Endocannabinoids in TNF-α and Ethanol Actions

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Abstract
During marijuana and alcohol consumption as well as during inflammation the reproductive axis is inhibited, mainly through the inhibition of luteinizing hormone-releasing hormone release. In male rats, this inhibitory effect is mediated, at least in part, by the activation of hypothalamic cannabinoid type 1 receptors (CB1). During inflammation, this activation of the endocannabinoid system seems to be mediated by an increase in TNF-α production followed by anandamide augmentations, similarly the effect of intragastic administration of ethanol (3 g/kg) seems to be due to an increase in anandamide. On the other hand, a number of different actions mediated by the endocannabinoid system in various organs and tissues have been described. Both cannabinoid receptors, CB1 and CB2, are localized in the submandibular gland where they mediate the inhibitory effect of intrasubmandibular injections of the endocannabinoid anandamide (6 × 10⁻⁵ M) on salivary secretion. Lipopolysaccharide (5 mg/kg/3 h) injected intraperitoneally and ethanol (3 g/kg/1 h) injected intragastrically inhibited the salivary secretion induced by the sialogogue metacholine; this inhibitory effect was blocked by CB1 and/or CB2 receptor antagonists. Similar to the hypothalamus, these effects seem to be mediated by increased anandamide. In summary, similar mechanisms mediate the inhibitory actions of endocannabinoids and cannabinoids in both hypothalamus and submandibular gland during drug consumption and inflammation.

Role of the Endocannabinoid System in the Hypothalamic-Pituitary Reproductive Axis and Salivary Secretion

It is well established that the major active ingredient of marijuana, Δ⁹-tetrahydrocannabinol (THC), is capable of suppressing the reproductive function [1]. Previous studies indicate that the inhibitory effect of THC on the reproductive axis is exerted mainly at hypothalamic level by inhibiting luteinizing hormone-releasing hormone (LHRH) release with the consequent decrease in luteinizing hormone secretion by the pituitary, thereby inhibit-
CB1 receptors, which are mainly expressed in the brain

nabinoid (CB) receptors were identified: the CB1 central

pears to be particularly abundant in the immune system

tory effects of AEA were prevented by the selective CB1

AMP (cAMP) content and LHRH release. These inhibi-

demonstrated that the same concentration of AEA sig-

The CB1 receptors are localized in different areas of

the rat brain [8]. In particular, we showed the presence of

CB1 and CB2 peripheral receptor subtype, which ap-

opposed for CB receptors, such as AM251 and SR141716A for

and the CB2 peripheral receptor subtype, which ap-

around that time 2 subtypes of G protein-coupled cann

involvement of the endocannabinoid system. Moreover

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CB1 receptors release induced by endocannabinoids. Moreover

involvement of THC to the brain. THC was transported into and accumu-

the sialogogue metacholine (MC) injected through the

SMG physiology. Salivation is controlled by the autonomic nervous sys-

the sublingual and parotid glands. In vitro studies on rat SMG slices showed that 3 H-

and fluid, and ductal modification of the pri-

We have shown immunohistochemically that both

the enzymatic degradation of components of the

the blood-brain barrier and acts on central mus-

the brain-stimulating activity with a consequent decrease in cAMP

the role of endocannabinoids in regulating both cAMP and protein 

The CB1 receptors are present in afferent and efferent

We have recently demonstrated that THC (10–8 M) inhibited


effect of AEA on LHRH release. However, naltrexone (10–5 M), an opioid receptor


do not modify the inhibitory effect of AEA.

reactive neurons was observed. However, CB1 receptor-immuno-

rate and secretion of proteins. CB receptors activates the

malignant salivary secretion was associated with the 2 funda-

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We also demonstrated that AEA injected intraglandularly inhibited NE- and MC-induced salivary secretion in vivo and that AM251 and AM630 prevented this inhibition, suggesting that both CB1 and CB2 are implicated in the modulation of saliva secretion [13]. CB2 mainly located at the periphery of the acini could regulate the release of saliva from these cells to the salivary ducts. The function of CB1 in the SMG is more difficult to explain, since these receptors were not immunodetected in the vicinity of the acini. However, the immunodetection of CB1 in the ductal system suggests a paracrine effect from the duct cells to the acinar cells to reduce the volume of saliva.

