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Alcohol and endocannabinoids: Neuroendocrine interactions in the reproductive axis

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ABSTRACT

Marihuana and alcohol consumption affect adversely reproduction by inhibiting the hypothalamic–pituitary–gonadal axis. The endocannabinoid system, present in the central nervous system and in peripheral tissues, participates in the regulation of hormones involved in the reproductive physiology such as luteinizing hormone, prolactin and oxytocin. This system is activated in response to pathophysiological conditions such as stress and inflammatory/infectious states as well as alcoholism and drug consumption acting as a negative modulator of reproductive function. The secretion of luteinizing hormone from the adenohypophysis is reduced, mainly through hypothalamic inhibitory action of cannabinoids and alcohol on luteinizing hormone releasing hormone release from its nervous terminals in the median eminence. This inhibitory effect is mediated, at least in part, by the activation of cannabinoid type 1 receptors. Cannabinoids also inhibit prolactin release from the lactotropes in the adenohypophysis acting locally and by increasing the release of hypothalamic dopamine mainly from tuberoinfundibular dopaminergic neurons in the external layer of the median eminence. On the contrary, ethanol stimulates prolactin release from the adenohypophysis as well as oxytocin from the neurohypophysis. Besides, endocannabinoids modulate oxytocin synthesis and release from the hypothalamic magnocellular neurons and neurohypophysis. In summary, all the results exposed in the present review suggest that there is interplay between the endocannabinoid system, hormones and neuropeptides in the control of reproduction and that this system mediates, at least in part, ethanol adverse effects on reproductive function.

Contents

Introduction

The hypothalamus is the area of the brain where the majority of neuroendocrine factors involved in the control of reproduction are produced. Different pathophysiological conditions such as endotoxemia, stress, alcoholism and drug consumption show alterations in the normal synthesis and release of these neuroendocrine factors. It is very important to consider the effects of alcohol and the participation of the endocannabinoid system on reproductive regulatory mechanisms, because young people usually start with alcohol and cannabinoids consumption while they are still in puberty, period during which the neuroendocrine regulation of reproduction is very vulnerable. Also, other fragile life stages are pregnancy and nursing, since they are very important processes that involve fine tuning of hormonal secretion and could be altered by alcohol ingestion and cannabinoid consumption. As example is the well-known fetal alcohol syndrome, a disorder that can occur to the embryo when a pregnant woman ingests alcohol.

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Alcohol is one of the most abused drugs that produce depression of the central nervous system (CNS) and several morphological and physiological alterations in humans. Alcohol appears to act through a multitude of mechanisms, instead of a single fundamental process (Vengeliene et al., 2008). Molecular and pharmacological studies demonstrated that ethanol (EtOH) induces neurochemical changes in various brain areas, in particular on GABAergic, glutamatergic, cholinergic, serotonergic and catecholaminergic neuronal systems (Nevo and Hamon, 1995). The primary targets are the membrane-bound-ligand-gated ion channels and voltage-dependent ion channels, such as *N*-methyl-D-aspartic acid (NMDA), $GABA_A$, serotonin and nicotinic cholinergic receptors as well as L-type Ca^{2+} channels and G-protein-activated inwardly rectifying K^+ channels (Vengeliene et al., 2008; Dopico and Lovinger, 2009). Furthermore, it was suggested that EtOH could act directly on cell membranes rafts by nonspecific interactions with lipidic components and therefore disrupting protein–lipid interactions (Szabo et al., 2007). Also, the molecule of EtOH can easily cross the cell membranes affecting intracellular proteins such as protein kinase A and C and therefore their pathways. Following this first hit of EtOH on specific targets in the brain, a second wave of indirect effects on a variety of neurotransmitter/neuropeptide systems are initiated leading to behavioral effects of alcohol, ranging from disinhibition to sedation and even hypnosis depending on the amount of EtOH consumed (Davies, 2003; Jia et al., 2008).

Early studies reported the inhibitory effects of the main psychoactive ingredient of *Cannabis sativa*, Δ^9-tetrahydrocannabinol (THC) on luteinizing hormone (LH) and prolactin (PRL) secretion (Wenger et al., 1999a). Then, with the discovery of the presence of cannabinoid receptors and the production of endogenous cannabinoids in the hypothalamus and pituitary, it became evident that endocannabinoids (ECs) control the regulation of reproduction at hypothalamic and pituitary levels (Olah et al., 2008; Fernandez-Solari et al., 2004). Since the activity of the anterior pituitary is under the influence of circulating sex steroids and several differences in the EC system were registered between sexes (Scorticati et al., 2003; Scorticati et al., 2004), in this review we focused on the role played by the EC system in hormone secretion in male and ovariectomized female rats.

Cannabinoids affect different physiological functions by binding to specific receptors. THC exerts its effects through GTP-binding protein coupled receptors: the cannabinoid 1 (CB1) receptor subtype, which is mainly expressed in the brain (Devane et al., 1988; Herkenham et al., 1990; Pacher et al., 2006), and the cannabinoid 2 (CB2) receptor subtype, which appears particularly abundant in the immune system but also exist in the CNS (Munro et al., 1993). Both CB1 and CB2 receptors are functionally linked to inhibition of adenylyl cyclase (AC) (Howlett and Fleming, 1984). After the discovery of cannabinoid receptors, 20 years ago, endogenous substances that bind to these receptors were discovered. There are two main ECs, arachidonoyl ethanolamide, also called "anandamide" (AEA), and 2-arachidonoyl glycerol (2-AG), both derivatives of arachidonic acid (Pacher et al., 2006; Mechoulam et al., 1998). These ECs are synthesized and released upon demand and act as retrograde signaling messengers in GABAergic and glutamatergic synapses and as modulators of postsynaptic transmission interacting with several neurotransmitters. Anandamide and 2-AG are transported into cells by specific uptake systems and degraded by the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase, respectively. Anandamide binds with high affinity to CB1 and CB2 receptors and also produces different effects by the activation of transient receptor potential vanilloid type 1 (TRPV1) channels reported to be also located in nervous tissues as well as in vessels (Cristino et al., 2006). Additionally, selective antagonists have been developed for CB1 receptors, such as AM251 and SR141716A and for CB2, such as AM630 and SR144528. Endocannabinoids together with their receptors, transporters and enzymes that synthesize and degrade them constitute the EC system. The EC system may be a widespread tuning system of numerous finely regulated tasks, and its importance is not limited to the central nervous functions but may concern several peripheral processes (Pacher et al., 2006). The EC system participates in the modulation of neurotransmitters release and in the hormonal regulation of food intake, cardiovascular, gastrointestinal, immune, behavioral and reproductive functions (Mouslech and Valla, 2009). Recent advances have correlated the EC system with drug addiction and alcoholism. Exogenous and endogenous cannabinoids as well as EtOH were shown to alter the normal production and release of neurohormones from the hypothalamus (Fernandez-Solari et al., 2004; Rettori et al., 1987; Lomniczi et al., 2000; da Veiga et al., 2008). The activation of the EC system acts in parallel with changes in the activity of several neurotransmitters including GABA and dopamine (DA) (Pistis et al., 2002) that also could be altered by EtOH. Moreover, *in vitro* studies demonstrated that EtOH as well as THC increase GABA content in the medial basal hypothalamus (MBH) (de Miguel et al., 1998; Noto and Myers, 1984). Therefore, the similarities observed in EtOH and cannabinoids regarding neuroendocrine actions suggest a possible interplay between EtOH and the EC system in the inhibition of the reproductive function.

