HEP (2005) 168:555–571

Effects of Cannabinoids on Hypothalamic and Reproductive Function

M. Maccarrone[1] (✉) · T. Wenger[2]

[1]Department of Biomedical Sciences, University of Teramo, Piazza A. Moro 45, 64100 Teramo, Italy
Maccarrone@vet.unite.it

[2]Department of Human Morphology and Developmental Biology, Semmelweis University, PO Box 95, 1450 Budapest, Hungary

Abstract Marijuana and cannabinoids have been shown to exert profound effects on hypothalamic regulatory functions and reproduction in both experimental animals and humans. Here we review the role of (endo)cannabinoids in the regulation of appetite and food intake. There is converging evidence that the hypothalamic endocannabinoid system changes after leptin treatment. Cannabinoid administration decreases heat production by altering hypothalamic neurotransmitter production. Experimental and human data have also shown that the endocannabinoid system is involved in the regulation of reproductive function at both central and peripheral levels. We discuss also the role of fatty acid amide hydrolase (FAAH) in gestation, and in particular the regulation of the activity of FAAH by progesterone and leptin. We show that endocannabinoids inhibit the release of leukaemia inhibitory factor (LIF) from peripheral T lymphocytes. Taken together, endocannabinoids not only help to maintain neuroendocrine homeostasis, but also take part in immunological changes occurring during early pregnancy.

Keywords Appetite · Cytokines · Endocannabinoids · Hypothalamus · Lymphocytes · Pituitary · Pregnancy · Reproduction · Sex hormones · Thermoregulation

1 Historical Background

Cannabis was used as a drug as long ago as 2000 B.C. Hemp is mentioned in the Atharva Veda approx. 2000 B.C. (veda: saint book of Hindi religion). The ancient Hindus credited it as giving "vital energy", and Pliny the Elder first mentions its effect on the reproductive system (cited by Butrica 2002): "...*semen eius extinguere genitarum uirorum dicitur*... (Its seed is said to extinguish men's semen)". Aetius mentioned (sixth century A.D.) that it could be used on women as well, although he did not specify the conditions of use. Cannabis has been widely known since the first millennium in the Middle East, and the physician al-Badri (middle of the thirteenth century A.D.) already recommended hashish to stimulate appetite (cited by Peters et al. 1999).

As early as in the tenth century in Middle Eastern medicine, the hemp was used as an antipyretic agent (cited by Lozano 2001). Moreau (1845) also mentioned the hypothermic effect of marijuana.

Cannabis was introduced into the modern Western world as a medicine by O'Shaughnessy in 1830, who recommended it to cure menstrual disorders (in Crawford 2002), probably not because of its effects on hormonal secretion but as an anticonvulsive smooth muscle relaxant.

It was not until 1970 that marijuana was extensively investigated, as a result of the identification of the major psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (THC), by Mechoulam et al. (1965).

2 General Anatomical Features

The hypothalamus is a subdivision of the diencephalon. It is a multifunctional centre for the control of visceromotor and endocrine activity. The hypothalamus integrates and modulates responses to changes in temperature or osmolality or in the level of specific hormones in the general circulation. Anteriorly it is bordered by the lamina terminalis. The third cerebral ventricle is its medial boundary and the lateral border is formed by basal forebrain structures (Fig. 1). The fornix divides the hypothalamus into medial and lateral region. The hypothalamus has varied and complex connections with several other CNS areas (Levine 2000). It receives information from sensory nerves, peripheral hormone secretions, and pathways originating in limbic and cortical structures. The output structures control brainstem autonomic centres like gastrointestinal (appetite, vomiting) regulatory areas. The hypothalamus plays a major role in emotional behaviour, and is sensitive to changes of blood temperature. As such, it plays a role in the regulation of body

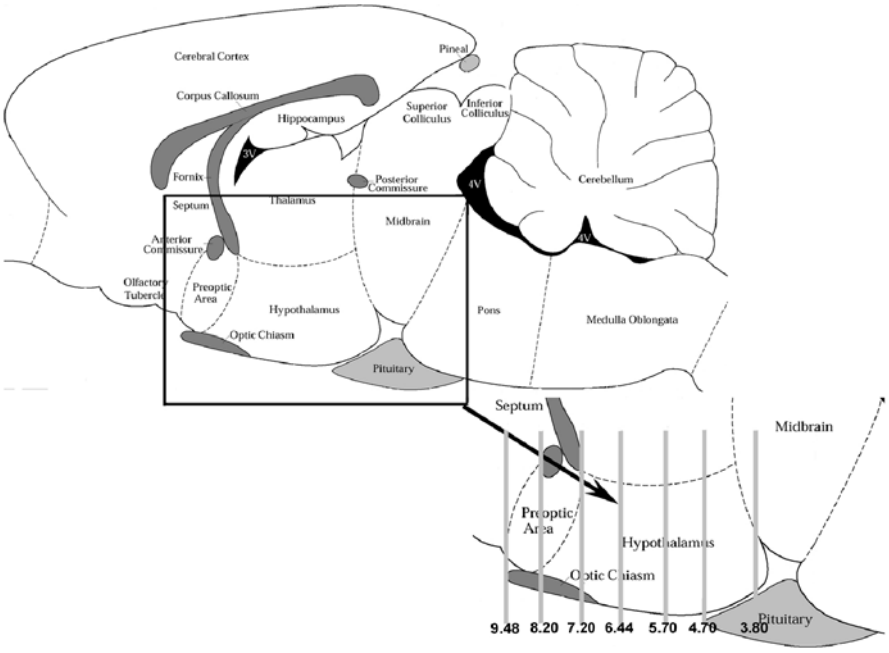

Fig. 1. Schematic drawing of the sagittal section of rat brain 0.4 mm lateral to the midline, according to the atlas of Paxinos-Watson (1997). The *box* shows a part of the forebrain. The *parallel lines* indicate frontal sections seen in Fig. 2. The *numbers* indicate the distance from the interaural line according to the Paxinos-Watson atlas. Only the main structures are labelled (for orientation)

temperature. Indeed, the hypothalamus is a brain area that is generally considered to be particularly important in maintaining homeostasis.

3
Cannabinoids in the Hypothalamus and Pituitary

The presence of endocannabinoids has been shown in the hypothalamus (Herkenham 1995) and in the anterior pituitary (Gonzales 1999). The central cannabinoid receptor (CB_1 receptor) is also present is these structures. The hypothalamus contains fewer cannabinoid binding sites than other areas of the CNS. Nevertheless the effects caused by the activation of CB_1 receptors in the hypothalamus are important, maybe because the receptors are more or less concentrated within specific hypothalamic nuclei-areas (Fig. 2). CB_1 receptors seem to be located on intrinsic hypothalamic neurons rather than on neurons with cell bodies located outside the hypothalamus, since hypothalamic deafferentation is not followed by any reduction in the number of cannabinoid receptor binding sites within this brain area (Romero 1998).