Additionally, since CB1 and CB2 receptor antagonists increased the salivary secretion induced by lower doses of sialogogues, these findings indicate that there is an endogenous endocannabinoid tone that could regulate salivary secretion.

These evidences suggest that the endocannabinoid system involves similar mechanisms in the brain and in peripheral organs such as the SMG. The FRSK-induced AC activity is a useful experimental procedure to study CB receptor biologic activity in the brain as well as in the periphery. At least in our experimental paradigm, CB receptors activation seems to promote inhibitory actions at brain level, such as LHRH release diminution, and peripherally, such as decrease in salivary secretion.

Role of the Endocannabinoid System in the Blockade of the Reproductive Axis and the Inhibition of Salivary Secretion during Infection

Lipopolysaccharide (LPS), an integral part of the outer membrane of Gram-negative bacteria, is a major pathogenic factor in septic shock. Macrophages are the primary targets of LPS, where the toxin interacts with the CD14 protein/Toll-like receptor-4 complex to activate multiple signaling pathways. LPS induces the expression and release of cytokines such as TNF-α, interleukin (IL)-1, IL-6 and IL-8, which have been implicated in the pathophysiology of septic shock [16]. Also, LPS induces the production of different lipid mediators in macrophages, such as prostaglandins, leukotrienes and AEA [17].

Cannabinoid agonists are known to decrease neurotoxicity and AEA is able to promote anti-inflammatory responses in astrocytes via CB1 receptors [18]. In addition to modulating cellular responsiveness to various cytokines, AEA was also reported to alter its production under different conditions. Studies in human peripheral blood mononuclear cells examining a wide variety of cytokines demonstrated that AEA increased or decreased cytokine release depending upon drug concentration [19]. Also, it was reported that both synthetic and endogenous cannabinoids inhibit the LPS-induced release of TNF-α from microglial cells [20] and that LPS increased AEA levels in mouse peritoneal macrophages by inducing AEA synthesis [17].

Regarding reproductive function, it is known that during endotoxemia induced by LPS, the hypothalamic-gonadotropin axis is inhibited. Also, LPS seems to activate similar mechanisms in the inhibitory pathway of LHRH as those exerted by endocannabinoids, principally by increasing GABAergic activity [21].

It was reported that LPS leads to the suppression of LHRH pulse generator activity through a mechanism involving TNF-α. This change was faithfully reflected in the luteinizing hormone secretory pattern [22]. Furthermore, we demonstrated that AEA (50 ng/5 μl) injected intracerebroventricularly reduced plasma luteinizing hormone levels in the rat [23], similarly to the reduction of gonadotropin levels observed in sheep during endotoxemia induced by LPS [24].

In this study, we demonstrated a connection between the neuroimmune and neuroendocannabinoid systems by showing the increase in AEA synthase activity in MBH removed from rats injected with LPS (5 mg/kg/3 h, intraperitoneally) compared to rats receiving vehicle. Additionally, we demonstrated that TNF-α (10 ng/5 μl) injected intracerebroventricularly increased the AEA synthase activity measured ex vivo 3 h after the injections. Therefore, it is possible that LPS, through an increase in TNF-α production, enhances AEA synthase activity, thereby activating the endocannabinoid system to inhibit the release of LHRH. To confirm this hypothesis we measured the effect of TNF-α on LHRH release from MBH incubated in vitro. TNF-α significantly reduced FRSK-stimulated cAMP content and LHRH release and these effects were blocked by the CB1 antagonist AM251. These results suggest that the endocannabinoid system participates in neuroendocrine responses and immune responses mediated by TNF-α.