Effects of ethanol, THC and endocannabinoids on hypothalamic–adenohypophyseal reproductive axis

Effects on luteinizing hormone releasing hormone (LHRH) release from the hypothalamus and luteinizing hormone (LH) release from the adenohypophysis

Luteinizing hormone releasing hormone (LHRH) is the key neuropeptide that triggers reproductive behavior and physiology. This neuropeptide is synthesized in hypothalamic neurons, mostly found in the preoptic area and in the arcuate nucleus, whose axonal terminals are located in the median eminence containing LHRH vesicles that respond with exocytosis to depolarizing stimuli on the neuronal membrane (Rettori et al., 1993).

It has been shown that glutamic acid acts on NMDA receptors on noradrenergic neurons increasing the release of norepinephrin (NE) in the preoptic area of the hypothalamus. Thus, NE activates neural nitric oxide synthase (nNOS) in nitridergic neurons and the nitric oxide (NO) released diffuses to LHRH terminals, where it induces the release of LHRH (Lomniczi et al., 2000; Rettori et al., 1993; Canteros et al., 1995). It has been shown that NO activates cyclooxygenase (COX) that increases prostanoids release (Rettori et al., 1992). Prostaglandin E2 (PGE2) by activating AC with the consequent increase in cAMP evokes exocytosis of LHRH granules by activation of protein kinase A. The released LHRH diffuses into the hypophyseal portal vessels that deliver it to the adenohypophysis where it acts on its receptors on gonadotropes to release LH and follicle stimulating hormone (FSH) (McCann and Rettori, 1996). In males, the primary role of FSH in spermatogenesis is stimulation of testicular Sertoli cell proliferation during prepubertal development, whereas LH acts on regulation of testosterone synthesis within the testis. Testosterone plays a main role in the maintenance of male fertility acting on morphological development and reproductive function (Battista et al., 2008).

Ethanol and THC can suppress the reproductive function in humans, monkeys and small rodents, such as the rat (Cicero et al., 1982; Dees et al., 1985; Murphy et al., 1994). On the basis of *in vitro* experiments using incubation of MBH of male rats, we have demonstrated that EtOH (50–100 mM) inhibits the NMDA-stimulated release of LHRH by increasing the release of two inhibitory neurotransmitters: β-endorphin and GABA (Lomniczi et al., 2000), which was confirmed since this inhibition was reversed by naltrexone, a μ-opioid receptor antagonist, and by bicuculline, a $GABA_A$ receptor antagonist. Furthermore, β-endorphin inhibits NOS activity while naltrexone increases basal NO levels (Faletti et al., 1999), indicating a tonic inhibitory β-endorphin

action on LHRH release. Also, the primary action of GABA appears to be to inhibit nitridergic activation of cyclooxygenase (Seilicovich et al., 1995). Therefore, the lower level of prostaglandins decreases AC activity with the consequent decrease of LHRH release. In *in vivo* experiments, using ovariectomized rats, which received EtOH via a permanent intragastric cannula, we showed marked decreases in the plasma concentration of LH and changes in its pattern of secretion (Dees et al., 1985). In contrast, EtOH did not significantly alter the pattern of FSH secretion in the same animals.

Regarding to the inhibitory effect of THC on the reproductive axis, other studies indicate that it is exerted mainly at hypothalamic level by inhibiting LHRH release with the consequent decrease of LH secretion by the adenohypophysis, but without effect on FSH (Ayalon et al., 1977). *In vitro* studies performed by our group in 1990 showed that THC (10^{-8} M) inhibited the NE (5×10^{-5} M) as well as the DA (5×10^{-5} M)-stimulated LHRH release from MBH explants of male rats (Rettori et al., 1990). Also, we demonstrated that a single dose of THC (2 μl of 10^{-6} M), injected into the third cerebral ventricle, decreased serum LH temporarily but did not alter serum FSH levels. The hypothalamic LHRH content was elevated by 30 min after the injection and persisted for 1 h, indicating that THC alters pituitary LH release by inhibiting the release of LHRH (Wenger et al., 1987). Furthermore, we showed the presence of CB1 receptors in the preoptic hypothalamic area and in the periventricular MBH of male rats, regions that contain the neuronal somas and terminals involved in the synthesis and release of LHRH, respectively (Rettori et al., 2002). Using double immunohistochemical techniques, no co-localization of CB1 receptors with LHRH immunoreactive neurons was observed. However, CB1-receptor immunoreactive neurons were shown adjacent to the third ventricle, an area that contains axons from LHRH neurons (Rettori et al., 2002).

Having demonstrated the presence of CB1 receptors in the MBH, we studied the effect of AEA on LHRH release. Firstly, we showed that AEA (10^{-9} M) decreases by 70% the NMDA-stimulated LHRH release from MBH incubated *in vitro* (Fernandez-Solari et al., 2004). Secondly, we demonstrated that the same concentration of AEA significantly decreases the forskolin-induced cAMP content and LHRH release. These inhibitory effects of AEA were prevented by the selective CB1 receptor antagonist, AM251 (10^{-5} M), confirming the participation of the EC system as inhibitor of LHRH release in male rats. Reports by others, working with immortalized hypothalamic LHRH neurons, support our findings since they demonstrated that the cannabinoid agonist, WIN 55,212-2, reduces LHRH release by acting through CB1 receptors, since AM251 totally blocked this inhibitory effect (Gammon et al., 2005). AEA (10^{-9} M) also significantly increased the release of GABA from MBH but had no effect on β-endorphin release. Moreover, bicuculline (10^{-4} M), a GABAergic antagonist, completely blocked the inhibitory effect of AEA on NMDA-stimulated LHRH release. However, the opioid receptor antagonist, naltrexone (10^{-6} M), did not modify the inhibitory effect of AEA (Fernandez-Solari et al., 2004). These data confirm that GABA mediates this ECs induced inhibition of LHRH release. Moreover, CB1 receptors have been observed on GABAergic neurons of the hypothalamus as well as of the corpus striatum (Hohmann and Herkenham, 2000). Supporting our findings, de Miguel et al. (1998) reported that an acute intraperitoneal administration of THC (5 mg/kg of body weight) reduced LH as well as PRL plasma levels and that the specific CB1 receptor antagonist, SR141716A (3 mg/kg of body weight, ip) attenuated both decreases. This was in parallel to an increase in the content of GABA in the MBH. Also, no changes in FSH levels were observed. On the other hand, chronic THC caused a marked reduction of CB1 receptor mRNA transcripts in the adenohypophysis of orchidectomized male rats which was reversed by replacement of dihydrotestosterone, suggesting the involvement of androgens feedback in CB1 receptor signaling system (Battista et al., 2008; Gonzalez et al., 1999).

Based on the demonstration that EtOH and AEA inhibited *in vitro* NMDA-stimulated LHRH release by increasing the release of GABA from MBH (Lomniczi et al., 2000), we studied the interplay between EtOH and the EC system. In fact, we have shown that EtOH (100 mM) inhibits forskolin-stimulated cAMP increase and LHRH release; inhibitory effects that are at least partially blocked by AM251, suggesting the involvement of CB1 receptors in the alcohol-induced blockade of reproductive function (Fernandez-Solari et al., 2004). The incomplete inhibition could be explained by the presence of the opioid system as another inhibitory pathway since, as was described above, EtOH increases the release of β-endorphin which also inhibits LHRH release (Lomniczi et al., 2000; Rettori et al., 2002).

Although, we did not observe co-localization of CB1 receptors with LHRH neurons as described above (Ayalon et al., 1977), CB1 receptors are present in different neurons of the hypothalamic area, such as GABAergic neurons. Furthermore, it has been shown that $GABA_A$ receptors co-localize with LHRH neurons in the hypothalamus (Spergel et al., 1999) suggesting that GABA could be the mediator between ECs and the LHRH secretion. In our studies both EtOH and AEA inhibit forskolin stimulation of cAMP and increase GABA release from the MBH, which is in agreement with Obrietan and van den Pol that demonstrate that an increase in cAMP can inhibit GABA release (Obrietan and van den Pol, 1997). Therefore, AEA and EtOH might inhibit LHRH release by decreasing cAMP content and increasing GABA release, a well-known inhibitor of LHRH release in adult rats.