Unlike the hypothalamus, the anterior pituitary, which is regulated by hypothalamic releasing and inhibiting factors, contains a large number of CB_1 recep-

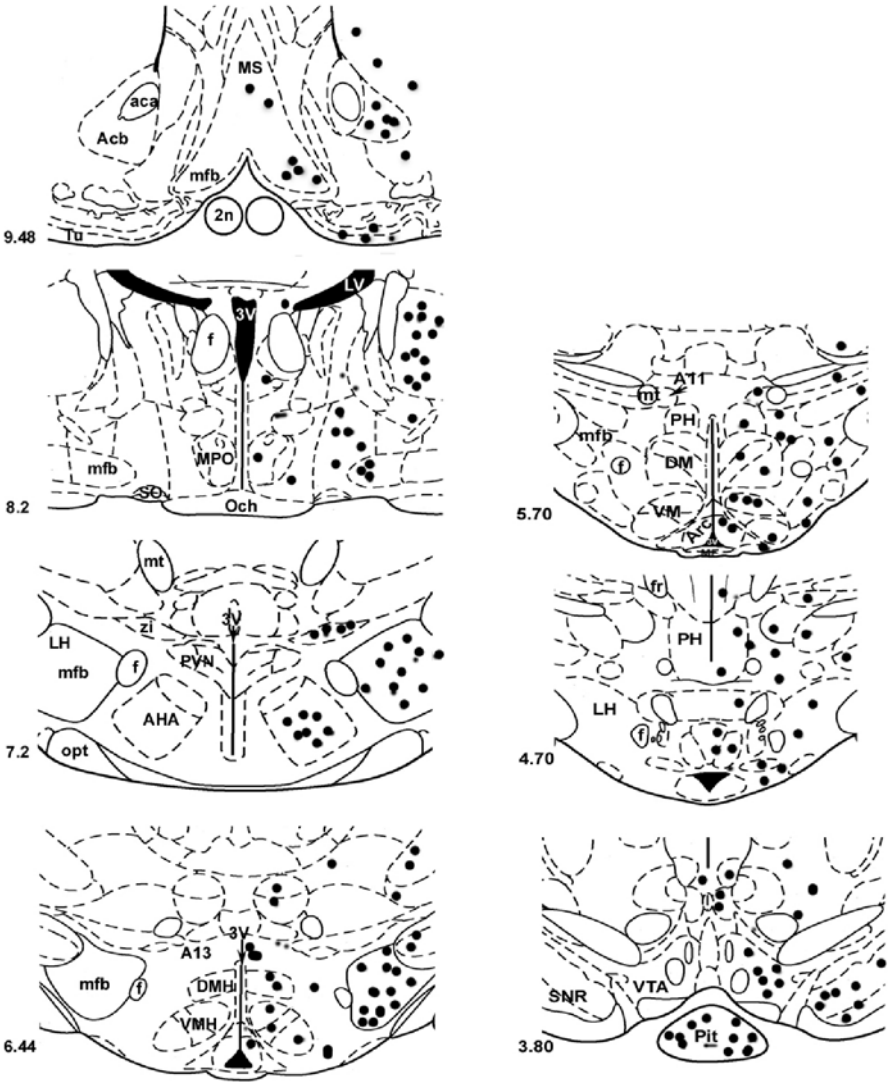

Fig. 2. Schematic drawing of different forebrain and midbrain areas to show the presence of cannabinoid receptor immunoreactivity (*dark spots*). Differences between fibres and cell bodies are not shown. Also not shown are quantitative differences between different areas in CB_1 receptor density. Only structures expressing CB_1 receptor immunoreactivity are labelled. The *numbers* indicate the distance from the interaural line (according to the Paxinos-Watson atlas, see also Fig. 1 for explanations). Abbreviations of structures labelled in this and in consecutive figures (in alphabetical order): *2n*, optic nerve; *3V*, third ventricle; *A11*, A11 dopamine cells; *A13*, A13 dopamine cells; *aca*, anterior commissure; *Acb*, accumbens nucleus; *AHA*, anterior hypothalamic area; *DMH*, dorsomedial hypothalamic nucleus; *f*, fornix; *fr*, fasciculus retroflexus; *LH*, lateral hypothalamic area; *LV*, lateral ventricle; *mfb*, median forebrain bundle; *MPO*, medial preoptic nucleus; *MS*, medial septal nucleus; *mt*, mamillothalamic tract; *Och*, optic chiasma; *opt*, optic tract; *Pit*, pituitary gland; *PVN*, paraventricular nucleus; *SNR*, substantia nigra; *SO*, supraoptic nucleus; *Tu*, olfactory tubercle; *VMH* ventromedial hypothalamic nucleus; *zi*, zona incerta. (Details from Moldrich and Wenger 2000, with the kind permission of Elsevier Science Publishing)

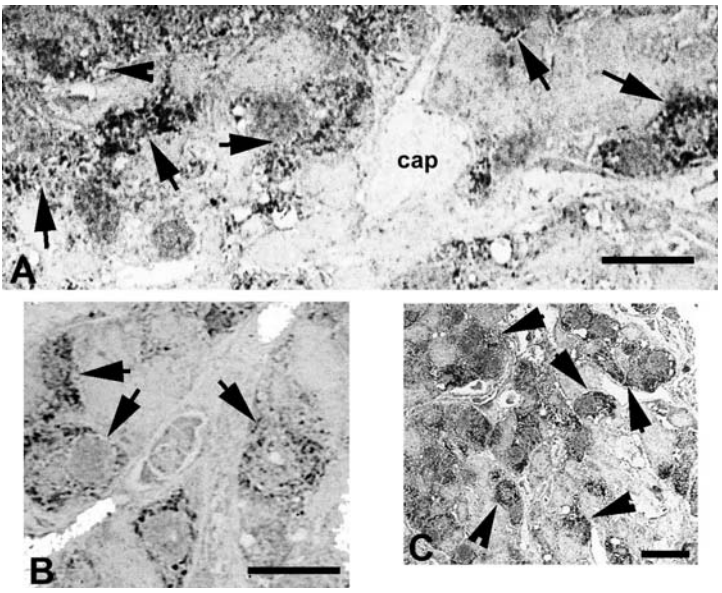

Fig. 3A–C. Immunohistochemical expression of CB_1 receptors in the anterior pituitary of rat. The *arrows* show intensely stained cells. **A** and **B** Gonadotrope cells. **C** Lactotropes. Note that the immunoreactive granules are present mainly at the periphery of the cells. *cap*, sinusoid capillary; *scale bars* = 35 μm in **A** and **B**, 25 μm in **C**

tors, mainly on lactotropes and gonadotropes (Wenger et al. 1999) (Fig. 3). *N*-Arachidonoylethanolamine (AEA) is also present in the pituitary (Gonzales et al. 1999).

