only limited information is available, the presence of CB receptors in the reproductive system has been reported and their functional importance has been proposed. In humans, protein expression of both isoforms of CB receptors has been detected, *albeit* at a low level, in post-meiotic germ, Leydig, and peritubular cells [35]. Transcripts encoding both isoforms of the CB receptors were also found in germ cells, and their differential distribution has been noted [35].

2. Endocannabinoid molecules

While THC and other synthetic CB receptor agonists hijack the endogenous CB receptor-mediated signaling to exert their pharmacological effects, ECB molecules serve homeostatic roles through activating CB receptors to respond to various challenges to the CNS and the periphery. To achieve this, the production and deactivation/hydrolysis of ECB molecules is tightly controlled through precise enzymatic actions [10,13].

AEA and 2-AG are the two best-characterized ECB molecules found in mammalian tissues [36-38]. During the 1990s, the first ECB molecule was discovered and named ‘anandamide’ after the Sanskrit word ‘nanda’, meaning ‘happiness’ [36]. Later, it was further discovered that another lipid molecule, 2-AG, which is present in large amounts in the brain, also binds and activates CB receptors to play a major role in neuronal synapses [37,38].

As shown in Fig. 1, AEA formation starts with the transfer of an arachidonate group from the *sn*-1 position of 1,2-diarachidonoyl-phosphatidylcholine to the free amino group of phosphatidylethanolamine (PE), which produces the AEA precursor *N*-arachidonoyl-

PE (NAPE) [39,40]. This reaction is catalyzed by the calcium-dependent *N*-acyl transferase (NAT) activity of an isoform of phospholipase A₂, PLA2G4E [41]. Hydrolytic cleavage of NAPE by an isoform of phospholipase D (PLD), the NAPE-PLD, produces AEA [42,43]. After biosynthesis, AEA diffuses out of the cell into the external milieu and activates CB receptors. AEA is deactivated through internalization into cells followed by intracellular hydrolysis catalyzed by the serine amidase fatty acid amide hydrolase (FAAH) [44] (Fig. 1).

Like AEA, 2-AG is also produced upon demand (Fig. 1). The membrane phospholipid that serves as its precursor, phosphatidylinositol-4,5-bisphosphate (PIP₂), is first hydrolyzed by phospholipase C (PLC), probably PLC-β and/or PLC-ε [45,46], to produce 1,2-diacylglycerol (DAG). DAG is then cleaved by the α or β isoform of diacylglycerol lipase (DGL or DAGL) to generate 2-AG [47-49]. In excitatory glutamatergic neurons of the brain, PLC and DGL-α are physically and functionally linked to type-5 metabotropic glutamate receptors in a multimolecular complex (the 'endocannabinoid signalosome') that enables efficient retrograde signaling from the postsynaptic dendritic spine to the axon terminal [49]. 2-AG is inactivated by enzymatic activities of the lipid hydrolases, monoacylglycerol lipase (MGL or MAGL) and, to a lesser extent, α/β-hydrolase domain-containing protein 6 (ABHD-6) [50-52] (Fig. 1).

The reproductive organs of mammals contain the entire repertoire of proteins needed to produce and degrade ECB molecules [35,53]. The main biosynthesizing enzymes, DGL and NAPE-PLD, were detected in germ cells and somatic cells, respectively [35,54-56]. In addition, abundant expression of the 2-AG-hydrolyzing enzyme, MGL, was observed in Sertoli cells, whereas the AEA-hydrolyzing enzyme, FAAH, was found in late spermatocytes and post-meiotic germ cells [35]. The presence of ECB molecules was also confirmed in the human testis [35].

PHYSIOLOGICAL ROLES OF ENDOCANNABINOID IN MALE REPRODUCTION

The ECS has been described as a critical modulator in the control of male and female reproduction at multiple stages of the HPG axis through CB receptors distributed in the hypothalamus, pituitary, and reproductive organs such as the testis. Centrally, the ECS affects neuronal

activities of hypothalamic gonadotropin-releasing hormone (GnRH)-secreting neurons and secretion of pituitary hormones, and locally, produces direct effects on the gonads, affecting the synthesis and secretion of sex hormones and spermatogenesis [22] (Fig. 2).