Finally, AEA synthase activity did not change after expositing MBH to EtOH (100 mM) *in vitro* for 30 min. However, AEA synthase activity increases in MBH dissected 1 hour after intragastric administration of EtOH (3 g/kg) to male rats (Rettori et al., 2007). Therefore, the mechanism by which EtOH activates the EC system remains to be elucidated.

In summary, EtOH could increase AEA release from LHRH neurons which acts presynaptically on CB1 receptors located on GABAergic neurons. An action of EtOH on CB1 receptors could not be discarded. The activation of CB1 receptors respond by inhibiting AC activity with the consequent decrease in cAMP production and increase in GABA release. GABA binds to $GABA_A$ receptors located on LHRH neurons, hyperpolarizing their cell membranes by increasing chloride influx and therefore decreasing the amount of LHRH that reaches the adenohypophysis to stimulate gonadotropins release (see Diagram 1).

Additionally, is very important to consider that pituitary mechanisms have to be taken into consideration when looking at the effects of cannabinoids and alcohol on reproductive functions. The presence and expression of ECs and CB1 receptor genes have been described in the gonadotropes and lactotropes of the adenohypophysis (Gonzalez et al., 1999; Wenger et al., 1999b). A study has demonstrated that male animals have higher levels of CB1 receptor transcripts than females (Wenger et al., 1999b) while the pituitary AEA content is higher in females than in males (Gonzalez et al., 1999). Both exogenous and endogenous cannabinoids exert mainly inhibitory actions on the hormone secretion from gonadotropes (Wenger et al., 1999a). A very recent study demonstrates that CB1 receptors located in the anterior pituitary cells are involved, at least in part, in the neuroendocrine effects of cannabinoids (Olah et al., 2008).

Numerous studies showed that EtOH suppresses reproductive activity through the inhibition of LHRH secretion from the hypothalamus; however, the intracellular content of LH in cells of the adenohypophysis of male rats is also reduced by EtOH (Uddin et al., 1994). Therefore, the inhibition of LH synthesis and secretion may be additionally due to a direct effect of EtOH on gonadotropes. EtOH suppression of LH secretion from gonadotropes is also mediated at least in part through a decrease in PKC translocation to its active site at the pituitary cell membrane (Steiner et al., 1997).

Effects on hypothalamic dopamine and prolactin (PRL) release from the adenohypophysis

Prolactin, that is mainly synthesized and secreted by lactotropes of the adenohypophysis, was first characterized and named due to its

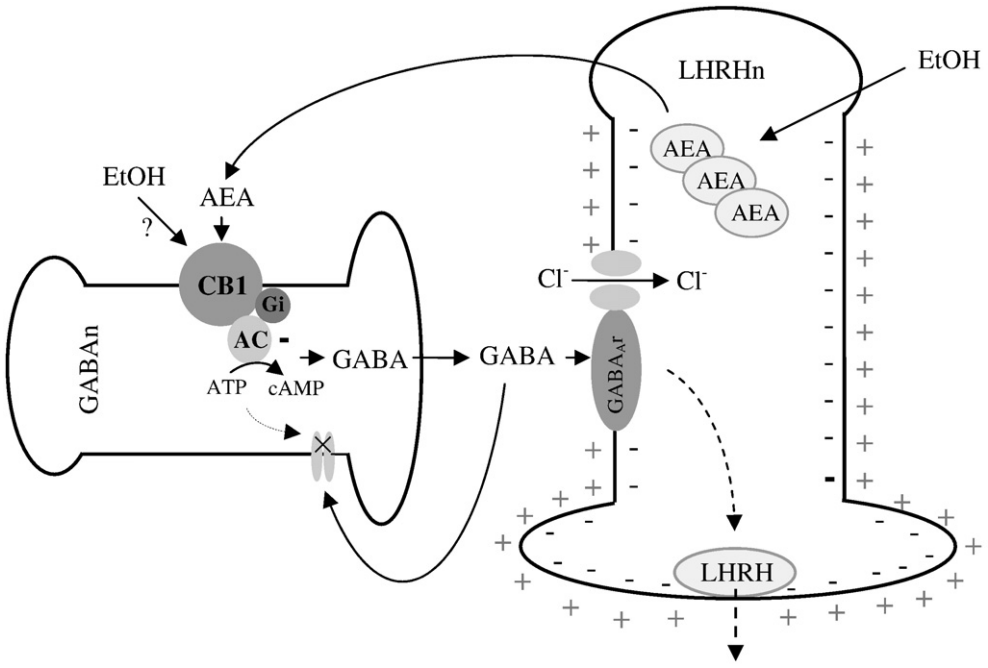

Diagram 1. Postulated mechanism of action of ethanol (EtOH) and anandamide (AEA) to suppress LHRH release. Ethanol (EtOH) could induce the synthesis and release of anandamide (AEA) from LHRH neurons (LHRHn). AEA binds to CB1 receptors located on GABAergic neuron (GABAn) inhibiting adenylyl cyclase (AC) activity with the consequent decrease in cAMP production and increasing GABA release. Then GABA binds to GABA type A receptors ($GABA_Ar$) located on LHRHn, hyperpolarizing its cell membranes by increasing chloride influx and therefore decreasing the secretion of LHRH. (Gi), GTP inhibitory binding protein. Solid arrows indicate stimulation. Dashed arrows indicate inhibition.

function to stimulate milk production. However, later was found to be produced in other tissues including the brain, mammary glands, the decidua and the immune system. It is considered now a pleiotropic hormone with a range of neuroendocrine functions, which include stimulation of maternal behavior, modulation of the stress response, osmoregulation, angiogenesis, stimulation of myelination in the CNS and participation as mediator in the immunoendocrine network (Grattan and Kokay, 2008). Dopamine is the major physiological inhibitory factor of PRL that inhibits the basal high-secretory tone of lactotropes. This inhibition is exerted by hypothalamic dopaminergic neurons of the periventricular and arcuate nuclei of the MBH. The released DA diffuses into the hypophysial portal capillaries and is transported by the long portal vessels to the anterior pituitary gland, where it acts on DA type 2 receptors (D2R) on lactotropes to inhibit PRL release (Ben-Jonathan et al., 2008). Prolactin is another hormone that is affected by ECs and EtOH.

Cannabinoids can affect brain DA concentrations and DA receptors (Tanda et al., 1997). We have demonstrated by *in vitro* and *in vivo* experiments that THC inhibits PRL release from the adenohypophysis of male rats by acting at hypothalamic level (Rettori et al., 1988). Moreover, we have shown that AEA, microinjected intracerebroventricularly (icv), inhibits PRL release from the adenohypophysis of male rats. Anandamide acts on CB1 receptors located on DAergic neurons in the MBH, activates DA release into the portal vessels and therefore inhibits PRL release (Scorticati et al., 2003). This effect of AEA on DA release from the MBH is similar to acute effects of THC on DAergic neurons in several other brain areas of the rat (Rodriguez de Fonseca et al., 1992). The presumed effect of AEA to increase synthesis and release of DA from the MBH was confirmed by incubating MBH explants in the presence of AEA (10^{-9} M), in which the release of DA was increased in the face of an increase in the DOPAC/DA ratio, indicating increased DA turnover (Scorticati et al., 2003).

