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The role of endocannabinoids in the hypothalamic regulation of visceral function

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Summary The hypothalamus plays an important role in the regulation of several visceral processes, including food intake, thermoregulation and control of anterior pituitary secretion.

Endogenous cannabinoids and CB_1 cannabinoid receptors have been found in the hypothalamus. In the present review, we would like to clarify the role of the endocannabinoid system in the regulation of the above-mentioned visceral functions.

There is historical support for the role of marihuana (i.e. exogenous cannabinoids) in the regulation of appetite. Endocannabinoids also stimulate food intake. Furthermore, the specific CB_1 receptor antagonist SR141716 reduces food intake. Leptin treatment decreases endocannabinoid levels in normal rats and ob/ob mice. These findings provide evidence for the role of the hypothalamic endocannabinoid system in food intake and appetite regulation.

Cannabinoids can change body temperature in a dose-dependent manner. High doses cause hypothermia while low doses cause hyperthermia. Cannabinoid administration decreases heat production. It seems that the effects of cannabinoids on thermoregulation is exerted by altering some neurochemical mediator effects at both the presynaptic and postsynaptic level.

THC and endocannabinoids have mainly inhibitory effects on the regulation of reproduction. Administration of anandamide (AEA) decreases serum luteinizing hormone (LH) and prolactin (PRL) levels. AEA causes a prolongation of pregnancy in rats and temporarily inhibits the postnatal development of the hypothalamo-pituitary axis in offspring. The action of AEA on the reproductory parameters occurs at both the hypothalamic and pituitary level. CB_1 receptors have also been found in the anterior pituitary. Further, LH levels in CB_1 receptor-inactivated mice were decreased compared with wild-type mice.

Taken together, all these observations suggest that the endocannabinoid system is playing an important part in the regulation of the mentioned visceral functions and it provides the bases for further applications of cannabinoid receptor agonists and/or antagonists in visceral diseases regulated by the hypothalamus.

INTRODUCTION

The hypothalamus is an area of the diencephalon situated ventrally to the thalamus. It is limited anteriorly by the lamina terminalis and is continuous posteriorly with the mesencephalon. The hypothalamus is seen to be bordered medially by the third cerebral ventricle and laterally by the subthalamus. It is divided into lateral and medial regions by the fornix. The medial region has a cluster of nuclei in a rostrocaudal orientation. The lateral hypothalamus contains mainly longitudinally oriented fibre bundles, among which are scattered neurones of the lateral nucleus.[1]

These regions of the hypothalamus have varied and complex connections with several other CNS areas.[2] The hypothalamus plays an important role in the regulation of visceral processes and responses of the autonomic nervous system. Hypothalamic structures receive information from sensory pathways, peripheral hormone secretions and pathways originating in limbic and cortical structures. The output includes the *control of neurohypophysis and adenohypophysis*. It *controls* brain stem

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autonomic centres like the *gastrointestinal regulatory areas*. Some regions of the hypothalamus are sensitive to changes in the temperature of blood and as such *regulate the body temperature*. The hypothalamus plays a major role in *emotional behaviour*. This latter is intimately related to the limbic system with which the hypothalamus has important connections. Experimental and clinical data suggest the presence of *satiety and feeding centres* in the ventromedial and lateral hypothalamus. In addition, the hypothalamus is believed to play a role in the daily sleep-wakefulness cycle.

The integrative activity of the hypothalamus can often be considered as a fundamental component of homeostatic systems, including neuroendocrine regulatory mechanisms. The question arises: how does signal integration occur in specific hypothalamic neurones?

The hemp as a medicine has been used for several centuries.[3] The first description as an appetite enhancer dates several hundreds years ago before the physiologists could reveal that the hypothalamus is the main 'eating centrum' (see Ref. 4).

Since the description of the main psychoactive component of marihuana, the Δ^9-tetrahydrocannabinol (THC), it has been of interest to investigate the effects of this and other cannabinoids on different hypothalamic functions (see Ref. 5 for review).

The effects of cannabinoids on hypothalamic functions are particularly important for two reasons:

- The possibility that marihuana can be used as a medicine is a very actual medical–political issue.
- Many young people start using marihuana at the age of puberty, when several important changes occur in the regulation of reproductive functions, including hypothalamic regulatory functions.

