Minireview: Endocannabinoids and Gonadal Hormones: Bidirectional Interactions in Physiology and Behavior

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Endocannabinoids act as a major neuromodulatory system in a variety of physiological and behavioral functions. Three major lines of evidence suggest that the endocannabinoid system interacts with gonadal hormones. First, the endocannabinoid system is implicated in behaviors and physiological functions that are known to be regulated in part by gonadal hormones. Second, receptors and metabolic enzymes of the endocannabinoid system are localized extensively on structures in the hypothalamic-pituitary-gonadal axis. Third, changes in levels of gonadal hormones alter endocannabinoid signaling. Here we reviewed and summarized the current evidence regarding the interaction between the endocannabinoid system and androgens, estrogens, and progesterone. Overall, it appears that bidirectional interactions characterize the relationship between endocannabinoids and gonadal hormones, with endocannabinoids down-regulating hypothalamic-pituitary-gonadal activity and gonadal hormones modulating protein expression in the endocannabinoid system. An understanding of these interactions will have implications for elucidating the neuroendocrine mechanisms underlying a number of behavioral and physiological functions as well as potential pharmaceutical treatments for disorders of these functions. **(*Endocrinology* 153: 1016–1024, 2012)**

Cannabis sativa has historically been a widely consumed plant known for its psychoactive properties and its reported effects on motivation, metabolism, and sexual functioning. The primary active component of cannabis was identified in the 1960s as Δ^9-tetrahydrocannabinol (THC) (1). Conclusive evidence for the site of action of THC and other cannabinoids remained elusive until the discovery of the presence of a cannabinoid receptor (2). Cannabinoid receptors have since been discovered to be part of a major neuromodulatory system known as the endocannabinoid system. The endocannabinoid system is widespread throughout the central nervous system (CNS) and peripheral regions and regulates a large array of physiological functions and behaviors. The same can be said for gonadal hormones, and there are several major lines of evidence suggesting that the two systems interact extensively. First, components of the endocannabinoid system are present throughout the hypothalamic-pituitary-gonadal (HPG) axis, and perturbations to this system cause changes in the HPG. Second, changes in the HPG axis alter the expression and function of proteins of the endocannabinoid system. Third, the endocannabinoid system is implicated in many behavioral and physiological functions, such as sexual behavior, that are known to be regulated by gonadal hormones. The current review seeks to summarize the findings relating to interactions between endocannabinoids and gonadal hormones.

The endocannabinoid system contains two types of G protein-coupled cannabinoid receptors: the CB_1 receptor and the CB_2 receptor. CB_1 receptors are found throughout the central nervous system and some peripheral tissues but are most densely expressed in the neurons of the cerebral cortex, hippocampus, amygdala, hypothalamus, basal ganglia outflow tracts, and cerebellum (3), whereas CB_2 receptors are mostly expressed in peripheral tissues and immune cells (4). The endogenous cannabinoid ligands

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Abbreviations: 2-AG, 2-Arachidonoylglycerol; CNS, central nervous system; FAAH, fatty acid amide hydrolase; HPA, hypothalamic-pituitary-adrenal; HPG, hypothalamic-pituitary-gonadal; OVX, ovariectomized; THC, Δ^9-tetrahydrocannabinol.

of these receptors (endocannabinoids) include arachidonoylethanolamide (anandamide) (5) and 2-arachidonoylglycerol (2-AG) (6). CB_1 receptors are located on the axon terminals of presynaptic neurons (7), whereas endocannabinoids are synthesized and released on demand (rather than stored in vesicles) by postsynaptic neurons (8). The binding of anandamide or 2-AG to CB_1 receptors inhibits the further release of neurotransmitters by the presynaptic cell and therefore allows the postsynaptic cell to regulate the level of incoming neurotransmission (9, 10). The mechanism of CB_2 receptor functionality is currently not well understood. Anandamide and 2-AG are eliminated from the synapse via cellular uptake followed by intracellular enzymatic breakdown (11). Fatty acid amide hydrolase (FAAH) is primarily responsible for the breakdown of anandamide (12), whereas monoacylglycerol lipase is primarily responsible for the breakdown of 2-AG (13). The scope of this review is limited to research on mammals, but the endocannabinoid system is present in a diverse array of taxa (*e.g.* see Ref. 14 for a review on endocannabinoids and amphibians).