1. The endocannabinoid system in the hypothalamic control of the male hypothalamus-pituitary-gonadal axis

In the brain, ECBs are produced in a neuronal activity-dependent manner in the post-synapses, and their primary role is to control the release of excitatory and inhibitory neurotransmitters by activating CB₁ receptors located at presynaptic axon terminals, serving as

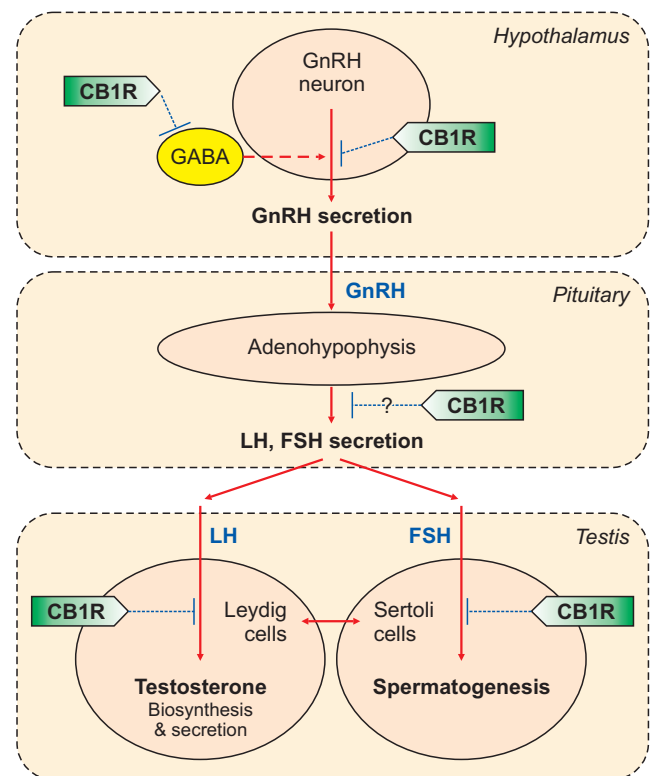


Fig. 2. Endocannabinoid (ECB) signaling in the hypothalamus-pituitary-gonadal (HPG) axis. The HPG axis is a tightly regulated endocrine system, and the gonadotropin-releasing hormone (GnRH), released in a pulsatile manner from the hypothalamus, is the prime modulator of the system. GnRH stimulates the release of pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, which are actively involved in gametogenesis regulation while also driving the synthesis and release of gonadal steroid hormones. The ECB system, through the activation of the cannabinoid CB₁ receptor signaling, is involved in the regulation of the HPG axis at multiple stages: (1) ECBs suppress the release of GnRH in the hypothalamus; (2) reduction of GnRH, in turn, suppresses the release of LH and FSH in the adenohypophysis where CB₁ receptor may play a role; and (3) direct action of ECBs on the Leydig and Sertoli cells reduces testosterone release and modulates spermatogenesis.

retrograde messengers [10-13]. The ECS may operate using a similar synaptic negative-feedback mechanism in the HPG axis. The HPG axis is a tightly regulated endocrine system, and the decapeptide hormone, GnRH, is released in a pulsatile manner from the hypothalamus as the prime modulator of reproduction. GnRH stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, which are actively involved in gametogenesis regulation while also driving the synthesis and release of gonadal steroid hormones [22]. LH up-regulates testosterone secretion in the testis, and high levels of testosterone down-regulates GnRH in the brain to eventually lower the release of LH. The negative feedback loop mechanism of testis on the hypothalamo-pituitary unit has an imperative role in the maintenance of testosterone levels [57].