The following main steps helped to understand the hypothalamic action of cannabinoids:

1. The description of the chemical structure of THC.[6]
2. The finding of high-affinity binding sites for synthetic cannabinoids in the brain, suggesting the existence of specific receptors.[7]
3. The identification and cloning of cannabinoid receptors (CB_1 receptors) first in the brain, then in some peripheral tissues, and of the CB_2 receptors in immune cells.[8–10]
4. The discovery of the endogenous cannabinoids, ligands for both CB_1 and CB_2 receptors, arachidonyl ethanolamide, anandamide (AEA) and 2-arachidonylglycerol (2-AG).[11–13]
5. The development of specific receptor antagonists (SR141716A for CB_1 and SR144528 for CB_2).[14,15]
6. And, finally, the recent availability of CB_1 receptor-inactivated mice.[16]

The presence of endocannabinoids has been shown in the hypothalamus.[17] The central cannabinoid receptors (CB_1 receptor) have also been described in the hypothalamus, which contains, however, fewer cannabinoid-binding sites than other CNS areas.[18,19] Nevertheless, these effects are likely to be caused by the activation of CB_1 receptors in the hypothalamus since these receptors are very much concentrated in certain nuclei.[20]

THE ENDOCANNABINOID SYSTEM AND APPETITE AND FEEDING

It has been described that cannabinoids can stimulate hunger in man, particularly for palatable foods.[21] Further studies confirmed that THC may cause overeating in laboratory animals and in humans.[22,23] Cannabis and THC can stimulate food intake.[21,23] AEA also induces overeating.[24] The effect of THC on feeding is dose-dependent. Low doses have stimulatory effects on food consumption while higher doses cause inhibition, which might mean that the dose–response relationship is biphasic. Electrical stimulation of the lateral hypothalamus, the 'food centre', increases spontaneous food intake in Lewis rats.[25]

Administration of AEA provoked hyperphagia and overeating in satiated rats. Attenuation of this effect by SR141716 was dose dependent.[24] All these data indicate that the effects of AEA on eating behaviour is mediated by CB_1 and not by CB_2 receptors. Moreover, SR141716 suppresses food intake in animals.[26–28] It is possible that SR141716 reverts a food-intake stimulating tone by endocannabinoids. Endocannabinoids seem to be involved in the control of appetite and in the feeling of reward originating from the consumption of palatable food.[29] In the limbic areas, THC is thought to induce the release of dopamine. This may be the cause of the increase in AEA levels described in the limbic system after THC treatment.[30] Such a phenomenon suggests the presence of a correlation between limbic AEA and dopamine levels and craving of palatable food.[31] Recently, Di Marzo et al. observed that food intake after prolonged fasting is lower in CB_1 receptor-inactivated mice than in their wild-type controls.[32] On the other hand, hypothalamic endocannabinoid signalling is constitutively stimulated in obese mice and Zucker rats.[32]

It is of interest that treatment with leptin (which is the primary signal through which the hypothalamus senses nutritional state) reduces AEA content in the hypothalamus.[32]

Taken together, these data suggest that the endocannabinoid system contributes to the stimulation of

appetite by activating the CB_1 receptors present in hypothalamus and that it may be a part of the appetite-triggering network controlled by leptin.

THE ENDOCANNABINOID SYSTEM AND THERMOREGULATION

As early as the 10th century, Arab physicians described that hemp (marihuana) is a good antipiretic agent.[33] Later in 1845, Moreau mentioned the hypothermic effect of hashish.[3] Pertwee's experimental works (1985) demonstrated that cannabis can produce decrease or increase in body temperature.[34] These changes in body temperature are dose dependent. Higher doses produce hypothermia while lower doses induce hyperthermia. It was suggested that a differential involvement of Gs and Gi protein activation at low or high doses, respectively, could explain these findings.[35]

Neurotransmitters present in the hypothalamus, like dopamine, GABA and opioid peptides, are implicated as mediators of hypothermia.[36] Data suggest that cannabinoid-induced hypothermia in rats is mediated by dopaminergic pathways.[37] There is some evidence that cannabinoids can interact with hypothalamic thermoregulatory centres, although CB_1 receptors are not very dense in the hypothalamus.[19,38] High doses of AEA (10 and 20 mg/kg) decreased rectal body temperature in rats.[39,40] This effect was not counteracted by the CB_1 antagonist SR141716A, although the hypothermia produced by THC and WIN55,212-2 (synthetic cannabinoid) was reversed by this antagonist. This raises the possibility that AEA may not be producing all of its effects by a direct interaction with CB_1 receptor.[41] However, in disagreement with these data, Costa et al. found that SR141716A blocks the hypothermic effect of AEA.[42]

N-vanillyl-arachidonic-amide (arvanil), a potential agonist of CB_1 receptor, was 100 times potent than AEA in producing hypothermia and this effect was not blocked by SR141716, which also indicates that some effects caused by cannabimimetic compound, are not (only) due to the activation of CB_1 receptor.[43]

On the other hand, *N*-arachidonoyl-dopamine, a novel synthetic CB_1 receptor ligand, induced hypothermia, indicating the involvement of CB_1 receptor in this process.[44] Finally, recently Hanus et al. demonstrated that an ether-type endocannabinoid (2-arachidonyl-glyceryl ether), which binds to the CB_1 receptor, also causes hypothermia.[45]

Hypothermia is a very characteristic effect of cannabinoids. Yet, it is questionable that this effect occurs only because of the activation of CB_1 receptors present in the hypothalamic regulatory centres, or other mechanisms should also be taken into consideration.