On the other hand, EtOH acutely administered to male and ovariectomized rats caused an increase in serum PRL levels (Dees et al., 1985; Seilicovich et al., 1985). This effect of EtOH was associated mainly with the increased release of β-endorphin from MBH that inhibits DAergic activity with the consequent increase in PRL serum levels (Lomniczi et al., 2000). Besides, EtOH acts directly inhibiting hypothalamic DAergic neurons (Seilicovich et al., 1985). However, the stimulatory pathway mediated by β-endorphin is the predominant one. Therefore, the opposite effects of AEA and EtOH on PRL release could be explained by the lack of interaction of ECs with the opioid system. Also, it is interesting to point out that all these effects are dependent on the dose of EtOH administrated and the time when the measurements take place.

The research groups of Wang et al. (2003) and Basavarajappa et al. (1998) have found that cannabinoids and alcohol activate the same reward pathways. Indeed, both cannabinoids and alcohol cause the release of DA in the nucleus accumbens of CB1 receptor +/+ mice. CB1 receptor −/− mice (knockout for CB1 receptors) exhibited a complete lack of alcohol-induced dopamine release as compared to wild type mice (Hungund et al., 2003). Interference with DA-mediated neurotransmission could underlie some of the effects of AEA and EtOH on the hypothalamic–pituitary axis.

As we mentioned before, the demonstration of CB1 gene expression and immunoreactivity as well as ECs synthesis in the adenohypophysis (Gonzalez et al., 1999; Wenger et al., 1999b; Gonzalez et al., 2000) also explain why endocannabinoids were able to affect directly the release of anterior pituitary hormones, in particular PRL. One study evidences that AEA inhibits PRL secretion from dispersed anterior pituitary cells by binding to CB1 receptors (Wenger et al., 1999c), and expression of these receptors is subjected to sex steroids influence (Gonzalez et al., 2000). A very recent work confirms by *in vivo* studies that AEA diminished PRL plasma levels in CB1 receptor inactivated mice by binding to TRPV1 (Olah et al., 2008).

Whether or not alcohol modulates cellular prolactin synthesis and secretion is a controversial topic. PRL response to EtOH is dependent on the gender and age/sexual maturity of the animals as well as on the mode of administration (Emanuele et al., 2001).

Effects of ethanol, THC and endocannabinoids in the hypothalamic–neurohypophyseal reproductive axis

The hypothalamic–neurohypophyseal system is a neuroendocrine system essential for survival. It consists of the hypothalamic supraoptic nuclei (SON) that are situated lateral to the optic chiasm and the paraventricular nuclei (PVN) on each side of the third ventricle that contains many neuropeptides. Magnocellular neurons in these nuclei synthesize oxytocin (OXT) and vasopressin (VP) and send axonal projections to the posterior pituitary. Neuronal activity stimulates the release of these hormones into the blood, regulating a number of important physiological functions, mainly reproduction and homeostasis. In addition, in the PVN another population of OXT-staining cells, the parvocellular neurons, has been identified. These neurons terminate in different regions of the CNS. Oxytocin and VP are also released from perikarya, dendrites and/or axon collaterals of magnocellular neurons and act as neurotransmitters modulating its own activity and/or binding to its receptors located in more distant brain structures such as amygdala, hippocampus, nucleus accumbens and others (McDonald et al., 2008). OXT is a hormone best known for its role in parturition and lactation while VP is well known by its homeostatic functions since is the anti-diuretic hormone (Scorticati et al., 2003). In addition, OXT and VP have been shown to act as neurotransmitters in various regions of the CNS, thus controlling complex neuroadaptive processes including memory, learning and social behaviors (McDonald et al., 2008).

In 1991, Herkenham et al. (1991) first reported that CB receptors are localized in the PVN of the hypothalamus as well as in anterior and posterior pituitary lobes. Endocannabinoids have also been found in these tissues (Pagotto et al., 2006). It has been reported that ECs are released as retrograde messengers in the SON by magnocellular neurons (Murphy et al., 1998) and that CB1 receptors are localized within the SON (Wenger and Moldrich, 2002), suggesting that ECs could modulate OXT and VP neurons in this region. Recent reports have highlighted the interaction between the ECs and the modulation of the physiology of magnocellular neurons, since OXT and ECs cooperate to shape the electrophysiological properties of SON neurons (McDonald et al., 2008). Very recently, we studied the effect of ECs on OXT release. We performed *in vitro* studies testing the effect of AEA on OXT release from neurohypophysis (NH) (De Laurentiis et al., 2010) and MBH *in vitro* in normal conditions (Steiner et al., 1997). Several doses of AEA from 10^{-11} to 10^{-8} M significantly decreased OXT release from NH, being 10^{-9} M the most effective inhibitory dose. On the contrary, AEA 10^{-9} M stimulated OXT release from MBH and this effect was mediated by CB1 receptor since it was blockaded by AM251, its selective antagonist. Moreover, the inhibition of FAAH, the enzyme that degrades AEA, by URB-597 (10^{-10} and 5.10^{-10} M) increased OXT release from MBH (Steiner et al., 1997).

Nitric oxide acts as a local modulator of magnocellular neuronal activity. In fact, we have demonstrated that NO donors reduce OXT secretion from both NH and MBH *in vitro* (Rettori et al., 1997). Therefore, we studied the interrelation between the EC system, nitric oxide and OXT release. Our study showed that AEA increases NOS activity in the NH as well as in the hypothalamus. These results are in concordance with other findings suggesting that AEA binding to cannabinoid receptors leads to NO production in several cell types (Maccarrone et al., 2000; Fimiani et al., 1999). We found that the inhibitory effect of AEA on OXT secretion from the NH is mediated by NO since the scavenging of NO by hemoglobin or the inhibition of NOS by L-NAME completely blocked this inhibitory effect. On the other hand, the production of NO induced by AEA could act as negative feedback on OXT release from MBH. In fact, in the presence of hemoglobin the stimulatory effect of AEA on OXT release from MBH is much bigger (Steiner et al., 1997). Little is known about the expression and function of cannabinoid receptors in the posterior pituitary lobe. We have performed pharmacological experiments *in vitro* in which the NH were incubated in the presence of AEA and cannabinoid or vanilloid receptors selective antagonists. The CB2 and the TRPV1 receptors antagonists, AM630 and capsazepine, respectively, completely blocked the inhibitory effects of AEA on OXT release from NH. However, in the presence of the CB1 receptor antagonist AM251, the inhibitory effect of AEA persisted, suggesting that this subtype of cannabinoid receptor does not participate in OXT release from the NH (De Laurentiis et al., 2010).

In summary, AEA, acting through CB2 and TRPV1 receptors, increases the activity of NOS, increasing NO production that inhibits OXT release from the NH. At hypothalamic level, AEA, acting through CB1 receptor, increases OXT release and NOS activity, and the consequent NO increase produces a negative feedback, ending the stimulatory effect of AEA on OXT release from the MBH (See Diagram 2).

There still exists the belief that alcohol improves lactation, and lactating women have sometimes been encouraged to drink low or moderate doses of alcohol as a way to increase milk production. The production, secretion and ejection of milk are the results of highly synchronized neuroendocrine processes, which are governed also in part by suckling. Breast stimulation results in transient release of both, PRL and OXT. Prolactin is involved in mammary gland development, initiation of lactation and is essential for its maintenance. Oxytocin plays a key role in the milk let down during nursing. These two hormones behave in tandem in normal conditions, but alcohol consumption disrupts this hormonal milieu. The vast majority of studies reported that EtOH administration decreased suckling-induced PRL. The later findings suggest that alcohol increases PRL levels in maternal circulation and that OXT, rather than PRL, may be the principal way by which alcohol induces detrimental effects in lactation (Mennella et al., 2005). In fact, the efficacy of alcohol in blocking uterine contractions is due in part to its inhibition of OXT, a hormone that is also involved in contraction of cells that causes the ejection of milk from mammary gland. As well as alcohol, THC produces a transient suspension of milk ejection because interferes with the release of OXT in response to suckling (Tyrey and Murphy, 1988). Moreover, the EC system plays a vital role in milk suckling and hence in growth and development during early stages of mouse life. The blockade of CB1 receptors in newborn mouse pups had a devastating effect on milk ingestion and growth (Fride et al., 2001). A further study of the ECs signaling in the SON is necessary to understand aspects of the physiological function of OXT–ECs interactions. OXT facilitates its own release during delivery and lactation and ECs have an inhibitory action. However, the CB1 knockout mouse is able to deliver pups and to provide them with milk, indicating that ECs are not of major importance in the regulation of OXT during lactation (McDonald et al., 2008).