No cannabinoid receptors have been found in pituitary corticotrope cells (Wenger at al. 1999).

3.1 Cannabinoids and Appetite and Feeding

Leptin, the 16-kDa product of the *obese* gene, has been implicated in the maintenance of feeding behaviour and energy balance (Campfield et al. 1995). Leptin is regarded as an "appetite-reducing" protein, and as the primary signal through which the hypothalamus regulates food intake and energy balance (Friedman and Halaas 1998). It is known that neurons in the ventromedial hypothalamic nucleus (VMH) and in the lateral hypothalamus (LHY) play a central role in the regulation of feeding and energy homeostasis (Oomura et al. 1969). The leptin receptor (LR) was first demonstrated in the choroid plexus and hypothalamus (Tartaglia 1995). Strong LR immunoreactivity was described in the hypothalamic arcuate nucleus (ARC), VMH and dorsomedial nucleus (DMN), and moderate immunoreactivity in the LHY (Maruta et al. 1999; Funahashi et al. 1999) (Fig. 4). It is interesting that leptin does not only regulate appetite and feeding, but also takes part in hypothalamic neuroendocrine regulation (Takashi et al. 2002).

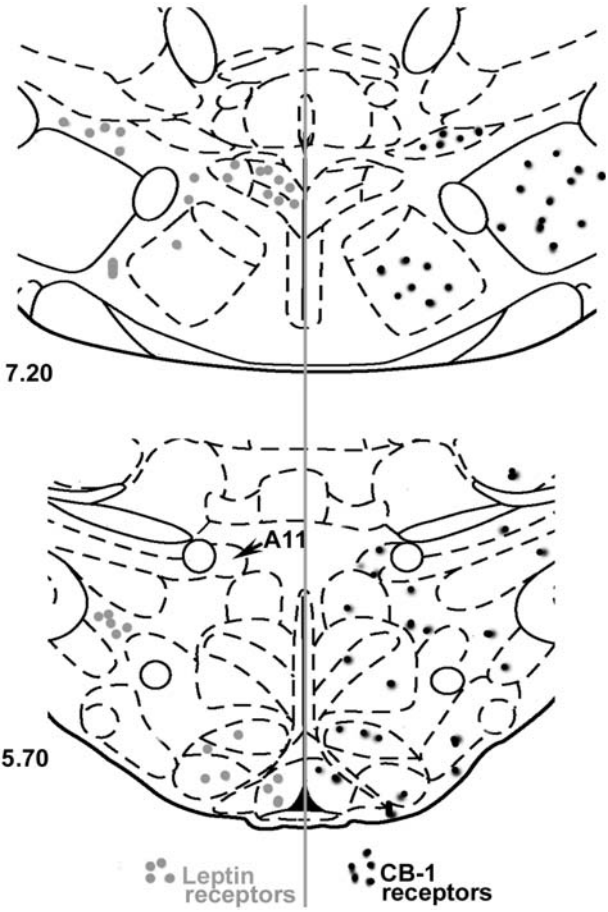

Fig. 4. Schematic drawing of two different forebrain areas to show the presence of CB_1 receptors (*right side*) and leptin receptors (*left side*). The structures are labelled as in Fig. 2. Note that there are sites such as the zona incerta, anterior hypothalamic area, ventromedial nucleus, and dorsomedial nucleus where both receptors are present. (Drawings concerning leptin were made using the data of Maruta et al. 1999)

On the other hand, studies confirmed that THC and AEA might cause overeating (Williams et al. 1998; Williams and Kirkham 1999). Administration of AEA caused hyperphagia and overeating in rats. Attenuation of this effect by the CB_1 receptor antagonist SR 141716A was dose dependent (Williams and Kirkham 1999). Di Marzo et al. (2001) reported that hypothalamic endocannabinoid signalling is constitutively stimulated in obese mice and Zucker rats. They also observed that food intake is lower in CB_1 receptor knockout (KO) mice than in their wild-type littermates. Hypothalamic endocannabinoids appear to be under negative control by leptin. AEA content is reduced in the hypothalamus after leptin treatment (Di Marzo et al. 2001), and AEA-hydrolase (fatty acid amide hydrolase, FAAH) activity is enhanced by leptin in human T cells (Maccarrone et al. 2003a).

It can be concluded that endocannabinoids contribute to the stimulation of appetite by activating the CB_1 receptors present in the hypothalamus (the presence of both leptin and CB_1 receptors in the hypothalamus is shown on Fig. 4) and that the CB_1 receptor antagonist SR 141716A can be considered to be an appetite-suppressing drug.

3.2 Cannabinoids and Thermoregulation

One of the characteristic pharmacological properties of CB_1 receptor agonists is an ability to induce hypothermia (Pertwee 1985). The changes of body temperature caused by cannabinoids are dose dependent. According to Pertwee, higher doses of THC cause hypothermia by lowering the thermoregulatory "set point", while lower doses are hyperthermic. It has been postulated that differential G_s and G_i protein activation by CB_1 receptors could explain these findings (Sulcova et al. 1998).

Cannabinoid-induced hypothermia is mediated by dopaminergic pathways (Pertwee 1992). It was proposed that AEA might not produce all of its effect on thermoregulation by a direct interaction on CB_1 receptors present in hypothalamic thermoregulatory centres. SR 141716A did not block hypothermia caused by AEA (Adams et al. 1998), although this CB_1 receptor antagonist reversed the hypothermia caused by WIN 55,212-2. The endocannabinoids *N*-arachidonoyl-dopamine and 2-arachidonoylglycerol (2-AG)-ether both caused hypothermia (Bisogno et al. 2000; Hanus et al. 2001), supporting the involvement of CB_1 receptors in this process.

On the other hand, *N*-vanillyl-arachidonyl-amide (arvanil), a VR1 receptor agonist, was 100 times more potent than AEA in producing hypothermia (Di Marzo et al. 2000), which indicates that hypothermia caused by cannabimimetic compounds may not (only) be due to the activation of CB_1 receptors.

It is possible that the endocannabinoid system is taking part in thermoregulation, too. However, it is still questionable whether this effect occurs by the activation of cannabinoid receptors and/or vanilloid receptor, or by other mechanisms.

3.3 Cannabinoids and Regulation of the Hypothalamo-Pituitary-Adrenal Cortical Axis

Both exogenous and endogenous CB_1 receptor agonists stimulate adrenocorticotrophic hormone (ACTH) and corticosterone secretion (Dewey 1986; Weidenfield et al. 1994; Wenger et al. 1997; Manzanares et al. 1999).

Chronic administration of THC increased corticotropin-releasing hormone (CRH) and proopiomelanocortin (POMC) gene expression in the rat hypothalamus (Corchero et al. 2001). Circulating gonadal steroids facilitate the latter effect.