The endocannabinoid system and androgens

The endocannabinoid system appears to regulate serum levels of gonadal hormones and gonadotrophins. Several earlier studies investigated whether use of marijuana in human males is associated with changes in levels of gonadal hormones or gonadotropins. Chronic marijuana use reduces levels of circulating testosterone, FSH, and LH levels (15, 16). Acute administration of marijuana also can reduce testosterone and LH levels (15, 17). However, others have reported no effects on circulating testosterone, LH, or FSH levels in response to either chronic marijuana or chronic THC hormones (18–22). The discrepancies among studies using human participants may be due to individual differences among the participants in the amounts consumed, and methodological differences between studies. The influence of cannabinoids on androgens appears to be more consistent in animal models. *In vitro* exposure to a THC medium caused a decrease in testosterone production by whole decapsulated mouse testes (23) and preparations of testosterone-secreting rat Leydig cells (24). Chronic administration of THC to male mice caused a regression in Leydig cell tissues and elimination of spermatogenesis, and the effects were reversed by cessation of THC treatment (25). Similarly, chronic administration of high doses of THC to male dogs caused testicular degeneration (26). Acute administration of THC was also effective in reducing serum testosterone levels (27) and in blocking testosterone's ability to reverse castration-induced changes in accessory sex structures in male rats (28, 29). THC and other cannabinoids also inhibited dihydrotestosterone binding to androgen receptors on *in vitro* rat prostate cells, perhaps via receptor-level conformational changes, suggesting that the effects of cannabinoids on androgens are not unique to testosterone (30).

More recent studies confirm that the effects of THC reflect the influence of the endocannabinoid system on the testes in regulating testosterone release and gonadal function. CB_1 receptors have been shown to be expressed in Leydig cells in mice and rats (31, 32), whereas significant concentrations of anandamide have been found in the testes (33). Like THC, anandamide administration was effective in reducing testosterone levels in wild-type mice; however, this effect was not seen in knockout mice for the CB_1 receptor gene (34). This suggests that THC acts on the testes by mimicking anandamide and that the CB_1 receptor is the direct site of action. The expression of CB_1 receptors and the activity of the endocannabinoid system also appear to play an important role in the differentiation and maturation of adult Leydig cells during postnatal development (31). Furthermore, CB_1 knockout mice show reduced serum testosterone levels (34), perhaps due to abnormal Leydig cell function induced by a lack of endocannabinoid regulation during development. In addition, CB_2 receptors, 2-AG, associated synthesis enzymes, FAAH, and monoacylglycerol lipase have all been detected in sperm-producing Sertoli cells, suggesting that endocannabinoids are directly involved in modulating the androgen-mediated process of spermatogenesis (35, 36). The endocannabinoid system also appears to regulate aspects of sperm motility and capacitation independent of direct androgen action (see Refs. 35 and 37 for reviews).

In addition to testicular actions, there is evidence that the endocannabinoid system interacts with gonadal androgens via effects on the hypothalamus and the anterior pituitary. THC, as well as the cannabinoids cannabinol and cannabidiol, lowered not only circulating testosterone levels but also levels of LH and FSH (38). One study revealed that acute THC administration caused significant reductions in circulating LH levels but only nonsignificant reductions in circulating testosterone in human males, suggesting a stronger effect in the pituitary (17). In rodents, serum LH decreased in response to anandamide administration in wild-type mice, whereas CB_1 knockouts were unresponsive to the treatment (34). Acute administration of THC decreased GnRH levels in the preoptic area and the mediobasal hypothalamus of the rat brain in a dose-dependent manner (27), whereas suppression of GnRH release by lipopolysaccharide or TNF-α was associated with increased anandamide synthesis in the mediobasal hypothalamus in *ex vivo* rat brains (39). This is consistent with other studies showing the hypothalamus as a