Research demonstrated a role of the ECS at both the hypothalamus and pituitary level along the gonadal axis [53] (Fig. 2). First, evidence indicates that hypothalamic GnRH neurons produce and secrete at least two different ECBs, 2-AG and AEA [58]. These lipid messengers, in turn, activate hypothalamic CB₁ receptors and inhibit the release of GnRH to regulate diverse functions of GnRH, including the onset of puberty, ovulation, lactational infertility, and menopause [58]. In mice, systemic or intracerebroventricular administration of AEA produced significant reductions in circulating levels of LH and testosterone [59,60]. The effect of AEA on LH secretion was mediated by activation of CB₁ receptors expressed in GnRH neurons, whose activation leads to the inhibition of pulsatile GnRH release [53,59]. Interestingly, an alternative neuronal circuit mechanism by which the ECS may affect GnRH neuron activity by modifying GABAergic synaptic activity has been proposed [61] (Fig. 2). Neuronal GABA is typically an inhibitory neurotransmitter; however, research found that it exerts a paradoxical excitatory effect on mature GnRH neurons [62], and that GABAergic afferent into kisspeptin neurons and/or GnRH neurons is an important positive regulator for the HPG axis [62-66]. Accordingly, ECS-mediated activation of CB₁ receptors, expressed on GABAergic inputs into GnRH neurons, resulted in decreased GnRH neuron firing rate and consequent reduction of GnRH release [58,64]. Therefore, activation of CB₁ receptors in the hypothalamus may inhibit GnRH release either through direct inhibition of GnRH neuronal activity or indirect effects on

the GABAergic activation of GnRH neurons (Fig. 2).

Next, the existence of CB₁ receptors, both the mRNA and proteins, and ECB molecules in the anterior pituitary of rodents has been observed [67-69]. The inhibitory effect of the ECS on hormonal secretion in the anterior pituitary has been also proposed [21]. These results suggest the role of the ECS as a neuromodulator at the pituitary level, but more research is needed to have a clear understanding on the underlying mechanism.

2. Endocannabinoid signaling in spermatogenesis

The presence of complete enzymatic machinery to synthesize and metabolize ECBs has been demonstrated in male reproductive organs [21,35]. Earlier studies have reported the presence of NAPE-PLD and FAAH, the biosynthetic and degradative enzymes for AEA, respectively, in human testis [55]. A recent study also found expression of NAPE-PLD in Leydig cells, Sertoli cells, and round spermatid nuclei, which indicates that these cells synthesize AEA [35]. Therefore, these data suggest a role of the ECS, mainly mediated by AEA, in reproductive regulation of late spermatocytes and spermatids.

Both CB₁ and CB₂ receptors have been found in post-meiotic germ, Leydig, and peritubular cells [35], and their functional relevance has been proposed [53]. In Leydig cells, activation of CB₁ receptors negatively affects testosterone biosynthesis by decreasing Leydig cell responsiveness to LH. Within Sertoli cells, the ECS, specifically the production and degradation of AEA, plays an important role in controlling spermatogenic output by maintaining a balance between the cell's survival and death [53]. Apoptosis *via* transient receptor potential vanilloid 1 (TRPV1) channels is induced through the orchestrated biosynthesis of AEA by NAPE-PLD [70]. This effect is antagonized by FSH, which increases the expression of FAAH through the activation of adenylyl cyclase (AC) and cAMP/protein kinase A (PKA) signaling [70-73]. FSH also triggers the phosphatidylinositol-3-kinase (PI3K) pathway, which in turn induces the expression of aromatase and leads to increased production of estradiol from testosterone [70,72]. Increased levels of estradiol act to an estrogen-responsive element in the *Faah* promoter, resulting in elevation of FAAH protein expression [73].