AEA activates the CRH-producing neurons in the hypothalamic paraventricular nucleus (Wenger et al. 1997). This effect of AEA may be mediated by a different

and as-yet-uncharacterized G protein-coupled cannabinoid receptor (CB_X), the presence of which in the CNS has been proposed (Wenger et al. 1997; Di Marzo et al. 2002; Wenger et al. 2003).

4 Cannabinoids and Reproduction

In the 1980s a great number of papers dealt with the effects of THC on reproduction and on neuroendocrine function. THC increased gonadotropin-releasing hormone (GnRH) (Collu 1976). Kumar et al. (1983) found that hypothalamic GnRH content was increased in ovariectomized rats after a single dose of THC. An accumulation of GnRH in dense-core vesicles was observed in the hypothalamic median eminence after THC treatment, (Doms et al. 1981). Ayalon et al. (1977) and Tyrey (1984) postulated that THC acted primarily through central neuroendocrine mechanisms, since its effects could be reversed by administration of exogenous GnRH. In contrast, Wenger et al. (1987) found no changes in GnRH content in the (anterior) hypothalamus after THC administration, and in in vitro studies it was demonstrated that THC did not alter the release or storage of gonadotropins and did not modify the responsiveness of cultured anterior pituitary cells to GnRH.

Since the early studies by Marks (1973) on the inhibitory effects of THC on pituitary luteinizing hormone (LH) secretion, a number of papers reported similar effects (Smith et al. 1980; Steger et al. 1980; Wenger et al. 1987). THC suppresses the tonic circulating level of LH in male rats (Chakravarty et al. 1982) and episodic LH secretion in female animals (Tyrey 1980).

Studies by Chakravarty et al. (1975) in intact female rats, by Kramer (1974) in male rats, by Dalterio et al. (1981) in male mice, and by Rettori et al. (1988) in male rats in vitro, have shown that administration of THC can lower prolactin (PRL) release.

AEA has similar effects on reproductive hormones as THC. AEA temporarily decreases serum LH level, and this effect lasts up to 2–3 h (Gonzales et al. 2000; Wenger et al. 1999). PRL levels can also be decreased by endocannabinoid treatment (Wenger et al. 1999). Sexual differences in CB_1 receptor density have been detected in the medial basal hypothalamus (MBH) (Rodriguez de Fonseca 1994). The density was higher in diestrus and decreased in oestrus. Gonzales et al. (2000) reported that AEA content in both the hypothalamus and anterior pituitary might be controlled by circulating sex steroids. AEA effects on the control of the regulation of reproduction are mediated by CB_1 receptors located in the hypothalamus and in the anterior pituitary (Fernandez-Ruiz et al. 1997; Romero et al. 1998; Wenger et al. 1997). Recently it has been demonstrated that AEA changes dopaminergic turnover, thus altering inhibitory dopaminergic effects on PRL secretion (Scorticati et al. 2003).

CB_1 receptor inactivation suppresses reproductive hormone secretion (Wenger et al. 2001). Serum LH and testosterone (T) levels significantly decreased in mutant ($CB_1^{-/-}$) mice (Table 1). Results from this investigation also indicated that cannabinoids regulate neuroendocrine function through the activation of CB_1

Table 1. Luteinizing hormone (LH) and testosterone (T) content in central cannabinoid receptor (CB_1 receptor) knockout mice (data from Wenger et al. 2001)

	LH mg/pituitary	LH ng/ml serum	T nmol/testis
$CB_1^{+/+}$	0.71±0.24	5.15±0.8	39.57±4.23
$CB_1^{-/-}$	0.76±0.3	2.6±0.24*	19.89±3.2**

$^{+/+}$, Wild-type mice; $^{-/-}$, CB_1 receptor knockout mice.
n=8–10 in all groups.
*p<0.01 vs +/+ (±SEM).
**pLT0.001 vs +/+ (±SEM).

Table 2. Anterior pituitary hormone content changes after AEA administration[1]

LH	FSH	PRL	ACTH
↓↓	–	↓↓	↑↑

↓↓, Significant decrease (p<0.01 or higher); ↑↑, significant increase (p<0.01 or higher); ACTH, adrenocorticotrophic hormone; AEA, anandamide; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin.
[1] One dose (0.1 mg/kg), i.p. administration.

receptors. This regulation seems to be mainly through inhibition of hormone release at the pituitary level and may or may not also involve the hypothalamus. Table 2 summarizes the effects of endocannabinoids on anterior pituitary hormone content. Interestingly, cannabinoids do not affect the secretion/release of follicle-stimulating hormone (FSH), and it remains to be ascertained whether or not they may modulate the purported FSH-releasing factor (Samson et al. 1980).

A direct regulatory role for endocannabinoids in normal human anterior pituitary gland and pituitary adenomas has also been postulated (Pagotto et al. 2001). Pituitary adenomas had higher AEA and 2-AG concentrations, pointing to a role for endocannabinoids in the development of pituitary adenomas too.

4.1 The Endocannabinoid System and Female Reproductive Function

Adverse effects of cannabinoids, and in particular of THC, on reproductive functions include retarded embryo development, foetal loss and pregnancy failure. They have been known for a long time (Geber and Schramm 1969; Kolodney et al. 1974; Das et al. 1995; Ness et al. 1999), and were recently reviewed (Paria and Dey 2000; Maccarrone et al. 2002).

THC has been reported to account for the majority of the reproductive hazards of marijuana use, and in males it leads to impotence by suppressing spermatogenesis, reducing the weight of reproductive organs, and decreasing the plasma concentration of circulating hormones like testosterone (Kolodney et al. 1974). In

females, THC inhibits ovulation by prolonging the oestrous cycle and decreasing the pro-oestrous surge of luteinizing hormone. In addition, exposure to natural cannabis extracts during pregnancy has been linked to embryotoxicity and to the production of specific teratological malformations in rats, hamsters and rabbits (Geber and Schramm 1969).

Also, AEA has been shown to impair pregnancy and embryo development in mice (Paria et al. 1996), suggesting that endocannabinoids might regulate fertility in mammals. Consistently, down-regulation of AEA levels in mouse uterus has been associated with increased uterine receptivity, which instead decreased when AEA was up-regulated (Yang et al. 1996; Schmid et al. 1997). The higher level of AEA in the nonreceptive uterus correlates well with the embryotoxic effect of the nonreceptive uterine environment, and also with the in vitro observation that AEA inhibits embryo development and zona-hatching of blastocysts (Paria et al. 1996; Yang et al. 1996; Schmid et al. 1997). In the mouse, mRNAs of AEA-binding CB_1 and CB_2 receptors are expressed in the preimplantation embryos, and the levels of CB_1 receptors are much higher than those found in brain (Das et al. 1995; Yang et al. 1996; Schmid et al. 1997). A recent study has also shown cross-talk between cannabinoid receptors and progesterone receptors in THC-induced modulation of sexual receptivity (Mani et al. 2001), further demonstrating that dysregulation of cannabinoid signalling disrupts uterine receptivity for embryo implantation (Paria et al. 2001).