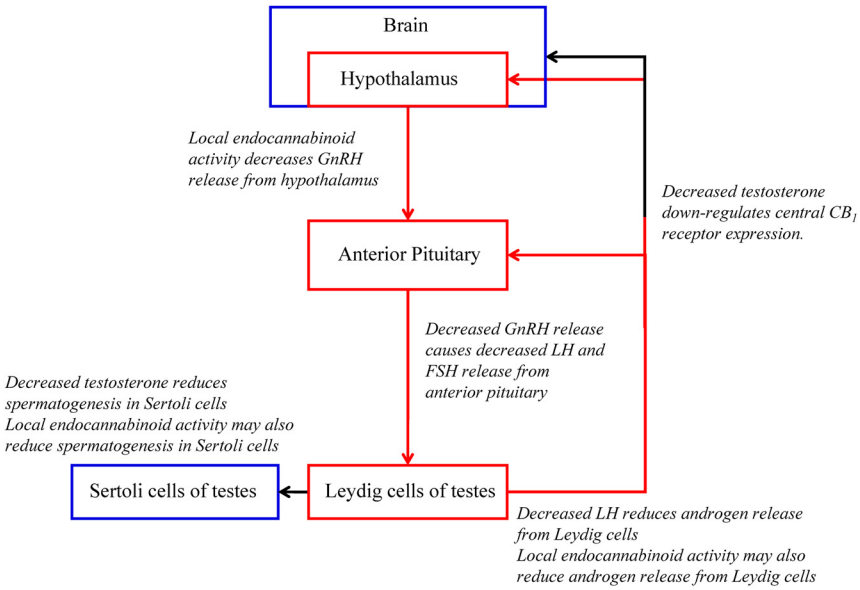

FIG. 1. Summary of major interactions of the endocannabinoid system with androgens. Endocannabinoids suppress release of GnRH, LH, and FSH. *Red arrows* and *boxes* represent interactions in the primary feedback loop of androgens and the endocannabinoid system. *Black arrows* and *blue boxes* represent other interactions occurring beyond the primary feedback loop.

region of dense CB_1 receptor localization and endocannabinoid signaling; anandamide, 2-AG, FAAH, CB_1 receptors, and CB_2 receptors have been detected in hypothalamic GnRH-releasing neurons (40). The secretion of γ-aminobutyric acid on GnRH-releasing neurons appears to be excitatory; treatment with the CB_1 agonist, WIN 55,212, decreased the excitatory signals, whereas the CB_1 antagonist, AM-251, blocked the effect of the agonist and increased the signals (41). These results suggest that endocannabinoids may mediate gonadal activity by down-regulating GnRH release via γ-aminobutyric acid activity, although action on other neurotransmitter systems cannot be ruled out.

Conversely, castration of male rats reduced CB_1 receptor density in the parotid gland, and this was reversed with the administration of testosterone (42). Castration also reduced the transcription of CB_1 mRNA in the rat anterior pituitary (35). Chronic THC administration typically causes a down-regulation in CB_1 receptor expression; this down-regulation, however, is not seen in the anterior pituitary of dihydrotestosterone-replaced castrated males, suggesting that reduced androgen levels may mediate the THC-induced down-regulation of CB_1 receptors in the anterior pituitary. These studies provide evidence that the endocannabinoid system and gonadal androgen release are reciprocally regulated via a negative feedback loop. This is unlike classical negative feedback in the HPG axis, in which LH up-regulates testosterone release and a high testosterone level down-regulates GnRH and LH release. Instead, endocannabinoids appear to directly inhibit the release of androgens from Leydig cells as well as down-regulating the release of LH from the anterior pituitary and GnRH from the hypothalamus; a low testosterone level reduces CB_1 receptor expression, and hence endocannabinoid signaling, in the hypothalamus and pituitary. Future research is still required to confirm this model. These interactions, in relationship to each other, are illustrated in Fig. 1.

Behaviorally, the endocannabinoid system is known to interact with male sexual function. Acute administration of THC to male rats reduced mount latency, reduced ejaculation latency, and increased the length of the refractory period after ejaculation (43) and reduced the number of sexual approaches a male rat made to a female rat (44). Chronic administration appears to have a similar deleterious influence on sexual behavior (45, 46). Administration of anandamide or the selective CB1 agonist HU-210 impairs sexual functioning (47, 48), whereas CB1 antagonists such as AM-251 facilitate sexual functioning (49). Therefore, it appears that activation of the endocannabinoid system in male rodents inhibits sexual behavior (for review see Ref. 50). In human males, chronic THC use may be associated with erectile dysfunction (15, 51, 52). A more recent study using venoocclusive plethysmography showed that chronic THC use may be linked to erectile dysfunction via early epithelial damage (53). However, marijuana users often report subjectively increased sexual pleasure and duration (54–56). These differential effects of THC on sexual behavior in human males appear to be dependent on the dose of THC consumed, with low doses increasing sexual desire and pleasure and higher doses decreasing sexual potency (57, 58). However, the influence of the endocannabinoid system on sexual behavior is due at least in part to the system's influence over CNS neurotransmission because testosterone administration does not attenuate the THC-induced reduction in sexual behavior in rodents (59). Further research is needed to identify the roles of central signaling *vs.* the above-described putative model of endocannabinoid-androgen interaction in regulating male sexual behavior.