THE ENDOCANNABINOID SYSTEM AND NEUROENDOCRINE REGULATION

One of the main functions of the hypothalamus is the regulation of pituitary hormone secretion. The hypothalamus is also a relay area between other CNS centres (e.g. limbic system[1]) and the endocrine system.

Axons from the magnocellular nuclei of the hypothalamus (supraoptic and paraventricular nucleus [PVN]) located in the pituitary stalk reach the posterior lobe of the pituitary, transporting neurosecretory material (vasopressin, antidiuretic hormone and oxytocin) synthesized in these nuclei and stored in the posterior lobe. At present, there is no evidence to suggest that either exogenous or endogenous cannabinoids act on posterior pituitary hormone regulation.

Several trophic factors are produced in the hypothalamus and influence the production of hormones in the anterior pituitary. These releasing or inhibiting factors reach the anterior pituitary through hypophyseal portal circulation.[2] As mentioned above, the hypothalamus contains a small amount of CB_1 receptors while there is a large amount of CB_1 receptors in the anterior pituitary, mainly on lactotroph and gonadotroph cells.[46] AEA is also present in these organs.[47]

The effects of cannabinoids on reproductory function have been investigated since the early 1970s. In 1973, Marks reported a marked depression within 1 h in luteinizing hormone (LH) secretion following THC administration.[48] As a result of this early report, a number of studies have begun examining the effects of other cannabis derivatives on reproductive functions.[49–52] It became evident that THC can produce mainly inhibitory effects on the reproductive system which can be reversible or irreversible. It was believed that the actions of THC are mediated primarily through the inhibition of hypothalamic regulatory centres by a direct action of THC or its metabolites within the hypothalamus and/or other CNS areas.[5,52]

It is unclear as to whether cannabinoids have direct toxic effects on the developing embryo and foetus. However, the effects on placental function and on delivery mechanisms may cause an increase of stillbirths in rats.[5,53] This question will be discussed elsewhere in this issue by Maccarrone et al.

AEA, when administered during the third week, affects pregnancy similarly to THC, i.e. pituitary and serum LH and prolactin (PRL) levels are decreased in pregnant rats.[5,52] This may indicate that in pregnant rats AEA causes an inhibition of hormone release, probably at the pituitary level. Offspring of AEA-treated rats were also affected. AEA (or its metabolites) caused mainly inhibitory effects on the measured parameters. This inhibition was most pronounced immediately after delivery,

whereas at the end of the juvenile period (late juvenile period, 20th postnatal day) no more differences were observed.[54] CB_1 receptors are present in the early postnatal period,[55] which supports the view that AEA may also play a role in normal pregnancy.

Results indicated that endocannabinoids, particularly AEA, possess a pharmacological activity similar to exogenous cannabinoids.[56] Because CB_1 receptors, present in the hypothalamus, show the highest density in the arcuate nucleus and the medial preoptic area, two important regulatory centres of anterior pituitary functions,[17,57] it became evident that the endocannabinoid system plays a role in the regulation of reproductive functions. Figures 1 and 2 show a comparison between the effects of THC and AEA on serum LH, follicle stimulating hormone (FSH) and (PRL). Both types of cannabinoids significantly decreased serum hormone levels with the exception of FSH in accordance with several reports.[5,51,52] In vitro investigations have shown that AEA also has direct action on dispersed pituitary cells.[58] Since all these effects were prevented by SR141716A, it is likely that the action of AEA is mediated by the activation of CB_1 receptors.