4.2 The Endocannabinoid System and Male Reproductive Function

Despite the knowledge that chronic administration of THC to animals lowers testosterone secretion and reduces the production, motility and viability of sperm (Hall and Solowij 1998), it is not yet known whether the endocannabinoid system has any role in the control of male fertility in mammals. The binding of AEA to a cannabinoid receptor present on spermatozoa of sea urchin (*Strongylocentrotus purpuratus*) has been shown to reduce their fertilizing capacity (Chang et al. 1993; Schuel et al. 1994), and evidence that AEA regulates human sperm functions required for fertilization has been recently reviewed (Schuel et al. 2002a). In addition, a recent in vitro study has demonstrated that *N*-palmitoylethanolamine, a homologue of AEA, may affect the time-course of capacitation of human spermatozoa by modulating the properties of their membranes (Ambrosini et al. 2003). On the other hand, rat testis is able to synthesize AEA (Sugiura et al. 1996), and this compound has been detected in human seminal plasma at nanomolar (10 nM) concentrations (Schuel et al. 2002b). More recently, the presence of CB_1 receptors in Leydig cells and their involvement in testosterone secretion have been demonstrated in mice (Wenger et al. 2001). Also, the function of Sertoli cells has been shown to be altered by THC, though the molecular basis for this alteration has not been established (Newton et al. 1993). As Sertoli cells of the mammalian seminiferous epithelium are involved in the regulation of germ cell development by providing nutrients and hormonal signals needed for spermatogenesis (Griswold

1995), we recently sought to investigate whether Sertoli cells were able to bind and degrade AEA, and whether this endocannabinoid might induce apoptosis in these cells. In this context, the effect of FSH was also checked, because it dramatically impacts fetal and early neonatal Sertoli cell proliferation and is critical in determining spermatogenic capacity in the adult mammal (Orth et al. 1998).

We found that Sertoli cells have the biochemical machinery to degrade AEA and express functional CB_2 receptors on their surface (Maccarrone et al. 2003b). In addition, FSH dose-dependently inhibited apoptosis induced by AEA in these cells through a remarkable (four- to fivefold) increase in FAAH activity (Maccarrone et al. 2003b). Taken together, these data extend to male fertility the potential for FAAH to regulate the activity of AEA. Additionally, the finding that Sertoli cells belong to the peripheral endocannabinoid system opens new perspectives to the understanding and treatment of male fertility problems.

4.3 Sex Hormones, Th_1/Th_2 Cytokines, Leukaemia Inhibiting Factor and Endocannabinoids

Human reproductive fluids, such as seminal plasma, mid-cycle oviductal fluid, follicular fluid, amniotic fluid, as well as human amniotic fluid and human milk have been reported to contain AEA, *N*-palmitoylethanolamine (PEA) and *N*-oleoylethanolamine (OEA) in the low nanomolar range, i.e. from 3 nM of AEA in the follicular fluid to 67 nM of OEA in human milk (Schuel et al. 2002b). This suggests that endocannabinoids might regulate multiple physiological and pathological reproductive functions in humans, implying that exogenous cannabinoids delivered by marijuana smoke could impact these processes. Consistent with the hypothesis that endocannabinoids adversely affect human fertility, we have recently found a fall in FAAH activity and expression in the T lymphocytes of women experiencing miscarriage (Maccarrone et al. 2000) and a rise (4-fold) in blood AEA levels of the same subjects, compared to women with normal gestation (Maccarrone et al. 2002). The other components of the endocannabinoid system, like the AEA membrane transporter (AMT) and CB_1 receptors, were not affected (Table 3).

Table 3. FAAH activity, AMT activity and CB_1 receptor binding in women who miscarried and those who did not (data from Maccarrone et al. 2001)

Parameter	Pregnant women	Miscarrying women
FAAH activity[a]	133 ± 9 (100%)	48 ± 5 (36%)*
AMT activity[b]	50 ± 4 (100%)	49 ± 4 (100%)
CB_1 binding[c]	20,380 ± 1,930 (100%)	20,400 ± 1,795 (100%)

AMT, anandamide membrane transporter; FAAH, fatty acid amide hydrolase.
[a]Expressed as $pmol.min^{-1}.mg\ protein^{-1}$.
[b]Expressed as $pmol.min^{-1}.mg\ protein^{-1}$.
[c]Expressed as $cpm.mg\ protein^{-1}$.
*$p<0.0001$ vs pregnant women ($p>0.05$ in all other cases).

Peripheral T lymphocytes regulate fertility at the feto-maternal interface, by producing type 1 T helper (Th_1) and type 2 T helper (Th_2) cytokines (Piccinni et al. 1998). Th_2 cytokines, such as interleukin (IL)-3, IL-4 and IL-10, favour blastocyst implantation and successful pregnancy by promoting trophoblast growth either directly or indirectly through the inhibition of natural killer (NK) cell activity and the stimulation of natural suppressor cells. Conversely, Th_1 cytokines, such as IL-2, IL-12 and interferon-γ (INF-γ), impair gestation by causing direct damage to the trophoblast, by stimulating NK cells and by enhancing tumour necrosis factor-α (TNF-α) secretion by macrophages. Also, trophoblasts

stimulate release of pro-fertility Th_2 cytokines from T lymphocytes (so-called "Th_2 bias"), while suppressing the anti-fertility Th_1 bias. Progesterone (P) favours the Th_2 bias, thus stimulating the release from T lymphocytes of LIF, which in turn favours fetal implantation and survival (Szekenes-Bartho and Wegmann 1996; Stewart and Cullinan 1997; Duval et al. 2000).

P also stimulates FAAH activity and expression in human T lymphocytes (Maccarrone et al. 2001) by enhancing the promoter activity of the *FAAH* gene (Maccarrone et al. 2003c). Regulation of FAAH expression was observed also upon lymphocyte treatment with Th_1/Th_2 cytokines: IL-4 and IL-10 enhanced FAAH, while IL-2 and INF-γ reduced it (Maccarrone et al. 2001). Unlike FAAH, the other proteins of the endocannabinoid system were not affected by P or by any of the cytokines tested (Maccarrone et al. 2001), pointing to FAAH as the "check point" for AEA degradation during pregnancy.

4.4 Perspectives

The reported findings clearly show that in mammals ligand-receptor signalling with endocannabinoids is intimately associated with embryo–uterine interactions during implantation, and that in humans low FAAH in lymphocytes correlates with spontaneous abortion. This calls for attention to AEA-hydrolase as a key point in the control of the endocannabinoid system during pregnancy. Moreover, the results seem to add the endocannabinoids to the hormone-cytokine networks responsible for embryo–uterine interactions, and might represent a useful framework for the interpretation of novel interactions between progesterone, FSH, leptin, Th_1/Th_2 cytokines and (endo)cannabinoids, which appear to regulate both female sexual receptivity and male reproduction.

