

Endocannabinoid System Participates in Neuroendocrine Control of Homeostasis

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Key Words

Oxytocin · Vasopressin · Cannabinoid receptors · Nitric oxide · Hypothalamus · Neurohypophysis

Abstract

The hypothalamo-neurohypophyseal system plays a role in homeostasis under a variety of stress conditions, including endotoxemia. Oxytocin (OXT) and vasopressin (VP) are important hormones synthesized by neurons in the hypothalamic paraventricular and supraoptic nuclei and released into different brain regions and from the neurohypophyseal terminals into the blood in response to many patho-physiological stimuli. However, the mechanism that controls OXT and VP secretion has not been fully elucidated. Nitric oxide (NO) is a known mediator that regulates the release of these hormones. The endocannabinoid system is a new intercellular system that modulates several neuroendocrine actions. Endocannabinoids (eCB) are released as retrograde messengers by many neurons, including hypothalamic magnocellular neurons and cannabinoid receptors are localized within these neurons, as well as in the anterior and posterior pituitary lobes, suggesting an eCB role in the production and release of OXT and VP. Lipopolysaccharide (LPS) injection is a model used as immune challenge. LPS causes a neuroendocrine response that is mediated by cytokines, tumor necrosis factor- α being one of them. We focused on NO and

endocannabinoid system participation on OXT and VP production and secretion during basal and stress conditions and found that eCB affect basal OXT and VP secretion by acting differently at each level of the hypothalamo-neurohypophyseal system. After LPS, there is an increase in eCB synthesis that enhances OXT secretion.

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The hypothalamic-neurohypophyseal system is a neuroendocrine system essential for survival. It consists of the hypothalamic supraoptic nuclei (SON) situated lateral to the optic chiasm, and the paraventricular nuclei (PVN) on each side of the third ventricle. Magnocellular neurons in those nuclei synthesise oxytocin (OXT) and vasopressin (VP) and send axonal projections to the posterior pituitary. The neuronal activity stimulates the release of hormones into the blood, regulating a number of important physiological functions, mainly reproduction and homeostasis. In addition, OXT and VP have been shown to have neurotransmitter functions in various regions of the central nervous system (CNS), thus controlling complex neuroadaptive processes [1].

The endocannabinoid system has been recognized as a major neuromodulatory system, whose function is to maintain brain homeostasis. Endocannabinoids (eCB) are lipophilic arachidonic acid derivatives produced in

brain and peripheral tissues. Several eCB have been identified. The first discovered, and more studied endogenous ligand for cannabinoid receptors is anandamide (AEA). Anandamide formation via energy-independent condensation of arachidonic acid and ethanolamine is attributed to an enzymatic activity termed 'anandamide synthase'. It has been proposed that this reaction is catalyzed by fatty acid amide hydrolase (FAAH), the enzyme that hydrolyzes AEA acting in reverse. Another pathway of AEA biosynthesis involves the hydrolysis of a phospholipid precursor catalyzed by phospholipase D. Depolarization of the postsynaptic cells generates eCB that act retrogradely to inhibit subsequent neurotransmitter release by its binding to a seven transmembrane $G_{i/o}$ -coupled cannabinoid receptors. Two subtypes of cannabinoid (CB) receptors, CB_1 and CB_2 , have been identified and cloned. The CB_1 receptor is the predominant subtype in the CNS, whereas the CB_2 receptor is primarily expressed in immune cells, although it is also found in glial cells of the CNS [2]. The eCB have affinity not only for CB_1 and CB_2 receptors, but also for the transient potential vanilloid type 1 receptor (TRPV1). Anandamide is involved through TRPV1 in physiological processes such as blood pressure control, pain sensation and airway responsiveness [8]. The eCB action is terminated by its removal from the extracellular space. The eCB are transported across the cell membrane by a cannabinoid transporter and degraded by the enzyme FAAH.

Recent reports have highlighted the interaction between the endocannabinoid system and the modulation of the physiology of magnocellular neurons, since OXT and eCB cooperate to shape the electrophysiological properties of SON neurons [1]. To date, most studies in this field focus on the relationship of these systems and their roles in the hypothalamus; however, there are no studies about the eCB action and function in the neurohypophysis (NH) to understand its participation in the regulation of OXT and VP release. By testing several concentrations of AEA (10^{-11} to 10^{-8} M), we have recently demonstrated that it decreases OXT and VP secretion in incubated neurohypophyses from adult male Sprague-Dawley rats, 10^{-9} M being the most effective inhibitory concentration.