THERAPEUTIC POTENTIAL OF CANNABINOIDS FOR MALE GENITOURINARY SYSTEM DISORDERS

The *Cannabis sativa* plant contains more than 100 compounds that share the CB chemical scaffold [10,14]. Among them, THC is a main terpenophenolic constituent of cannabis and is responsible for the majority of the plant's reinforcing (intoxicating) effects, by binding to and activating CB₁ receptors [10,11,16,18,19] (Fig. 3). THC also activates CB₂ receptors that contribute to other less-well understood effects such as those exerted on the immune system [10,11]. Recent research has documented that a variety of human disorders are accompanied by dysfunction in the ECS; therefore, pharmacological interventions that normalize dysfunctional ECB signaling, *i.e.* temporally activating CB receptors by THC could be potential therapeutics for diseases associated with hypo-cannabinergic pathology [10] (Fig. 3).

CBD is a major non-psychoactive compound found in

cannabis plant and proposed to have anti-inflammatory, analgesic, anxiolytic, neuroprotective, and anti-seizure effects [74,75] (Fig. 3). In 2018, the CBD-based drug (Epidiolex[®]) was approved by the FDA for the treatment of seizures associated with two rare and severe forms of epilepsy, Lennox-Gastaut syndrome and Dravet syndrome, in patients two years of age and older. Then in 2020, the FDA approved Epidiolex[®] oral solution for the treatment of seizures associated with tuberous sclerosis complex in patients one year of age and older [76-78]. Beneficial effects of CBD in brain disorders include neuroprotective activity *via* anti-inflammatory and anti-oxidative properties [79-83], sedative effects that decrease anxiety [87,88], and as described, an anti-epileptic effect that reduces seizure frequency [86-89]. The therapeutic potential of CBD has also been proposed further for treating ischemic stroke [90], schizophrenia [91,92], and Alzheimer's disease [93]. Although the exact cellular and/or molecular targets of CBD in the body remain unclear, current research postulates that CBD may serve as a negative allosteric modulator of CB₁ receptors or increase ECB tone by elevating AEA levels, among other broad-spectrum mechanisms [82,83,94].

According to Clinicaltrials.gov run by the National Institute of Health (NIH) in the United States, as of August 8, 2022, about 407 clinical studies have been initiated and/or completed to test the therapeutic applications of CBD for neuropsychiatric diseases, cancer, and chronic disorders accompanied with inflammation and pain, such as osteoarthritis pain [95]. With regard to human genitourinary diseases, two main areas of therapeutic interest are treating pain associated with various conditions and lower urinary tract symptoms (LUTS) [96] (Table 1).

A phase 2 study is currently underway to test hemp-based CBD for chemotherapy-induced neuropathy (CIPN) among non-metastatic breast, colorectal, uterine, and ovarian cancer patients who received neoadjuvant or adjuvant therapy that included neurotoxic chemotherapeutic agents. Also, another ongoing study investigates the efficacy of ASP3652, a peripherally restricted FAAH inhibitor that elevates tissue levels of AEA, in the treatment of patients with chronic abacterial prostatitis/chronic pelvic pain syndrome (CP/CPPS). A recently completed Phase 2 study assessed the effect of CBD oil on pain after ureteroscopy for kidney stones. In addition, AEA and palmitoylethanolamide (PEA),

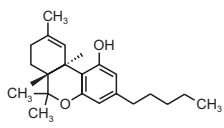
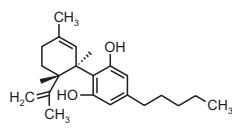
Δ^9 -Tetrahydrocannabinol THC	Cannabidiol CBD
	
Psychoactive (intoxicating)	Non-psychoactive
Direct agonist for CB ₁ and CB ₂ receptors	Multiple bodily targets
Appetite stimulant Anxiogenic in some cases Drowsiness Anti-nausea Analgesic Muscular spasticity	Anti-seizure Anxiolytic Anti-depressant Anti-inflammatory Analgesic (chronic pain) Anti-psychotic Neuroprotective Anti-tumor property

Fig. 3. Two main phytocannabinoids, THC and CBD. THC and CBD are two of the most well-known cannabinoids in the *Cannabis* plant with potential therapeutic utilities. They have distinct pharmacological properties and targets in the body. The intoxicating effects of THC, such as euphoria, relaxation, and sometimes paranoia, is associated with short-term memory deficits and increased risk for psychiatric disorders including psychosis, depression, anxiety, and substance use disorders. These problems are not caused by CBD, which displays anxiolytic, anti-psychotic, and anti-inflammatory and analgesic effects. Unanswered questions regarding potential side effects of phytocannabinoids along with the therapeutic potential of endocannabinoid modulation requires further investigation.