It was demonstrated that acute alcohol administration to lactating rats inhibits suckling-induced OXT release resulting in reduction of milk secretion (Subramanian, 1999). On the other hand, in prolonged EtOH exposure, numerous neurons of the hypothalamic magnocellular system degenerate, but the mRNA levels of OXT and VP are not decreased due to compensatory changes undergone by the surviving neurons (Silva et al., 2002).

In recent preliminary experiments, we showed that EtOH (100 mM) increased significantly OXT release from NH incubated *in vitro*. Moreover, AM630, a CB2 receptor selective antagonist, prevented this stimulatory effect, returning OXT to control levels. Therefore, the EC system could mediate the effects of alcohol in the regulation of hypothalamo–neurohypophyseal reproductive axis activity. However, additional experiments are needed to confirm this hypothesis.

It is well known that VP, also called anti-diuretic hormone, acts mainly in the homeostasis to maintain body fluids and also is co-secreted with the hypothalamic peptide corticotrophin releasing factor in response to stress (Rivier and Lee, 1996). Ingestion of EtOH is known to induce diuresis. The results of several studies performed about half a century ago strongly suggested that the enhanced diuresis

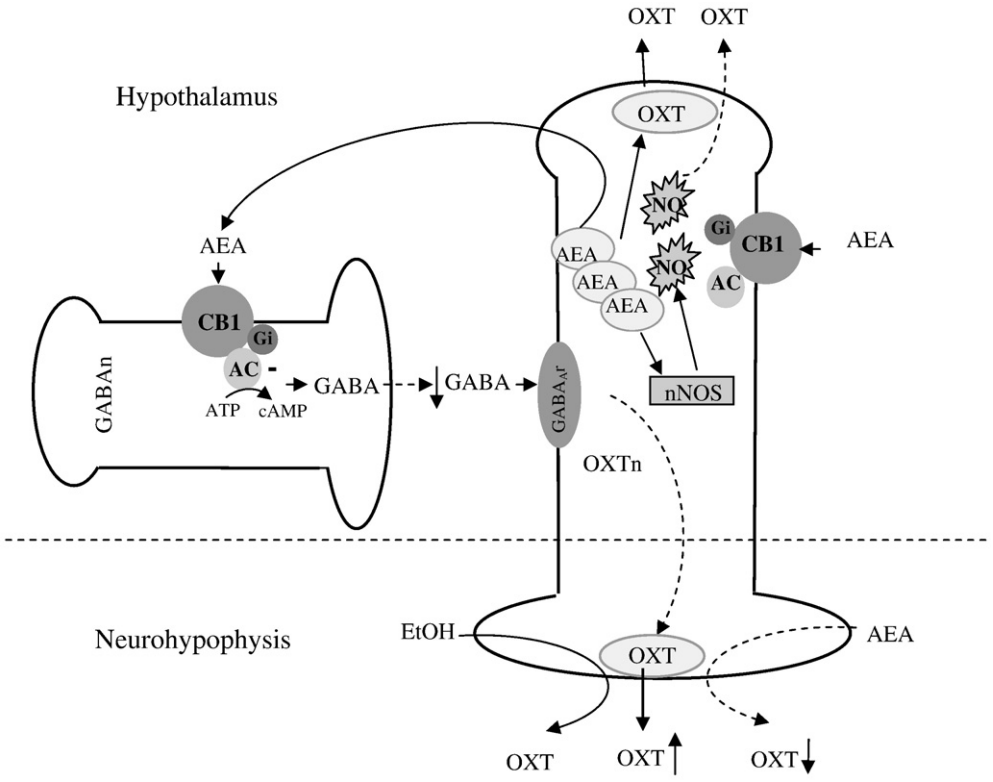

Diagram 2. Postulated mechanism of action of ethanol (EtOH) and anandamide (AEA) to suppress oxytocin (OXT) release. Activation of anandamide (AEA) synthesis and release from hypothalamic magnocellular oxytocin neurons (OXTn) could increase hypothalamic release of oxytocin (OXT). AEA activating neuronal nitric oxide synthase (nNOS) increases the production of nitric oxide (NO) that inhibits OXT hypothalamic release. Also, AEA could bind to presynaptically localized CB1 receptors located on GABAergic neurons (GABAn) resulting in a downregulation of GABA release. AEA signaling at GABAergic synapses is expected to result in increased secretion of OXT from axon terminals of the neurohypophysis. Finally, AEA could act at neurohypophyseal level decreasing OXT release. About ethanol effect, recent results demonstrate an increase in OXT release from neurohypophysis. Solid arrows indicate stimulation. Dashed arrows indicate inhibition. ↑: increase; ↓: decrease.

following alcohol administration is caused by a suppression of VP release and not by a direct effect of alcohol upon the kidneys (Parlesak et al., 2004). This effect was reported to be through EtOH inhibition of calcium currents in the nerve terminals and voltage-gated calcium channels might be the target of EtOH actions (Wang et al., 1991). It was also reported that *in vitro* EtOH concentrations (5–25 mM) induce inhibition of VP release from the median eminence, whereas higher EtOH concentrations (greater than 50 mM) potentiate its release (Brinton et al., 1986). *In vivo*, EtOH (126 mg %), a behaviorally relevant blood concentration, does not produce a significant difference in VP brain content, although there is a consistent trend towards an increase of VP in the hypothalamus and NH (Rivier and Lee, 1996). It could be postulated that in alcohol ingestion, at least two mechanisms work to maintain homeostasis within normal levels. First, neurohypophyseal system attempts to overcome with the inhibitory action of EtOH on the synthesis and release of VP, and second, the kidneys present an increase in their sensitivity to VP by changes in its receptors, so by this way there is a more efficient response to low levels of the circulating hormone (Zeballos et al., 2006).

On the other hand, recent results of our group demonstrated that AEA decreased VP secretion from incubated NH. AEA action was mediated by NO since the inhibition of NO synthesis completely blocked this inhibitory effect. CB2 and TRPV1 are involved in this inhibitory effect since AM630 and capsazepine, but not AM251, blocked AEA inhibitory effect (De Laurentiis et al., 2010), as was observed studying OXT release.

The similarities observed *in vitro* on the effect of EtOH and anandamide on OXT and VP secretion from the NH suggest that alcohol action could be mediated by the EC system.

Conclusions

From early studies, focusing upon the potent negative effects of Cannabis components on rodent, primate and human pregnancy, the role of cannabinoids stood out in the physiology and pathophysiology of reproduction. Marihuana and alcohol are among the main factors which negatively affect fertility in humans. The potent negative effects of cannabinoids and alcohol upon reproduction are largely documented, but the studies on the potential role of the EC system in the hormonal regulation related to alcohol impairment of reproductive functions are scarce.

According to literature and our experiments, we may conclude that the actions of endocannabinoids and EtOH on reproductive regulatory mechanisms occur through hypothalamic, pituitary and gonadal sites. The abovementioned observations suggest a role of the EC system in the fine tuning control of pituitary hormones secretion. In particular, the release of the LH, OXT and PRL may be inhibited by AEA that acts on both hypothalamic and pituitary levels. Moreover, ECs are synthesized in target reproductive organs, testis and ovary, where they act locally playing a physiological role in reproductive functions (Maccarrone, 2009). Similarly, alcohol intake suppresses reproductive function acting at several target sites at all levels of the hypothalamic–pituitary–gonadal axis. Although alcohol inhibits reproductive function acting mainly on the CNS, it can also directly suppress pituitary hormones synthesis and release and inhibit steroid hormone secretion from the gonads.