It seems that cannabinoid receptor-containing neurons in the hypothalamus are intrinsic constituents of this part of the CNS because cannabinoid-binding sites did not vary after hypothalamic deafferentation.[59] This, and the presence of CB_1 receptors in anterior pituitary and the fact that the pituitary AEA content is under sex steroid control,[60] emphasize the view that endogenous cannabinoids act at both pituitary and hypothalamic levels when taking part in the regulation of reproductive function. Recent works on CB_1 receptor-inactivated mice further show the importance of the endocannabinoid system in this regulation.[61,62] Serum LH and testosterone levels

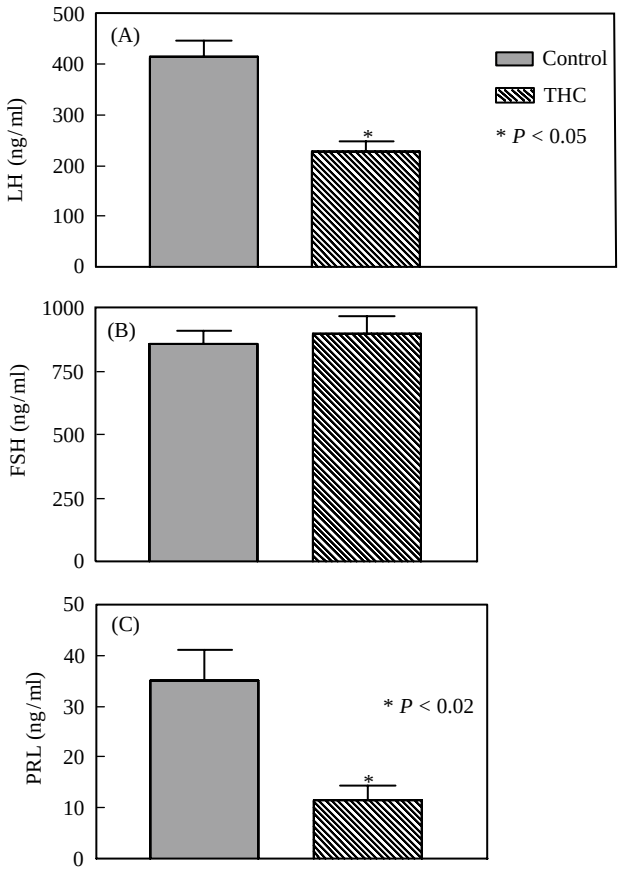

Fig. 1 The effects of THC on serum LH (A), FSH (B) and PRL (C) concentrations in ovariectomized (OVX) rats. The hormone contents were measured at least three weeks after OVX had been performed. The bars represent the average of 9–10 animals ± SEM (see in detail in Refs. 51 and 52).

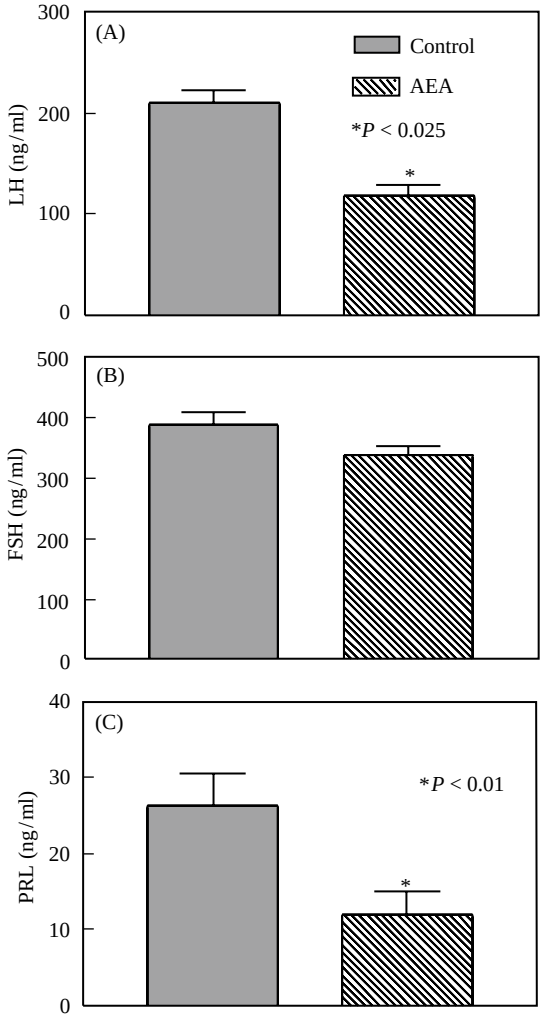

Fig. 2 The effects of AEA on serum LH (A), FSH (B) and PRL (C) concentration in ovariectomized rats. The hormone contents were measured at least three weeks after OVX had been performed. The bars represent the average of 9–10 animals ± SEM (see in detail in Ref. 54).