The endocannabinoid system and estrogens

Initial cannabinoid research suggested that THC may exert some of its effects by interacting directly with estradiol receptors. Some studies have suggested that both

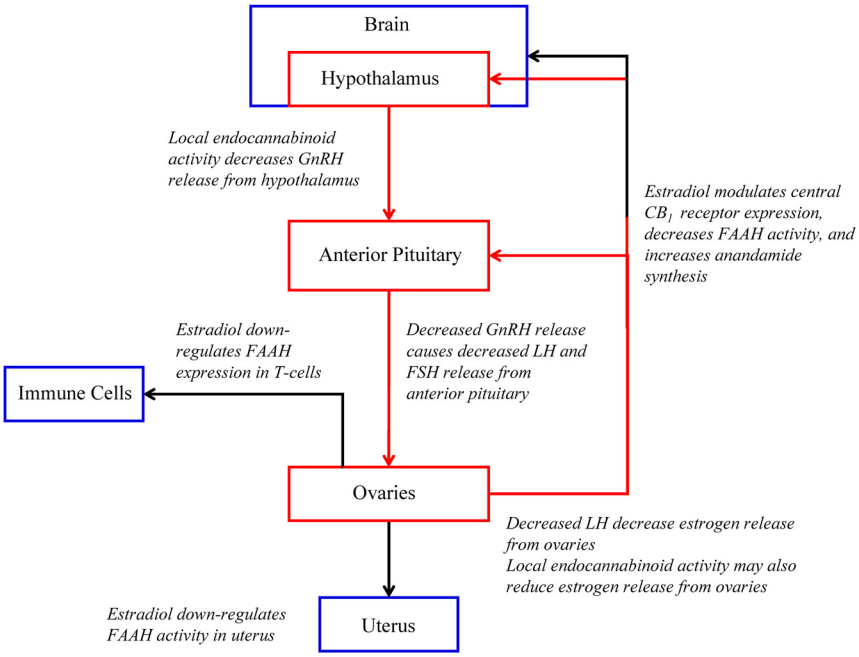

FIG. 2. Summary of major interactions of the endocannabinoid system with estrogens. Endocannabinoids suppress release of GnRH, LH, and FSH. Estrogens regulate functioning of FAAH, the principal catabolic enzyme for the endocannabinoid anandamide. *Red arrows* and *boxes* represent interactions in the primary feedback loop of androgens and the endocannabinoid system. *Black arrows* and *blue boxes* represent other interactions occurring beyond the primary feedback loop.

crude cannabis extract and THC inhibit the binding of estradiol to estradiol receptors *in vivo* (60–62). However, more recent studies have not been able to replicate these estradiol binding effects using either THC, other cannabinoids such as cannabinol, or THC metabolites (63, 64). Notwithstanding the inconsistencies in the literature, interest in direct THC binding to estrogen receptors decreased after the characterization of the CB_1 and CB_2 receptors. However, there is substantial evidence for direct and indirect interactions between the endocannabinoid system and estrogens. As is the case for androgens, the endocannabinoid system appears to modulate the release of estrogens via the central down-regulation of LH and GnRH. Acute THC administration has been shown to decrease serum LH levels, as well as abolish the pulsatile fluctuation of serum LH levels, in ovariectomized (OVX) female rats (65, 66). These effects were reversed by administration of GnRH, suggesting that the anterior pituitary remained sensitive to hypothalamic hormonal control and further suggesting that cannabinoids act on central neurotransmission to suppress LH release. The suppression of LH release by THC has also been seen in OVX female rhesus monkeys (67) as well as intact female mice (68).