An interesting possibility raised by the data is that quantitation of FAAH protein in lymphocytes, which is easy to measure in routine analyses, might become an accurate marker of spontaneous abortion in humans. Such markers have long been sought, because of their potential diagnostic value, but they are not yet available or are still restricted to specific clinical situations.

5 General Conclusions

The endocannabinoid system contributes to the control of hypothalamic regulatory mechanisms. We do not know (yet) in which part of the hypothalamus the endocannabinoids are synthesized, but it is possible that cannabinoid receptors, present in the hypothalamus, are activated by AEA or other endocannabinoids released/synthesized quite far away. There is also the possibility that endocannabinoids act on presynaptic membranes to modulate the release of various neurotransmitters. Also of interest is the hypothesis that endocannabinoids may participate in hormone-cytokine networks that regulate reproduction, as this opens new perspectives for the development of novel medicines for human infertility.

Acknowledgements.
M.M. wishes to thank Prof. A. Finazzi-Agrò (Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata") for continuing interest and support, and all colleagues who have contributed to the research on endocannabinoids and fertility performed in his laboratory. This investigation was partly supported by Ministero dell'Istruzione, dell'Università e della Ricerca (Cofin 2002 and 2003 to M.M.), Rome. The research grants of Medical Research Council of Hungary (ETT) 003/03 and Hungarian Research Foundation (OTKA) No. T-034365 to T.W. are gratefully acknowledged.

References

Adams IB, Compton DR, Martin BR (1998) Assessment of anandamide interaction with the cannabinoid brain receptor: SR 141716A antagonism studies in mice and autoradiographic analysis of receptor binding in rat brain. J Pharmacol Exp Ther 284:1209–1217

Ambrosini A, Zolese G, Wozniak M, Genga D, Boscaro M, Mantero F, Balercia G (2003) Idiopathic infertility: susceptibility of spermatozoa to in-vitro capacitation, in the presence and the absence of palmitylethanolamide (a homologue of anandamide), is strongly correlated with membrane polarity studied by Laurdan fluorescence. Mol Hum Reprod 9:381–388

Ayalon D, Nir L, Cordova T, Bauminger S, Puder M, Naor Z, Kashi B, Zor U, Harrell A, Lindner HR (1977) Acute effects of Δ9-tetrahydrocannabinol on the hypothalamo-pituitary-ovarian axis in rat. Neuroendocrinology 23:31–42

Bisogno T, Melck D, Bobrov Myu, Grestaya NM, Bezuglov VV, De Petrocellis L, Di Marzo (2000) N-acyl-dopamines: novel synthetic CB1-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. Biochem J 351:817–824

Butrica JL (2002) The medical use of cannabis among the Greek and Romans. J Cannabis Ther 2:51–70

Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P (1995) Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546–549

Chakravarty I, Sheth AR, Ghosh JJ (1975) Effects of acute Δ9-tetrahydrocannabinol treatment of serum luteinizing hormone and prolactin levels in adult female rats. Fertil Steril 26:947–948

Chakravarty I, Sheth PR, Sheth AR, Ghosh JJ (1982) Delta-9-tetrahydrocannabinol: its effect on hypothalamo-pituitary system in male rats. Arch Androl 8:25–27

Chang MC, Berkery D, Schuel R, Laychock SG, Zimmerman AM, Zimmerman S, Schuel H (1993) Evidence for a cannabinoid receptor in sea urchin sperm and its role in blockade of the acrosome reaction. Mol Reprod Dev 36:507–516

Collu R (1976) Endocrine effects of chronic intraventricular administration of Δ9-tetrahydrocannabinol to prepuberal and adult male rats. Life Sci 18:223–230

Corchero J, Manzanares J, Fuentes JA (2001) Role of gonadal steroids in the corticotropin-releasing hormone and proopiomelanocortin gene expression response to Δ9-tetrahydrocannabinol in the hypothalamus of the rat. Neuroendocrinology 74:185–192

Crawford V (2002) A homelie herbe: medicinal cannabis in early England. J Cannabis Ther 2:71–80

Dalterio SL, Michael SD, Macmillan BT, Bartke A (1981) Differential effects of cannabinoid exposure and stress on plasma prolactin, growth hormone and corticosterone levels in male mice. Life Sci 28:761–766

Das SK, Paria BC, Chakraborty I, Dey SK (1995) Cannabinoid ligand-receptor signaling in the mouse uterus. Proc Natl Acad Sci USA 92:4332–4336

Dewey WI (1986) Cannabinoid pharmacology. Pharmacol Rev 38:151–178

Di Marzo V, Breivogel C, Bisogno T, Melck D, Patrick G, Tao Q, Szallasi A, Razdan RK, Martin BR (2000) Neurobehavioral activity in mice of N-vanillyl-arachidonoyl-amide. Eur J Pharmacol 406:363–374

Di Marzo V, Goparaju SK, Wang L, Liu J, Bátkai S, Járai Z, Fezza F, Miura GI, Palmiter RD, Sugiura T, Kunos G (2001) Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 410:822–825

Di Marzo V, De Petrocellis L, Fezza F, Ligresti A, Bisogno T (2002) Anandamide receptors. Prostaglandins Leukot Essent Fatty Acids 66:377–391

Doms RW Jr, Nichols SF, Harclerode J (1981) The effects of Δ9-tetrahydrocannabinol and phencyclidine hydrochloride on gonadotrophs in rat anterior pituitary. Fed Proc 40:279

Duval D, Reinhardt B, Kedinger C, Boeuf H (2000) Role of suppressors of cytokine signaling (Socs) in leukemia inhibitory factor (LIF)-dependent embryonic stem cell survival. FASEB J 14:1577–1584

Fernandez-Ruiz JJ, Munoz RM, Romero J, Villanua MA, Makryannis A, Ramos JA (1997) Time course of the effects of different cannabimimetics on prolactin and gonadotrophin secretion: evidence for the presence of CB1 receptors in hypothalamic structures and their involvement in the effects of cannabimimetics. Biochem Pharmacol 53:1919–1927

Friedman JM, Halaas JL (1998) Leptin and regulation of body weight in mammals. Nature 395:763–770

Funahashi H, Yada T, Muroya S, Takigawa M, Ryushi T, Horie S, Nakai Y, Shioda S (1999) The effect of leptin on feeding-regulating neurones in the rat hypothalamus. Neurosci Lett 264:117–120

Geber WF, Schramm LC (1969) Effect of marihuana extract on fetal hamsters and rabbits. Toxicol Appl Pharmacol 14:276

Gonzales S, Bisogno T, Wenger T, Manzanares J, Milone A, Berrendero F, Di Marzo V, Ramos JA, Fernandez-Ruiz JJ (2000) Sex steroid influence on cannabinoid CB1 receptor mRNA and endocannabinoid levels in the anterior pituitary gland. Biochem Biophys Res Commun 270:260–266