Table 1. Therapeutic utility of cannabinoids for genitourinary disorders

Condition	Disease	Test drug	Dose and duration	Status	Remark	Clinical Trials Identifier
Pain	CIPN	CBD	Various dosages, 3 times per day, 12 weeks	Clinical-ongoing	Hemp-based CBD	NCT04398446
	Pain after ureteroscopy	CBD oil (Epidiolex)	20 mg per day for 3 days	Clinical-completed	Ureteroscopy for kidney stones	NCT04387617
Chronic cystitis	CP/CPPS	ASP3652	Various dosages, twice daily for 12 weeks	Clinical-ongoing	Peripheral FAAH inhibitor	NCT01391338
		Nabilone and Dronabinol	1 mg Nabilone and 2.5 mg Dronabinol, once daily for 6 months	Case report	Synthetic THC analogues	Ref [119]
LUTS	Interstitial cystitis/painful bladder syndrome	AEA and PEA	25 mg/kg AEA and 10–30 mg/kg PEA, single dose	Preclinical	Rat model of visceral and somatic inflammatory pain	Ref [120]
	Detrusor overactivity	Sativex	THC and CBD; max daily doses set at 130 mg THC and 120 mg CBD, for 10 weeks	Clinical-completed	Multiple sclerosis patients	NCT00678795
Renal disease	Overactive bladder	Medical Cannabis	Inhaled dried buds or sublingual oil extract, for 8 weeks	Clinical-ongoing	Parkinson's disease patients	NCT05106504
	Pruritus	Nabilone	0.5 mg, 1–2 per day for 3 weeks	Clinical-ongoing	End-stage renal disease patients	NCT05180968
Cancer	Diabetic nephropathy	GFB-024	Ascending doses ranging from 5–300 mg for 10 weeks	Clinical-completed	Peripheral CB ₁ receptor agonist	NCT04880291
	Prostate cancer	CBD oil (Epidiolex)	600–800 mg, once daily for up to 12 weeks	Clinical-completed	Biochemically recurrent prostate cancer	NCT04428203

AEA: anandamide, CBD: cannabidiol, CIPN: chemotherapy-induced neuropathy, CP/CPPS: chronic abacterial prostatitis/chronic pelvic pain syndrome, FAAH: fatty acid amide hydrolase, LUTS: lower urinary tract symptoms, PEA: palmitoylethanolamide, THC: Δ^9 -tetrahydrocannabinol.

an ECB-related lipid molecule, were tested in animal model for interstitial cystitis/painful bladder syndrome.

In multiple sclerosis (MS) patients, use of the oral mucosal spray Sativex, a mixture of THC and CBD, displayed a significant effect on overactive bladder [97]. Cannabis-derivatives also demonstrated mixed degrees of improvement in incontinence, frequency, nocturia in multiple clinical trials with MS patients [96].

Additional conditions that CB medications have been implicated includes treating pruritus associated with end-stage renal dysfunction and various cancers. The anticancer effects of CBs against prostate cancer have been limited to preclinical *in vitro* studies so far, and translation to human conditions has been sluggish (Table 1).

PHYTOCANNABINOIDS: A DOUBLE-EDGED SWORD FOR MALE GENITOURINARY SYSTEM?