Anandamide, like THC, suppressed the release of LH in male and OVX female rats (69). However, in OVX females, administration of the CB_1 receptor antagonist AM-251 produced an even greater inhibition of LH release. The effect of anandamide on LH could be reversed in OVX females by priming with estradiol, but this reversal was blocked by coadministration of AM-251. OVX reduced CB_1 receptor density in the limbic forebrain; this effect was reversed by estradiol administration (70). Similarly, OVX female rats showed estradiol-reversible reductions in CB_1 density in the hippocampus and amygdala, whereas the opposite was seen in the hypothalamus (71). Furthermore, estradiol treatment after OVX reduced CB_1 mRNA levels in the anterior pituitary (72). Castrated males also exhibited a reduction in CB_1 mRNA levels in the anterior pituitary, which was not reversed by administration of dihydrotestosterone, suggesting that the influence of androgens on central CB_1 receptor expression is via aromatization of testosterone into estradiol (72). Estradiol administration decreased the ability of synthetic CB_1 agonists to suppress hypothalamic glutamatergic synaptic transmission and increased the ability of agonists to suppress the hypothalamic γ-aminobutyric acid synaptic transmission (73). These results collectively indicate that changes to estrogen functioning can influence central endocannabinoid signaling, and these can be region and synapse specific. Figure 2 illustrates the reciprocal effects of central endocannabinoid activity and estradiol levels in relation to each other.

Endocannabinoid activity and CB_1 receptor densities in the brain appear to fluctuate throughout the estrous and menstrual cycle. In the mediobasal hypothalamus of female rats, the density of CB receptors was highest during diestrus and lowest during estrus; in the limbic forebrain, the receptors' affinity to cannabinoids was highest during diestrus and lowest during estrus, but their densities did not fluctuate (70). CB_1 mRNA transcript levels were found to be highest during diestrus and lowest during estrus in the anterior pituitary of rats (72). Anandamide and 2-AG levels appeared to be highest during diestrus and lowest during estrus in the hypothalamus but showed the opposite pattern in the anterior pituitary (72, 74). Conversely, in humans, circulating anandamide levels were higher during the follicular phase and highest during ovulation and lower during the luteal phase; anandamide levels were positively correlated with serum estradiol, FSH, and LH, but not progesterone, levels (75, 76). The appar-

ently discrepant findings from animal and human studies may be the results of measuring central *vs.* serum endocannabinoid levels. One possibility is that the endocannabinoid system plays a role in regulating the estrous or menstrual cycle, and central changes in that system precede changes in peripheral endocannabinoid and estrogen content. This is consistent with the trend in the anterior pituitary being the opposite of that in the hypothalamus.

Related to changes across the estrous and menstrual cycle is the role that endocannabinoids play in fertility. CB_1 and CB_2 receptors, FAAH, and the anandamide synthesis enzyme *N*-acyl phosphatidylethanolamine phospholipase D have been identified in the human and rodent uterus (77–79), whereas FAAH and *N*-acyl phosphatidylethanolamine phospholipase D have been found in the ovaries (80). FAAH activity and protein content were highest, and serum anandamide content was lowest, during the proposed zygote implantation window in humans (81). This led to the suggestion that low anandamide levels are required to allow successful implantation and carrying offspring to term, but high anandamide facilitates the labor process. This is supported by reduced levels of circulating anandamide during pregnancy but a surge of anandamide near labor (76). Additionally, increased anandamide or treatment with cannabinoid agonists has been associated with miscarriages in humans (82) and disruptions to implantation and embryonic development in rodents (83, 84).

The FAAH enzyme also appears to be a major site of interaction between the endocannabinoid system and estrogens. Estrogens appear to decrease FAAH activity in the mouse uterus (78). The *faah* gene contains an estrogen response element; translocation of the estrogen receptor-α caused a down-regulation of *faah* transcription (85). This is consistent with the finding that a CB_1 receptor antagonist reversed the anxiolytic effect of estradiol in rats and that the FAAH inhibitor URB 597 produced an anxiolytic effect similar to that produced by estradiol (86). This suggests that estradiol recruits the endocannabinoid system in some of its behavioral effects and can down-regulate the FAAH activity in the CNS. However, estradiol administration in OVX female rats also increased the levels of synthesized anandamide in the medial basal hypothalamus, suggesting that estradiol may also directly interact with endocannabinoid synthesis (69). The interaction between FAAH, anandamide synthesis, and estradiol is also illustrated in Fig. 2. It can be seen that, as with androgens, estrogens have a bidirectional interaction with the endocannabinoid system. Endocannabinoid activity down-regulates HPG axis activity by reducing the release of GnRH by the hypothalamus, leading to reduced estrogen levels. Conversely, estrogen modulates endocannabinoid signaling via CB_1 expression in the CNS, as well as by up-regulating anandamide content by decreasing FAAH transcription in both peripheral and central regions. However, due to the differential effects of estrogens on endocannabinoid signaling in different tissues, multiple pathways of interaction likely exist.