Nitric oxide (NO) is a gaseous molecule and a highly reactive free radical produced from L-arginine by nitric oxide synthase (NOS) with multiple and complex roles within many biological systems. Although the involvement of NO in the regulation of OXT and VP release has been investigated, the complicated mechanisms involved remain unclear [3]. Neural NOS (nNOS) coexists with OXT and VP, not only in the cell bodies of hypothalamic

magnocellular neurons but also in the axon terminals in the neural pituitary lobe. In our previous work, we demonstrated in vitro that NO donors reduce OXT secretion from the neural pituitary lobe [4] and that neurokinin A increases neurohypophyseal NOS activity. Therefore, this increased production of NO inhibits OXT secretion from this tissue [5]. Very recently, we confirmed that NO reduces both OXT and VP secretion from NH of male adult rats and thus studied the interrelation between the endocannabinoid system, NO and OXT/VP release. Our in vitro studies performed in tissues from untreated adult male rats showed that AEA increased NOS activity in the NH as well as in the hypothalamus. We also found that the inhibitory effect of AEA on OXT and VP secretion from the NH seems to be mediated by NO since the scavenging of NO by hemoglobin or the inhibition of NOS by L-NAME completely blocked this inhibitory effect.

Little is known about the expression and function of cannabinoid receptors in the posterior pituitary lobe. In this study, we performed pharmacological experiments in vitro in which the NH was incubated in the presence of AEA and cannabinoid or vanilloid receptor-selective antagonists. We found that the CB_2 and TRPV1 antagonists, AM630 and capsazepine, respectively, completely blocked the inhibitory effects of AEA on OXT and VP release from the NH. However, in the presence of CB_1 antagonist (AM251), the inhibitory effect of AEA persisted, suggesting that this subtype of cannabinoid receptor does not participate in OXT and VP release. These results indicate that AEA acts through CB_2 and TRPV1 receptors to inhibit the release of both neuropeptides at the neurohypophyseal level.

Stress is a response of an organism to challenges (stressors) from the environment, aimed at re-establishing homeostasis. In general, infection and inflammation are considered physical stressors. In response to them, hypothalamic neurons trigger the release of corticotrophin-releasing hormone (CRH) which leads to the activation of the hypothalamic-pituitary-adrenal axis. Besides CRH, other neuropeptides such as OXT and VP may act as secretagogues of adrenocorticotrophic hormone (ACTH). These peptides act synergistically with CRH to stimulate ACTH release from the pituitary and therefore stimulate the adrenal gland. Recent data suggest that eCB may play a pivotal role in the regulation of this axis activity at hypothalamic level [2].

During infection, bacterial products such as lipopolysaccharide (LPS) cause the release of proinflammatory cytokines from immune cells. Also, LPS causes changes in the CNS, many of which are mediated by LPS-induced

cytokine production in the brain, resulting in metabolic changes, induction of fever, etc. The neuroendocrine response to infection can be mimicked by peripheral injection of LPS, which is a commonly used model of immune challenge [6].

In a previous work, we demonstrated the connection between the immune and the endocannabinoid systems at the hypothalamic level by showing the increase of AEA synthase activity in hypothalami obtained from adult male rats injected i.p. with LPS [7]. It is known that tumor necrosis factor- α (TNF- α) is one of the main cytokines that mediate the effects attributed to LPS on the hypothalamic pituitary axis. The i.c.v. administration of TNF- α and the incubation of hypothalamic fragments with this cytokine also increase AEA synthase activity [7]. In addition, we have recently evaluated the *in vitro* effect of AEA on OXT production in hypothalamic fragments from untreated adult male rats. To this end, we incubated hypothalamic fragments containing SON and PVN nuclei in the presence of AEA (10^{-6} to 10^{-9} M) and observed that the lowest concentration tested significantly increased OXT production. This result is contrary to that observed in the NH. We also observed that the incubation with TNF- α increased OXT production from hypothalamic fragments and that this effect was mediated by endocannabinoids since the presence of CB₁ antagonist (AM251) completely blocked this effect (fig. 1).