Since the ECS regulates major bodily functions, unwanted side effects may occur when manipulating its activity, which should be anticipated in advance and carefully considered during drug development. Indeed, strong evidence obtained from preclinical studies indicated that administration of cannabis extracts acts on the gonadal axis and reduces its function. Human studies also demonstrated that exposure to cannabis or its derivatives is associated with reduced sperm count and motility along with abnormal morphology, and may negatively impact male fertility [58, 98-100]. In addition, cannabis consumption decreased the levels of plasma testosterone in human users compared to non-users [98], which was associated with reduced plasma LH [58].

The reproductive toxicity of cannabis was mainly reproduced by administration with THC in animal models [58,101-103]. In these studies, exposure to THC altered homeostasis of the HPG axis, and long-term administration of THC significantly decreased spermatogenesis. This phenomenon can be presumed to be the result of THC over-activating CB receptors distributed in the CNS and testis. In contrast, CBD has been suggested to be generally well tolerated by humans because the reported adverse events are mild [74-76]. However, CBD is not risk-free [104]. Clinical studies found that CBD causes adverse effects, including drug-drug interactions, hepatic abnormalities, fatigue,

vomiting, diarrhea, somnolence, insomnia, and suicidal thoughts [104]. In addition, animal studies found that chronic high doses of CBD produce developmental toxicity and affect CNS function, among other peripheral effects including changes in organ weight, hepatocellular injuries, and hypotension [104]. Importantly, concerns have been raised about the adverse effects of CBD on male reproductive system [105]. Evidence indicates that exposure to CBD is associated with a reduction in mammalian testis size, the number of germ and Sertoli cells, fertilization rates, spermatogenesis, and plasma concentrations of hypothalamic, pituitary and gonadal hormones including a decrease in testosterone [100,105-108]. In sexually mature rhesus monkeys, oral administration of 30–300 mg/kg body weight/day CBD for 90 days caused reductions of testicular size and spermatogenesis [109]. A number of studies have reported that preincubation of sperm with CBD inhibited fertilization in sea urchins, a relevant model to study fertilization because of their similarity to human embryos in the early developmental stages [110-111]. The underlying mechanisms by which CBD negatively influences male reproductive system have not been elucidated, but may involve damages to Sertoli cells [112]. Finally, it is notable that CBs may interfere the ECB system required for normal development [113-116], and early life exposure to THC or CBD affected maturation of the HPG axis in rodents. In 21-day-old male Swiss mice, 15 or 30 mg/kg/day CBD administered orally for 34 consecutive days followed by a 35-day recovery period caused a decrease in the number of Sertoli cells, abnormalities in sperm morphology, and decreases in plasma testosterone levels [107,108,117,118]. Therefore, long-term adverse effects of chronic THC or CBD administration during early life could be a significant human health issue that needs scientific attention.

CONCLUSIONS

Preclinical and clinical evidence demonstrates the central role that the ECS plays in regulating many of the body's key processes, including homeostasis of the HPG axis and male reproductive functions. The ability of phytocannabinoids to reduce pain and inflammation provides promising opportunities for therapeutic intervention for genitourinary and degenerative disorders. However, more scientific evidence should be obtained to fully address the general public's interest in utiliz-

ing cannabis products for human disorders and as health supplements. Important knowledge gaps remain, including the role of ECS during early life development of the reproductive system and the underlying mechanisms by which CBD negatively influences male reproductive functions.

Despite these unanswered questions, it is clear that the therapeutic potential of ECB modulation calls for further basic and clinical investigation. Continued study to better understand the complexity of the ECS will provide new insights into the pathogenesis of reproductive and genitourinary disorders, allowing researchers to identify new ways to leverage this signaling system for therapeutic benefit. Drugs can be designed that selectively act on aspects of the ECS required for therapeutic purposes while avoiding unwanted side effects. Alternately, research exploring the uncontrolled use of cannabis (*i.e.*, recreational and substance use disorder) might provide further evidence of its deleterious impacts on human reproductive function.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: KMJ. Investigation: JL, ES, KMJ. Writing: JL, ES, KMJ.

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