Gonzalez S, Manzanares J, Berrendero F, Wenger T, Corchero J, Bisogno T, Romero J, Fuentes JA, Di Marzo V, Ramos JA, Fernandez-Ruiz J (1999) Identification of endocannabinoids and CB1 receptor mRNA in the pituitary gland. Neuroendocrinology 70:137–145

Griswold MD (1995) Interactions between germ cells and Sertoli cells in the testis. Biol Reprod 52:211–216

Hall W, Solowij N (1998) Adverse effects of cannabis. Lancet 352:1611–1616

Hanus L, Abu-Lafi S, Friede E, Breuer A, Vogel Z, Shalev DE, Kustanovich I, Mechoulam R (2001) 2-Arachidonyl glyceryl ether, an endogenous agonist of the CB1 receptor. Proc Natl Acad Sci USA 98:3662–3665

Herkenham M (1995) Localisation of cannabinoid receptors in brain and periphery. In: Pertwee RG (ed) Cannabinoid receptors. Academic Press, London, San Diego, p 145

Kolodney RC, Masters WH, Kolodner RM, Toro G (1974) Depression of plasma testosterone levels after chronic intensive marihuana use. N Engl J Med 290:872–874
Kramer J, Ben David M (1974) Suppression of prolactin secretion by acute administration of Δ9-THC in rats. Proc Soc Exp Biol Med 147:482–484
Kumar MSA, Chen CL (1983) Naloxone blocks the effects of Δ9-tetrahydrocannabinol on serum luteinizing hormone and prolactin in rats. Subst Alcohol Actions Misuse 4:347–353
Levine JE (2000) The hypothalamus as a major integrative center. In: Conn PM, Freeman ME (eds) Neuroendocrinology in physiology and medicine. Humana Press, Totowa, p 75
Lozano I (2001) The therapeutic use of Cannabis sativa (L.) in Arabic medicine. J Cannabis Ther 1:63–70
Maccarrone M, Valensise H, Bari M, Lazzarin N, Romanini C, Finazzi-Agrò A (2000) Relation between decreased anandamide hydrolase concentrations in human lymphocytes and miscarriage. Lancet 355:1326–1329
Maccarrone M, Valensise H, Bari M, Lazzarin N, Romanini C, Finazzi-Agrò A (2001) Progesterone up-regulates anandamide hydrolase in human lymphocytes: role of cytokines and implications for fertility. J Immunol 166:7183–7189
Maccarrone M, Falciglia K, Di Rienzo M, Finazzi-Agrò A (2002) Endocannabinoids, hormone-cytokine networks and human fertility. Prostaglandins Leukot Essent Fatty Acids 66:309–317
Maccarrone M, Di Rienzo M, Finazzi-Agrò A, Rossi A (2003a) Leptin activates the anandamide hydrolase promoter in human T lymphocytes through STAT3. J Biol Chem 278:13318–13324
Maccarrone M, Cecconi S, Rossi G, Battista N, Pauselli R, Finazzi-Agrò A (2003b) Anandamide activity and degradation are regulated by early postnatal ageing and follicle-stimulating hormone in mouse Sertoli cells. Endocrinology 144:20–28
Maccarrone M, Bari M, Di Rienzo M, Finazzi-Agrò A, Rossi A (2003c) Progesterone activates fatty acid amide hydrolase (FAAH) promoter in human T lymphocytes through the transcription factor Ikaros. Evidence for a synergistic effect of leptin. J Biol Chem 278:32726–32732
Mani SK, Mitchell A, O'Malley BW (2001) Progesterone receptor and dopamine receptors are required in Δ9-tetrahydrocannabinol modulation of sexual receptivity in female rats. Proc Natl Acad Sci USA 98:1249–1254
Manzanares J, Corchero J, Fuentes JA (1999) Opioid and cannabinoid receptor-mediated regulation of the increase in adrenocorticotropin hormone and corticosterone plasma concentrations induced by central administration of Δ9-tetrahydrocannabinol in rats. Brain Res 839:173–179
Marks BH (1973) Δ9-TetraHydroCannabinol and luteinizing hormone secretion. Prog Brain Res 39:331–338
Maruta O, Shioda S, Funahashi H, Nakai Y (1999) Immunocytochemical localisation of leptin receptor in rat hypothalamus. Showa Univ J Med Sci 11:75–82
Mechoulam R, Gaoni Y (1965) A total synthesis of dl-delta-tetrahydrocannabinol, the active constituent of hashish. J Am Chem Soc 87:3273–3275
Moldrich G, Wenger T (2000) Localization of the CB1 cannabinoid receptor in the rat brain. An immunohistochemical study. Peptides 21:1735–1742
Moreau JJ (1845) De hachish et de l'alienation mentale: etudes psychologiques. Fortin Masson, Paris
Ness RB, Grisso JA, Hirschinger N, Markovic N, Shaw LM, Day NL, Kline J (1999) Cocaine and tobacco use and the risk of spontaneous abortion. N Engl J Med 340:333–339
Newton SC, Murphy LL, Bartke A (1993) In vitro effects of psychoactive and non-psychoactive cannabinoids on immature rat Sertoli cell function. Life Sci 53:1429–1434
Oomura Y, Ono T, Ooyama H, Wayner MJ (1969) Glucose and osmosensitive neurones of the rat hypothalamus. Nature 222:282–284
Orth JM, McGuinness MP, Qiu J, Jester WF Jr, Li LH (1998) Use of in vitro systems to study male germ cell development in neonatal rats. Theriogenology 49:431–439

Pagotto U, Marsicano G, Fezza F, Theodoropoulou M, Grubler Y Arzberger T, Milone A, Losa M, Di Marzo V, Lutz B, Stalla GK (2001) Normal human pituitary gland and pituitary adenomas express cannabinoid receptor type 1 and synthesise endogenous cannabinoids: first evidence for a direct role of cannabinoids on hormone modulation at the human pituitary level. J Clin Endocrinol Metab 86:2687–2696

Paria BC, Dey SK (2000) Ligand-receptor signaling with endocannabinoids in preimplantation embryo development and implantation. Chem Phys Lipids 108:211–220

Paria BC, Deutsch DD, Dey SK (1996) The uterus is a potential site for anandamide synthesis and hydrolysis: differential profiles of anandamide synthase and hydrolase activities in the mouse uterus during the periimplantation period. Mol Reprod Dev 45:183–192

Paria BC, Song H, Wang X, Schmid PC, Krebsbach RJ, Schmid HH, Bonner TI, Zimmer A, Dey SK (2001) Dysregulated cannabinoid signaling disrupts uterine receptivity for embryo implantation. J Biol Chem 276:20523–20528