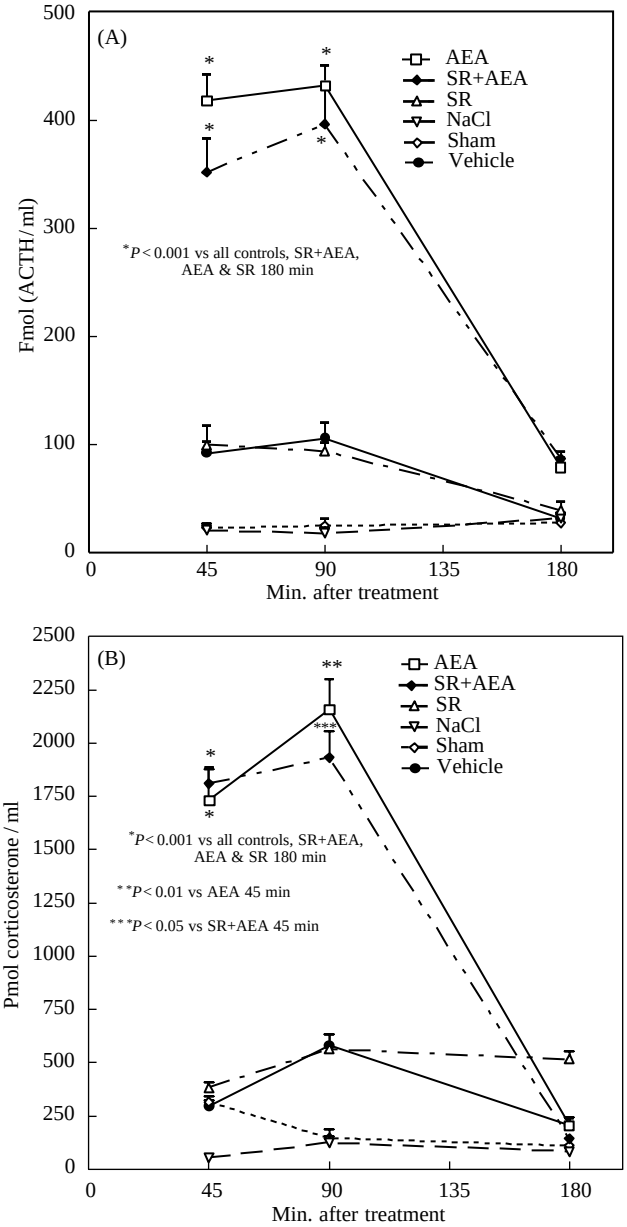

Fig. 3 The effects of AEA treatment on serum ACTH (A) and serum corticosterone (B) concentration in adult male rats. The animals were given 0.01 mg/kg AEA i.p. in time indicated and decapitated at every 45 min. Strict care was taken to avoid any stress (see detail in Ref. 65). Each point represents 8–10 animals ± SEM.

were significantly decreased in mutant ($CB_1^{-/-}$) mice, indicating that the CB_1 receptor activation is needed for the effects of cannabinoids on the regulation of reproductive functions.[62]

Wiedenfield and co-workers demonstrated that corticotropin releasing hormone (CRH) is directly involved in mediating the ACTH-corticosterone response to neuronal stimuli of hypothalamic paraventricular nucleus.[63] The parvocellular part of PVN is one of the main sites producing CRH (together with hippocampus).[2] Endogenous cannabinoids can stimulate adrenocortical function.[64] Experiments in male rats have shown that AEA injection activated the parvocellular part of PVN, an effect that was not prevented by SR141716A.[65] Also, ACTH and corticosterone levels were significantly increased as described before (Figs 3A and B). It was described that AEA activates the parvocellular part of the PVN when acting on hypothalamo-pituitary-adrenal axis, which postulates the presence of a not yet characterized central cannabinoid receptor.[16,65]

Table 1 The effects of anandamide[a] on pituitary hormone secretion in male rats[b]

	Serum	Pituitary
LH	Highly significant decrease	Significant decrease
FSH	No change	No change
PRL	Highly significant decrease	Highly significant decrease
ACTH	Highly significant increase	Low increase

[a]One dose of 0.02 mg/kg i.p.
[b]90 min after treatment.

Table 1 shows the schematic summary of AEA effects on anterior pituitary hormones, observed in male rats.

GENERAL CONCLUSIONS

The data and observations discussed here emphasize the role of the endocannabinoid system in hypothalamic regulatory mechanisms. They also strongly support the view that AEA is a neuromodulator and takes part in several CNS functions. AEA might activate cannabinoid receptors that are far from the site of release, and indeed there is evidence that CB_1 receptors are present in the cell body and in the processes of some neurons.[19] There is a possibility that AEA is a neurotransmitter acting on the same neuron where it is synthesized (autocrine action).[20] It also possible that AEA acts on presynaptic membranes to modulate the neuronal release of different neurotransmitters, in which case AEA functions as a neuromodulator.[66]

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