The results reported suggest that alcohol could trigger a relevant negative regulation of hypothalamic–adenohypophyseal and hypothalamic–neurohypophyseal axes by activating the EC system. The

Table 1
Summary of the main effects of Δ^9-tetrahydrocannabinol (THC), anandamide (AEA) and ethanol (EtOH) on luteinizing hormone releasing hormone (LHRH), luteinizing hormone (LH), prolactin (PRL) and oxytocin (OXT) in different rat models. ↑: increase; ↓: decrease; : no change.

Hormone	Compound/dose	Model	Effect	Reference
LHRH	THC, 10^{-8} M	Male rat, in vitro	↓ Hypothalamic release	Rettori et al. (1990)
	THC, 10^{-6} M, icv	Male rat, in vivo	↑ Hypothalamic content	Wenger et al. (1987)
	AEA, 10^{-9} M	Male rat, in vitro	↓ Hypothalamic release	Fernandez-Solari et al. (2004)
	EtOH 50 and 100 mM	Male rat, in vitro	↓ Hypothalamic release	Lomniczi et al. (2000)
LH	THC, 10^{-6} M, icv	Male rat, in vivo	↓ Plasma concentration	Wenger et al. (1987)
	AEA, 5 mg/kg, ip	Male rat, in vivo	↓ Plasma concentration	De Miguel et al. (1998)
	AEA, 20 ng/2 μl, icv	Male rat, in vivo	↓ Plasma concentration	Scorticati et al. (2004)
		OVX rat, in vivo	↓ Plasma concentration	Scorticati et al. (2004)
	EtOH, 3 g/kg, ig	OVX rat, in vivo	↓ Plasma concentration	Dees et al. (1985)
PRL	THC, 4 and 0.4 μg/2 μl, icv	Male rat, in vivo	↓ Plasma concentration	Rettori et al. (1988)
	THC, 5×10^{-8} M and 5×10^{-5} M	Male rat, in vitro	↓ Adenohypophyseal release	Rettori et al. (1988)
	AEA, 20 ng/2 μl, icv	Male rat, in vivo	↓ Plasma concentration	Scorticati et al. (2003)
		OVX rat, in vivo	= Plasma concentration	Scorticati et al. (2003)
	EtOH, 5 g/kg, ip	Male rat, in vivo	↑ Plasma concentration	Seilicovich et al. (1995)
	EtOH, 3 g/kg, ig	OVX rat, in vivo	↑ Plasma concentration	Dees et al. (1985)
OXT	AEA, 10^{-11} to 10^{-8} M	Male rat, in vitro	↑ Hypothalamic release	De Laurentiis et al. (2010)
			↓ Neurohypophyseal release	De Laurentiis et al. (2010)
	EtOH, 100 mM	Male rat, in vitro	↑ Neurohypophyseal release	Unpublished data

interactions between ethanol and the EC system are also demonstrated by other experimental approaches and in different brain areas not related to reproduction. For example, it was shown that self-administration of ethanol increased 2-AG in nucleus accumbens and that the pharmacological blockage of CB1 receptors decreases ethanol consumption (Caillé et al., 2007).

In conclusion, the EC system is tightly modulated in the hypothalamus, pituitary and gonadal tissues to ensure reproduction. Marihuana and alcohol exert potent effects on the homeostasis of this system. Furthermore, these substances disrupt the hypothalamic and pituitary control of hormones and sex steroids exerting powerful negative effects on reproductive health. The most relevant findings presented in this review are summarized in Table 1.

Now it is clear that Cannabis derivatives and alcohol are substances that should not be used, especially during pregnancy, since they can interact with ECs synthesis and metabolism. Further studies of the role of ECs in neuroendocrine regulation of reproduction will elucidate the degree of participation of these compounds in normal and pathophysiological conditions.

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References

Ayalon, D., Nir, I., Cordova, T., Bauminger, S., Puder, M., Naor, Z., Kashi, R., Zor, U., Harell, A., Lindner, H.R., 1977. Acute effect of Δ1-tetrahydrocannabinol on the hypothalamo–pituitary ovarian axis in the rat. Neuroendocrinology 23, 31–42.

Basavarajappa, B., Cooper, T., Hungund, B., 1998. Chronic ethanol administration down-regulates cannabinoid receptors in mouse brain synaptic plasma membrane. Brain Res. 793, 212–218.

Battista, N., Rapino, C., Di Tommaso, M., Bari, M., Pasquariello, N., Maccarrone, M., 2008. Regulation of male fertility by the endocannabinoid system. Mol. Cell. Endocrinol. 286, 17–23.

Ben-Jonathan, N., LaPensee, C.R., LaPensee, E.W., 2008. What can we learn from rodents about prolactin in humans? Endocr. Rev. 29, 1–41.

Brinton, R.E., Gruener, R., Deshmukh, P., Yamamura, H.I., 1986. In vitro inhibition of vasopressin release in brain by behaviorally relevant ethanol concentrations. Neurosci. Lett. 67, 213–217.

Caillé, S., Alvarez-Jaimes, L., Polis, I., Stouffer, D.G., Parsons, L.H., 2007. Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. J. Neurosci. 27, 3695–3702.

Caldwell, H.K., Young, W.S., 2006. Oxytocin and Vasopressin: genetics and behavioural implications. In: Springer-Verlag Berlin Heidelberg (Eds.): Handbook of Neurochemistry and Molecular Neurobiology, pp. 573-607.

Canteros, G., Rettori, V., Franchi, A., Genaro, A., Cebral, E., Faletti, A., Gimeno, M., McCann, S.M., 1995. Ethanol inhibits luteinizing hormone-releasing hormone (LHRH) secretion by blocking the response of LHRH neuronal terminals to nitric oxide. Proc. Natl. Acad. Sci. U. S. A. 92, 3416–3420.

Cicero, T.J., Newman, K.S., Gerrity, M., Schmoeker, P.F., Bell, R.D., 1982. Ethanol inhibits the naloxone induced release of luteinizing hormone-releasing hormone from the hypothalamus of the male rat. Life Sci. 31, 1587–1596.

Cristino, L., De Petrocellis, L., Pryce, G., Baker, D., Guglielmotti, V., Di Marzo, V., 2006. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience 139, 1405–1415.

da Veiga, M.A., Fonseca Bloise, F., Costa-E-Sousa, R.H., Souza, L.L., Almeida, N.A., Oliveira, K.J., Pazos-Moura, C.C., 2008. Acute effects of endocannabinoid anandamide and CB1 receptor antagonist, AM251 in the regulation of thyrotropin secretion. J. Endocrinol. 199, 235–242.

Davies, M., 2003. The role of $GABA_A$ receptors in mediating the effects of alcohol in the central nervous system. J. Psychiatry Neurosci. 28, 263–274.

De Laurentiis, A., Fernandez Solari, J., Mohn, C., Zorrilla Zubilete, M., Rettori, V., 2010. The endocannabinoid system participates in the neuroendocrine control of homeostasis. Neuroimmunomodulation 17 (3), 153–156.

De Laurentiis, A., Fernandez-Solari, J., Mohn, C., Burdet, B., Zorrilla Zubilete, M., Rettori, V., 2010. The hypothalamic endocannabinoid system participates in the secretion of oxytocin and tumor necrosis factor-alpha induced by lipopolysaccharide. J. Neuroimmunol. 221, 32-41.

de Miguel, R., Romero, J., Muñoz, R.M., García-Gil, L., Gonzalez, S., Villanua, M.A., Makriyannis, A., Ramos, J.A., Fernandez-Ruiz, J.J., 1998. Effects of cannabinoids on prolactin and gonadotrophin secretion: involvement of changes in hypothalamic gamma-aminobutyric acid (GABA) inputs. Biochem. Pharmacol. 56, 1331–1338.