The endocannabinoid system and progesterone

As is the case with both androgens and estrogens, the release of progesterone from the corpus luteum can be attenuated by endocannabinoid activity. Chronic administration of anandamide in pregnant rats decreased serum progesterone and LH content (87). Treatment with either CB_1 or CB_2 receptor agonists reduced levels of serum progesterone, corpus luteum weights, corpus luteum LH receptor mRNA content, and corpus luteum LH receptor density in sheep (88). This suggests that the release of progesterone is at least partially regulated by central endocannabinoid control over LH release but is also controlled by direct endocannabinoid binding onto receptor sites on the corpus luteum. Like androgens and estrogens, progesterone can also regulate endocannabinoid signaling. Progesterone up-regulated the FAAH expression in T cells by interacting with a transcription factor in the promoter region of the *faah* gene (89, 90). In addition, progesterone increased FAAH expression and activity in immortalized human lymphoma U937 cells but not in immortalized human neuroblastoma CPH100 cells (91). As with estradiol, progesterone down-regulated FAAH activity in the mouse uterus (78). Therefore, as with estrogens, progesterone appears to regulate endocannabinoid signaling in a cell type-specific manner in peripheral tissues via the control of FAAH expression and activity. The interaction between endocannabinoid signaling, progesterone, and FAAH activity is illustrated in Fig. 3.

Behaviorally, progesterone has been shown to interact with endocannabinoids in female sexual responding. Acute THC administration to rats increased sexual receptivity at low doses but decreased it at high doses (92) and increased both sexual receptivity and proceptivity in female hamsters (93). Conversely, the CB_1 receptor agonist HU-210 reduced sexual receptivity and proceptivity (94), whereas the antagonist AM-251 increased sexual motivation in female rats (95). The much higher potency and/or selectivity of synthetic CB_1 ligands over THC at the CB_1 receptor as well as methodological differences [for example, another report (95) used a novel runway apparatus to test for motivation] may explain these differential results. Acute central administration of the progesterone antagonist RU 38486 blocked the stimulatory effects of THC on female sexual behavior in rats (96). Intracerebroventricular administration of antisense progesterone receptor oli-

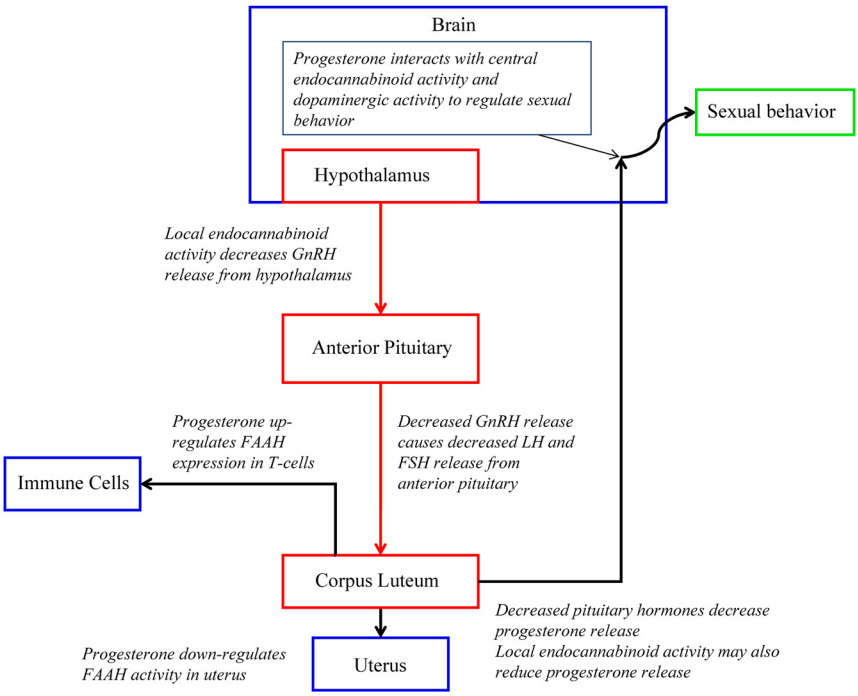

FIG. 3. Summary of major interactions of the endocannabinoid system with progesterone. Endocannabinoids suppress release of GnRH, LH, and FSH. Progesterone regulates functioning of FAAH, the principal catabolic enzyme for the endocannabinoid anandamide. *Red arrows* and *boxes* represent interactions within the HPG axis. *Black arrows*, *blue boxes*, and *green boxes* represent other interactions.