On the other hand, it is well known that salivary secretion is altered in different pathological states concomitantly with other physiologic parameters. We previously demonstrated that LPS injected intraperitoneally inhibits salivary secretion by increasing prostaglandin production [25]. Also, we recently demonstrated that LPS injected intraperitoneally increased AEA synthase activity in the SMG 3 h after the toxin injection. Therefore, we hypoth-
esized that the inhibition of salivary secretion observed during inflammation could be mediated by the activation of the endocannabinoid system in the SMG. In fact, both AM251 and AM630 (6 × 10⁻⁴ M), CB1 and CB2 receptor antagonists, respectively, injected intraglandularly, blocked, at least partially, the inhibitory effect of LPS on MC-induced salivary secretion. The biological activity of CB1 and CB2 receptors in the SMG was confirmed by the experimental procedure of FRSK-induced AC activity, showing that AM251 was more efficient than AM630 in blocking the inhibitory effect of TNF-α on FRSK-stimulated cAMP content in SMG incubated in vitro.

In summary, these results demonstrate that during endotoxemia induced by LPS the reproductive axis and salivary secretion are attenuated, at least in part, due to the activation of the endocannabinoid system that acts as an immunoprotector system.

Role of the Endocannabinoid System in the Ethanol-Induced Blockade of the Reproductive Axis and the Inhibition of Salivary Secretion

It is well known that ethanol (EtOH), similar to THC, can suppress reproductive function [26]. Much evidence exists that EtOH exerts its pharmacological effects in the central nervous system by modulating the function of intracellular signal transduction pathways by acting on several receptors. Since the actions of EtOH and THC on the hypothalamic-gonadotrophic axis are similar, we hypothesized that the effects of EtOH might be mediated by the endocannabinoid system.

On the basis of in vitro experiments with MBH, we demonstrated that EtOH (100 mM) inhibited the NMDA-stimulated release of LHRH by increasing the release of GABA [27] as well as AEA. Also, EtOH (100 mM) inhibited the FRSK-stimulated cAMP increase and LHRH release, inhibitory effects that were at least partially blocked by AM251, suggesting the involvement of CB1 receptors in the alcohol-induced blockade of reproductive function [10]. The incomplete inhibition could be due to the presence of a second inhibitory pathway such as the opioid system, since it has been shown that EtOH increases the release of β-endorphin which also can inhibit LHRH release [9].

In these in vitro experiments, AEA synthase activity did not change after exposing MBH to EtOH (100 mM) for 30 min. However, AEA synthase activity increased in MBH dissected 1 h after intragastric administration of EtOH (3 g/kg).

On the other hand, it is known that alcohol consumption decreases salivary secretion as does AEA. We hypothesized that EtOH might act through the endocannabinoid system to inhibit salivary secretion. Gastric administration of EtOH (3 g/kg) in adult male rats inhibited MC-stimulated saliva secretion, studied 1 h after injection, which was partially restored by intraglandular injection of AM251 and AM630 (6 × 10⁻⁴ M). Moreover, AEA synthase activity was increased significantly in SMG removed 1 h after intragastric administration of EtOH (3 g/kg). Also, EtOH (100 mM) significantly reduced the FRSK-increased cAMP content in SMG slices incubated in vitro for 30 min. This inhibitory effect was significantly blocked by the CB1 and CB2 receptor antagonists. Therefore, the hyposialia observed after alcohol consumption could be due to CB receptor activation in the salivary glands.

In summary, EtOH and the LPS-induced TNF-α release increase AEA synthesis in the MBH. This augmentation of AEA production activates CB1 receptors that reduce cAMP, thereby activating GABAergic neurons that respond by increasing the release of GABA. Finally, GABA acts on GABA_A receptors located on LHRH neurons to inhibit LHRH release. Similarly, the inhibitory effect produced by alcohol and inflammation on salivary secretion is mediated, at least in part, by the endocannabinoid system. In these cases, AEA synthase activity is increased and the endocannabinoid acts on both CB1 and CB2 receptors decreasing cAMP production, thereby conducing to the diminution of salivary secretion.

References