Dees, W.L., Rettori, V., Kozlowski, G.P., McCann, S.M., 1985. Ethanol and the pulsatile release of luteinizing hormone, follicle stimulating hormone and prolactin in ovariectomized rats. Alcohol 2, 641–646.

Devane, W.A., Dysarz III, F.A., Johnson, M.R., Melvin, L.S., Howlett, A.C., 1988. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. 34, 605–613.

Dopico, A.M., Lovinger, D.M., 2009. Acute alcohol action and desensitization of ligand-gated ion channels. Pharmacol. Rev. 61, 98–114.

Emanuele, N.V., Lapaglia, N., Emanuele, M.A., 2001. Impact of acute and chronic ethanol exposure on prolactin in both male and female rats. Endocrine 16, 29–37.

Faletti, A.G., Mastronardi, C.A., Lomniczi, A., Seilicovich, A., Gimeno, M., McCann, S.M., Rettori, V., 1999. beta-Endorphin blocks luteinizing hormone-releasing hormone release by inhibiting the nitricoxidergic pathway controlling its release. Proc. Natl. Acad. Sci. U. S. A. 96, 1722–1726.

Fernandez-Solari, J., Scorticati, C., Mohn, C., De Laurentiis, A., Billi, S., Franchi, A., McCann, S.M., Rettori, V., 2004. Alcohol inhibits luteinizing hormone-releasing hormone release by activating the endocannabinoid system. Proc. Natl. Acad. Sci. U. S. A. 101, 3264–3268.

Fimiani, C., Liberty, T., Aquirre, A., Amin, L., Ali, N., Stefano, G., 1999. Opiate, cannabinoid, and eicosanoid signalling converges on common intracellular pathways nitric oxide coupling. Prostaglandins Other Lipid Mediat. 57, 23–34.

Fride, E., Ginzburg, Y., Breuer, A., Bisogno, T., Di Marzo, V., Mechoulam, R., 2001. Critical role of the endogenous cannabinoid system in mouse pup suckling and growth. Eur. J. Pharmacol. 419, 207–214.

Gammon, C.M., Freeman Jr., G.M., Xie, W., Petersen, S.L., Wetsel, W.C., 2005. Regulation of gonadotropin-releasing hormone secretion by cannabinoids. Endocrinology 146, 4491–4499.

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

Gonzalez, S., Bisogno, T., Wenger, T., Manzanares, J., Milone, A., Berrendero, F., Di Marzo, V., Ramos, J.A., Fernandez-Ruiz, J.J., 2000. Sex steroid influence on cannabinoid CB1receptor mRNA and endocannabinoid levels in the anterior pituitary gland. Biochem. Biophys. Res. Commun. 270, 260–266.

Grattan, D.R., Kokay, I.C., 2008. Prolactin: a pleiotropic neuroendocrine hormone. J. Neuroendocrinol. 20, 752–763.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K. C., 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. U. S. A. 87, 1932–1936.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1991. Characterization and localization of cannabiniod receptors in rat brain: a quantitative in vitro autoradiographic study. J. Neurosci. 11, 563–583.

Hohmann, A., Herkenham, M., 2000. Localization of cannabinoid CB(1) receptor mRNA in neuronal subpopulations of rat striatum: a double-label in situ hybridization study. Synapse 37, 71–80.

Howlett, A., Fleming, R., 1984. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. Mol. Pharmacol. 26, 532–538.

Hungund, B.L., Szakall, I., Adam, A., Basavarajappa, B.S., Vadasz, C., 2003. Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. J. Neurochem. 84, 698–704.

Jia, F., Chandra, D., Homanics, G.E., Harrison, N.L., 2008. Ethanol modulates synaptic and extrasynaptic $GABA_A$ receptors in the thalamus. J. Pharmacol. Exp. Ther. 326, 475–482.

Lomniczi, A., Mastronardi, C.A., Falleti, A., Seilicovich, A., De Laurentiis, A., McCann, S.M., Rettori, V., 2000. Inhibitory pathways and the inhibition of luteinizing hormone-releasing hormone release by alcohol. Proc. Natl. Acad. Sci. U. S. A. 97, 2337–2342.

Maccarrone, M., 2009. Endocannabinoids: friends and foes of reproduction. Prog. Lipid Res. 48, 344–354.

Maccarrone, M., van der Stelt, M., Rossi, A., Veldink, G.A., Vliegenthart, J.F., Agrò, A.F., 2000. Anandamide uptake by human endothelial cells and its regulation by nitric oxide. J. Biol. Chem. 275, 13484–13492.

McCann, S.M., Rettori, V., 1996. The role of nitric oxide in reproduction. Proc. Soc. Exp. Biol. Med. 211, 7–15.

McDonald, N.A., Kuzmiski, J.B., Naderi, N., Schwab, Y., Pittman, Q.J., 2008. In: Neumann, I.D., Landgraf, R. (Eds.), Endogenous modulators of synaptic transmission: cannabinoid regulation in the supraopitc nucleus. : Progress in Brain Research, vol. 170. Elsevier, Oxford, UK, pp. 129–136.

Mechoulam, R., Fride, E., Di Marzo, V., 1998. Endocannabinoids. Eur. J. Pharmacol. 359, 1–18.

Mennella, J., Pepino, Y., Teff, K., 2005. Acute alcohol consumption disrupts the hormonal milieu in lactating women. J. Clin. End. Metab. 90, 1979–1985.

Mouslech, Z., Valla, V., 2009. Endocannabinoid system: an overview of its potential in current medical practice. Neuro Endocrinol. Lett. 30, 153–179.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365, 61–65.

Murphy, L.L., Gher, J., Steger, R., Bartke, A., 1994. Effects of delta 9-tetrahydrocannabinol on copulatory behavior and neuroendocrine responses of male rats to female conspecifics. Pharmacol. Biochem. Behav. 48, 1011–1017.

Murphy, L.L., Muñoz, R.M., Adrian, B.A., Villanúa, M.A., 1998. Function of cannabinoid receptors in the neuroendocrine regulation of hormone secretion. Neurobiol. Dis. 5, 432–436.

Nevo, I., Hamon, M., 1995. Neurotransmitter and neuromodulatory mechanisms involved in alcohol abuse and alcoholism. Neurochem. Int. 26, 305–336.

Noto, T., Myers, R., 1984. In vivo alteration in hypothalamic amino acid synthesis during perfusion of ethanol and morphine in unrestrained rat. Neurochem. Res. 9, 1653–1665.

Obrietan, K., van den Pol, A.N., 1997. GABA activity mediating cytosolic Ca2+ rises in developing neurons is modulated by cAMP-dependent signal transduction. J. Neurosci. 17, 4785–4799.

Olah, M., Milloh, H., Wenger, T., 2008. The role of endocannabinoids in the regulation of luteinizing hormone and prolactin release. Differences between the effects of AEA and 2AG. Mol. Cell. Endocrinol. 286, 36–40.

Pacher, P., Bátkai, S., Kunos, G., 2006. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol. Rev. 58, 389–462.

Pagotto, U., Marsicano, G., Cota, D., Lutz, B., Pasquali, R., 2006. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. Endocr. Rev. 27, 73–100.

Parlesak, A., Pohl, C., Bode, J.C., Bode, C., 2004. Water metabolism in rats subjected to chronic alcohol administration. Nephron Physiol. 97, 9–15.

Pistis, M., Ferraro, L., Pira, L., Flore, G., Tanganelli, S., Gessa, G.L., Devoto, P., 2002. Delta (9)-tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an in vivo microdialysis study. Brain Res. 948, 155–158.