gonucleotides, which suppress progesterone receptor expression, also produced a similar blockage of the effects of THC. Administration of the CB_1 receptor antagonist SR 141716A in turn blocked the facilitatory effects of progesterone on female sexual behavior. In addition, antisense dopamine 1 receptor nucleotides blocked the effects of THC and progesterone, whereas SR 141716A administration blocked the facilitatory effects of dopamine on female sexual behavior. These results suggest that sexual receptivity requires a bidirectional central interaction between progesterone and the endocannabinoids, and this interaction is associated with the dopaminergic signaling. Such an interaction is similar to what has been seen with the previously mentioned anxiolytic effects of estradiol and URB 597 and may also underlie other behaviors in which both gonadal hormones and the endocannabinoid system have been implicated. In humans, one recent study found increased female sexual arousal was correlated with an acute reduction in levels of anandamide and 2-AG (97). No other studies to date have investigated the role of the endocannabinoid system in physiological aspects of female sexual functioning. More research in this area is required to determine whether the principles found in animal models apply to human females .

Conclusions

From the described behavioral, physiological, and biochemical evidence, a picture of the overall reciprocal interactions between the endocannabinoid system and gonadal hormones in mammals is emerging. In the hypothalamus and the anterior pituitary, endocannabinoid signaling suppresses the release of GnRH and LH, which subsequently reduces gonadal hormone release. There is also evidence that endocannabinoid signaling directly reduces androgen release from Leydig cells. Changes in gonadal hormone levels feedback upon the hypothalamus, pituitary, and limbic regions and alter expression of CB_1 receptor activity, forming a feedback loop. In the periphery, estrogens and progesterone alter FAAH activity in reproductive tissues and immune cells. On the other hand, in the forebrain, bidirectional interactions regulate behaviors such as emotionality and sexual motivation. Overall, it appears that endocannabinoid signaling primarily acts on gonadal hormones to decrease their release, whereas gonadal hormones, especially estradiol, cause changes in endocannabinoid-linked protein expression. A parallel model of interaction between endocannabinoids and hormones can be seen in the relationship between the endocannabinoid system and the hypothalamic-pituitary-adrenal (HPA) axis mediated stress response (see Ref. 98 for a review). In this system, increased endocannabinoid activity suppresses the release of glucocorticoids, whereas chronic stress and HPA activation can induce long-term changes in the endocannabinoid system. Here endocannabinoids serve as a negative feedback loop to prevent maladaptive excess activation of the HPA axis, and tonic anandamide levels in particular appear to be a gatekeeper, which must be lowered before the HPA stress response can be occur. A similar process likely occurs in the case of the HPG axis; endocannabinoid activity forms a negative feedback loop, which maintains gonadal hormones at the correct physiological levels and prevents overactivation of this system. Gonadal hormone regulation of central endocannabinoid system protein activity may then serve to prevent excess inhibition of the HPG axis by endocannabinoids. Such an interaction may in fact be the primary pathway of endocannabinoid regulation of many global hormonal systems and subsequent feedback of these hormones on central endocannabinoid activity.

A clear understanding of the interplay between these two systems can have important implications for elucidating the mechanisms underlying a vast number of be-

havioral and physiological functions. Beyond the domains described previously, the influence of both endocannabinoids and gonadal hormones has been observed in areas as diverse as cancer (99), homeostasis (100), memory (101, 102), neurogenesis (103, 104), and drug addiction (105). Additionally, pharmacological agents targeting the endocannabinoid system have potential to provide novel treatments for dysfunctions and disorders modulated by gonadal hormones, for example, sexual disorders. Further research is required to fully characterize the interplay between the two systems. This is especially important in the forebrain because it appears that the specific nature of the interaction is variable and dependent on region, cell type, and function. In addition, interactions with other systems, such as the dopamine system (95), are also present and likely play a behavioral role. Understanding these interactions will be especially crucial as new medications targeting the endocannabinoid system are developed and enter the market. The proposed model of interaction between endocannabinoids and gonadal hormones may also help elucidate the interplay between the endocannabinoid system and other endocrine systems, which would widen the implications of current and future research in this area and advance our understanding of physiology and behavior.

Acknowledgments

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