Paxinos G, Watson C (1997) The rat brain in steretaxic coordinates. Academic Press, San Diego

Pertwee RG (1985) Effects of cannabinoids on thermoregulation: a brief review. In: Harvey DJ (ed) Marihuana '84. IRL Press, London, p 263

Pertwee RG (1992) In vivo interactions between psychotropic cannabinoids and other drugs involving central and peripheral neurochemical mediators. In: Murphy L, Bartke A (eds) Marijuana/cannabinoids. Neurobiology and neurophysiology. CRC Press, Boca Raton, p 165

Peters H, Nahas G (1999) A brief history of four millennia (B.C. 2000-A.D. 1974). In: Nahas GG, Sutin KM, Harvey DJ, Agurell S (eds) Marihuana and medicine. Humana Press, Totowa, p 3

Piccinni MP, Beloni L, Livi C, Maggi E, Scarselli G, Romagnani S (1998) Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. Nat Med 4:1020–1024

Rettori V, Wenger T, Snyder G, Dalterio S, McCann SM (1988) Hypothalamic action of delta9-tetrahydrocannabinol to inhibit the release of prolactin and growth hormone in the rat. Neuroendocrinology 47:498–503

Rodriguez de Fonseca F, Cebeira M, Ramos JA, Martin M, Fernandez-Ruiz JJ (1994) Cannabinoid receptors in the rat brain areas: sexual differences, fluctuations during estrous cycle and changes after gonadectomy and sex steroid replacement. Life Sci 54:159–170

Romero J, Wenger T, deMiguel R, Ramos JA, Fernández-Ruiz JJ (1998) Cannabinoid receptor binding did not vary in several hypothalamic nuclei after hypothalamic deafferentation. Life Sci 63:351–356

Samson WK, Snyder G, Fawcett CP, McCann SM (1980) Chromatographic and biological analysis of ME and OVLT LHRH. Peptides 1:97–102

Schmid PC, Paria BC, Krebsbach RJ, Schmid HHO, Dey SK (1997) Changes in anandamide levels in mouse uterus are associated with uterine receptivity for embryo implantation. Proc Natl Acad Sci USA 94:4188–4192

Schuel H, Goldstein E, Mechoulam R, Zimmerman AM, Zimmerman S (1994) Anandamide (arachidonylethanolamide), a brain cannabinoid receptor agonist, reduces sperm fertilizing capacity in sea urchins by inhibiting the acrosome reaction. Proc Natl Acad Sci USA 91:7678–7682

Schuel H, Burkman LJ, Lippes J, Crickard K, Forester E, Piomelli D, Giuffrida A (2002a) N-acylethanolamines in human reproductive fluids. Chem Phys Lipids 121:211–227

Schuel H, Burkman LJ, Lippes J, Crickard K, Mahony MC, Giuffrida A, Picone RP, Makriyannis A (2002b) Evidence that anandamide-signaling regulates human sperm functions required for fertilization. Mol Reprod Dev 63:376–387

Scorticati C, Mohn C, De Laurentiis A, Vissio P, Fernandez-Solari C, Seilicovich A, McCann SM, Rettori V (2003) The effect of anandamide on prolactin secretion is modulated by estrogen. Proc Natl Acad Sci USA 100:2134–2139

Smith CG, Besch NF, Asch RH (1980) Effects of marihuana on reproductive system. In: Thomas JA (ed) Advances in sex hormone research, vol. 4. Urban and Swarzenberg, Baltimore, p 273

Steger RW, Silverman AY, Siler-Khodr TM, Asch RH (1980) The effects of Δ9-tetrahydrocannabinolon the positive and negative feedback control of luteinizing hormone release. Life Sci 27:1911–1916

Stewart CL, Cullinan EB (1997) Preimplantation development of the mammalian embryo and its regulation by growth factors. Dev Genet 21:91–101

Sugiura T, Kondo S, Sukagawa A, Tonegawa T, Nakane S, Yamashita A, Waku K (1996) Enzymatic synthesis of anandamide an endogenous cannabinoid receptor ligand through N-acylphosphatidylethanolamine pathway in testis: involvement of Ca2+-dependent transacylase and phosphodiesterase activities. Biochem Biophys Res Commun 218:113–117

Sulcova E, Mechoulam R, Friede E (1998) Biphasic effects of anandamide. Pharmacol Biochem Behav 59:347–352

Szekenes-Bartho J, Wegmann TG (1996) A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. J Reprod Immunol 31:81–95

Takahashi N, Patel HR, Qi Y, Dushay J, Ahima RS (2002) Divergent effects of leptin in mice susceptible or resistant to obesity. Horm Metab Res 34:691–697

Tartaglia LA, Devos R, Richard GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Monarty A, Moore KJ, Smutko JS, Mays GG, Woof EA, Monroe CA, Tepper RI (1995) Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263–1271

Tyrey C (1980) Δ9-tetrahydrocannabinol: a potent inhibitor of episodic luteinizing hormone secretion. J Pharmacol Exp Ther 213:306–308

Tyrey C (1984) Endocrine aspects of cannabinoid action in female subprimates: search for site of action. In: Braude MC, Ludford JP (eds) Marijuana effects on the endocrine and reproductive systems. NIDA Research Monograph Ser 44, Rockville, p 65

Weidenfeld J, Feldman S, Mechoulam R (1994) Effect of the brain constituent anandamide, a cannabinoid receptor agonist, on the hypothalamo-pituitary-adrenal axis in the rat. Neuroendocrinology 59:110–112

Wenger T, Rettori V, Snyder G, Dalterio S, McCann S (1987) Effects of Δ9-tetrahydrocannabinol (THC) on the hypothalamic-pituitary control of LH and FSH secretion in adult male rats. Neuroendocrinology 46:488–493

Wenger T, Jamali KA, Juaneda C, Leonardelli J, Tramu G (1997) Arachidonoyl ethanolamide (anandamide) activates the parvocellular part of hypothalamic paraventricular nucleus. Biochem Biophys Res Commun 237:724–728

Wenger T, Fernandez-Ruiz J, Ramos J (1999) Immunohistochemical demonstration of CB1 receptors in the anterior lobe of the pituitary gland. J Neuroendocrinol 11:873–878

Wenger T, Ledent C, Csernus V, Gerendai I (2001) The central cannabinoid receptor inactivation suppresses endocrine reproductive functions. Biochem Biophys Res Commun 284:363–368

Williams CM, Kirkham TC (1999) Anandamide induces overeating meditation by central (CB1) receptors. Psychopharmacology (Berl) 143:315–317

Williams CM, Rogers PJ, Kirkham TC (1998) Hyperphagia in prefeed rats following oral Δ9-THC. Physiol Biol Behav 65:343–346

Yang Z-M, Paria BC, Dey SK (1996) Activation of brain-type cannabinoid receptors interferes with preimplantation mouse embryo development. Biol Reprod 55:756–761