Rettori, V., Wenger, T., Snyder, G., Dalterio, S., McCann, S.M., 1987. Effects of delta-9-tetrahydrocannabinol on the hypothalamic–pituitary control of luteinizing hormone and follicle-stimulating hormone secretion in adult male rats. Neuroendocrinology 46, 488–493.

Rettori, V., Wenger, T., Snyder, G., Dalterio, S., McCann, S.M., 1988. Hypothalamic action of delta-9-tetrahydrocannabinol to inhibit the release of prolactin and growth hormone in the rat. Neuroendocrinology 47, 498–503.

Rettori, V., Aguila, M.C., Gimeno, M.F., Franchi, A.M., McCann, S.M., 1990. In vitro effect of Δ9-tetrahydrocannabinol to stimulate somatostatin release and block that of luteinizing hormone-releasing hormone by suppression of the release of prostaglandin E2. Proc. Natl. Acad. Sci. U. S. A. 87, 10063–10066.

Rettori, V., Gimeno, M., Lyson, K., McCann, S.M., 1992. Nitric oxide mediates norepinephrine-induced prostaglandin E2 release from the hypothalamus. Proc. Natl. Acad. Sci. U. S. A. 89, 11543–11546.

Rettori, V., Belova, N., Dees, W.L., Nyberg, C.L., Gimeno, M., McCann, S.M., 1993. Role of nitric oxide in the control of luteinizing hormone-releasing hormone release in vivo and in vitro. Proc. Natl. Acad. Sci. U. S. A. 90, 10130–10134.

Rettori, V., Canteros, G., Reynoso, R., Gimeno, M., McCann, S.M., 1997. Oxytocin stimulates the release of luteinizing hormone-releasing hormone from medial basal hypothalamic explants by releasing nitric oxide. Proc. Natl. Acad. Sci. U. S. A. 94, 2741–2744.

Rettori, V., Lomniczi, A., Mohn, C., Scorticati, C., Vissio, P., Lasaga, M., Franchi, A., McCann, S.M., 2002. Mechanisms of inhibition of LHRH release by alcohol and cannabinoids. Prog. Brain Res. 141, 175–181.

Rettori, V., Fernandez-Solari, J., Prestifilippo, J.P., Mohn, C., De Laurentiis, A., Bornstein, S.R., Ehrhart-Bornstein, M., Elverdin, J.C., McCann, S.M., 2007. Endocannabinoids in TNF-alpha and ethanol actions. Neuroimmunomodulation 14, 188–192.

Rivier, C., Lee, S., 1996. Acute alcohol administration stimulates the activity of hypothalamic neurons that express corticotropin-releasing factor and vasopressin. Brain Res. 726, 1–10.

Rodriguez de Fonseca, F., Fernadez-Ruiz, J.J., Murphy, L.L., Cebeira, M., Steger, R.W., Bartke, A., Ramos, J.A., 1992. Acute effects of delta-9-tetrahydrocannabinol on dopaminergic activity in several rat brain areas. Pharmacol. Biochem. Behav. 42, 269–275.

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

Scorticati, C., Fernández-Solari, J., De Laurentiis, A., Mohn, C., Prestifilippo, J.P., Lasaga, M., Seilicovich, A., Billi, S., Franchi, A., McCann, S.M., Rettori, V., 2004. The inhibitory effect of anandamide on luteinizing hormone-releasing hormone secretion is reversed by estrogen. Proc. Natl. Acad. Sci. U. S. A. 101, 11891–11896.

Seilicovich, A., Rubio, M., Duvilanski, B., Muñoz Maines, V., Rettori, V., 1985. Inhibition by naloxone of the rise in hypothalamic dopamine and serum prolactin induced by ethanol. Psychopharmacology 87, 461–463.

Seilicovich, A., Duvilanski, B., Pisera, D., Theas, S., Gimeno, M., Rettori, V., McCann, S.M., 1995. Nitric oxide inhibits hypothalamic luteinizing hormone-releasing hormone release by releasing gamma-aminobutyric acid. Proc. Natl. Acad. Sci. U. S. A. 92, 3421–3424.

Silva, S.M., Madeira, M.D., Ruela, C., Paula-Barbosa, M.M., 2002. Prolonged alcohol intake leads to irreversible loss of vasopressin and oxytocin neurons in the paraventricular nucleus of the hypothalamus. Brain Res. 925, 76–88.

Spergel, D.J., Kruth, U., Hanley, D., Sprengel, R., Seeburg, P., 1999. GABA- and glutamate-activated channels in green fluorescent protein-tagged gonadotropin-releasing hormone neurons in transgenic mice. J. Neurosci. 19, 2037–2050.

Steiner, J., Kirsteins, L., LaPaglia, N., Lawrence, A., Williams, D., Emanuele, N., Emanuele, M., 1997. The effect of acute ethanol (EtOH) exposure on protein kinase C (PKC) activity in anterior pituitary. Alcohol 14, 209–211.

Subramanian, M.G., 1999. Alcohol inhibits suckling-induced oxytocin release in the lactating rat. Alcohol 19, 51–55.

Szabo, G., Dolganiuc, A., Dai, Q., Pruett, S.B., 2007. TLR4, ethanol, and lipid rafts: a new mechanism of ethanol action with implications for other receptor-mediated effects. J. Immunol. 178, 1243–1249.

Tanda, G., Pontieri, F.E., Chiara, G., 1997. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 276, 2048–2050.

Tyrey, L., Murphy, L.L., 1988. Inhibition of suckling-induced milk ejections in the lactating rat by delta 9-tetrahydrocannabinol. Endocrinology 123, 469–472.

Uddin, S., Emanuele, M.A., Emanuele, N.V., Redá, D., Kelley, M.R., 1994. The effect of in vitro ethanol exposure on luteinizing hormone and follicle stimulating hormone mRNA levels, content, and secretion. Endocr. Res. 20, 201–217.

Vengeliene, V., Bilbao, A., Molander, A., Spanagel, R., 2008. Neuropharmacology of alcohol addiction. Br. J. Pharmacol. 154, 299–315.

Wang, X.M., Lemos, J.R., Dayanithi, G., Nordmann, J.J., Treistman, S.N., 1991. Ethanol reduces vasopressin release by inhibiting calcium currents in nerve terminals. Brain Res. 551, 338–341.

Wang, L., Liu, J., Harvey-White, J., Zimmer, A., Kunos, G., 2003. Endocannabinoid signaling via cannabinoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice. Proc. Natl. Acad. Sci. U. S. A. 100, 1393–1398.

Wenger, T., Moldrich, G., 2002. The role of endocannabinoids in the hypothalamic regulation of visceral function. Prostaglandins Leukot. Essent. Fatty Acids 66, 301–307.

Wenger, T., Rettori, V., Snyder, G.D., Dalterio, S., McCann, S.M., 1987. Effects of delta-9-tetrahydrocannabinol on the hypothalamic–pituitary control of luteinizing hormone and follicle-stimulating hormone secretion in adult male rats. Neuroendocrinology 46, 488–493.

Wenger, T., Toth, B.E., Juaneda, C., Leonardelli, J., Tramu, G., 1999a. The effects of cannabinoids on the regulation of reproduction. Life Sci. 65, 695–702.

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

Wenger, T., Fernandez-Ruiz, J.J., Ramos, J.A., 1999c. Immunocytochemical demonstration of CB1 cannabinoid receptors in the anterior lobe of the pituitary. J. Neuroendocrinol. 11, 873–878.

Zeballos, G.A., Basalto, J., Munoz, C.A., Salinas-Zeballos, M.E., 2006. Chronic alcohol ingestion: effects on water metabolism. Ann. N. Y. Acad. Sci. 273, 343–350.