It is known that LPS activates magnocellular OXT and VP neurons in SON and PVN; however, little is known about the participation of the endocannabinoid system in this effect. Additionally, we performed *in vivo* experiments where adult male rats received a single i.p. dose of LPS. We observed that LPS i.p. injection increased plasma OXT and TNF- α levels after 1 h of administration, returning to basal levels at 3 h post injection. Blockade of hypothalamic cannabinoid receptors CB₁ and CB₂ by i.c.v. administration of selective antagonists, AM251 and AM630 respectively, attenuated the LPS-induced increases in plasma OXT and TNF- α levels. Also, enhancing hypothalamic endocannabinoid signaling by i.c.v. administration of a FAAH inhibitor (URB597) potentiated LPS-induced increases in circulating OXT and TNF- α levels. The amplification of endocannabinoid signaling at the hypothalamic level thus seems to facilitate the response of neuropeptides and cytokines after an acute immune challenge. These recent results suggest that endocannabinoids may signal through hypothalamic cannabinoid receptors to facilitate LPS-induced neuroendocrine response during infection and are in concordance with previous works of others [9] (fig. 1).

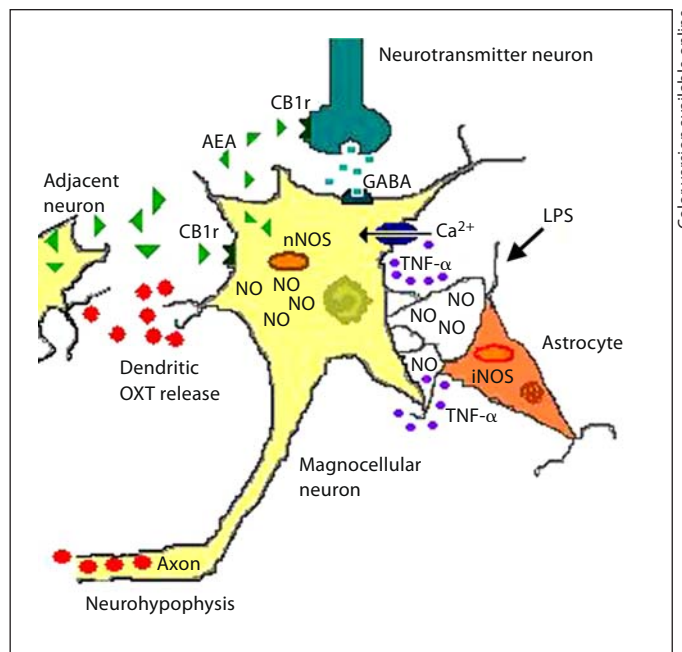


Fig. 1. Postulated mechanism for endocannabinoid participation in OXT release from PVN and SON hypothalamic nuclei after LPS challenge: LPS challenge increases the brain production of cytokines, between them TNF- α . There is also iNOS activation from astrocytes with an increase in NO production. These factors and Ca²⁺ depolarization induce the activation of magnocellular neurons increasing the release of OXT. Also, LPS increase the synthesis of AEA which stimulates nNOS activity, increasing NO stimulating OXT release. Furthermore, AEA acts as a retrograde messenger on CB₁ receptors present on presynaptic terminals of adjacent neurons. Since CB₁ are G_{i/o}-coupled receptors, AEA inhibition of inhibitory GABA adds to the augmentation of OXT release.

In our studies, we focused on the participation of NO and the endocannabinoid system on OXT and VP production and secretion during basal and stress conditions.

Our results are in agreement with most of the data in the literature since it is well known that cannabinoids exert inhibitory effects on hormone secretion from pituitary gland having inhibitory impact on neuroendocrine function. One notable exception to this cannabinoid inhibition of hypothalamic neuroendocrine function is the reported stimulatory effect of cannabinoids on the stress-induced hypothalamic pituitary adrenal axis. Activation of the axis is the main neuroendocrine response to stress and cannabinoids, rather than suppressing this axis, have been shown to enhance secretion of hypothalamic-pituitary hormones.

The results were assessed by modulating NO and endocannabinoid signaling by pharmacological means and sometimes differed depending at which level we performed the in vitro experiments. We consider that the results obtained in the in vivo model of LPS challenge show a real landscape of the situation since the hypothalamo-pituitary-adrenal axis is influenced by many neuroimmunoendocrine signals.

In summary, the present results are in agreement with the fact that the endocannabinoid system of the brain plays an important role in homeostasis by regulating hypothalamo-neurohypophyseal axis activity.

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