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THE ENDOCANNABINOID-CB RECEPTOR SYSTEM: A NEW PLAYER IN THE BRAIN-GUT-ADIPOSE FIELD

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The 1990's have witnessed the discovery of the 'endocannabinoid-cannabinoid (CB) receptor (ECBR) system' consisting of specific receptors (CB_1 and CB_2), at least 3 endogenous ligands (anandamide, 2-arachidonyl glycerol and noladin ether) and their enzymes. Subsequently, a series of discoveries were made on the involvement of the ECBR system in many physiological functions including immunity, inflammation, neurotoxicity and neurotrauma, epilepsy, depression and stress, appetite, food intake and energy homeostasis, cardiovascular regulation, reproduction, and bone remodeling. The brain-gut-adipose axis regulates digestive processes, food ingestion and energy balance and is closely associated with hormonal regulation by the hypothalamic-pituitary-adrenal stress axis and by the mesolimbic reward system. It is proposed to call these interfacing systems the "alimentary control system". ECBR presence in brain, gastrointestinal as well as adipose tissue explains its role in food intake, digestion and the regulation of adipose tissue mass. Moreover, the ECBR system's involvement in stress and emotional processing, makes it eminently suited to be (one of) the principle players in the alimentary control system. The ECBR system is present during the early embryonal and postnatal stages and we have discovered endocannabinoids in maternal milk. The ECBR system seems to be of critical value for newborn milk ingestion and suckling. Ghrelin, an orexigenic gastric hormone, exerts many effects similar to those of endocannabinoids. Therefore this review will compare some of the functions and anatomy of ghrelin and endocannabinoids. It is concluded that (i) the ECBR system is a major mediator between the brain and the alimentary system, and possibly, the adipose tissue, (ii) the role of the ECBR system in adult regulation of food processing is a remnant of its critical role for the initiation of feeding in the newborn, and (iii) the pervasive influence of the ECBR system in alimentary control make it a highly suitable target for therapeutic developments for pathophysiological conditions such as inflammatory bowel disease, irritable bowel syndrome, gastric ulcers, nausea, anorexia and failure-to-thrive. ***Biomed Rev* 2006; 17: 23-42.**

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INTRODUCTION: THE "ALIMENTARY CONTROL SYSTEM"

Appetite, hunger and satiety are regulated by a number of hormonal signals emanating from the central nervous system (CNS), and upper gastrointestinal (GI) tract (GIT) and adipose tissue (1,2). Thus it is impossible to separate the appetite-hunger-satiety signals which regulate food intake, from the digestive processes in the GIT and the adipose tissue secretions such as leptin. Therefore in this review, regulation of food intake, appetite and the brain-gut-adipose system will be considered as one system regulating *in*gestion and *di*gestion, and will be denoted the *"alimentary control system"*.

The rich bidirectional interactions between the brain and the local neuronal network (the enteric nervous system - ENS) in the intestinal system (the 'brain-gut axis'), have been extensively studied. Thus, for example, psychological stress as well as transcranial stimulation of the cortex in humans, have been shown to affect gut activities such as secretion and motility (3). Conversely, using brain imaging techniques such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET), have shown that visceral sensation resulting from stimulation of the oesophagus, stomach or rectum, resulted in activation of higher brain centers including the somatosensory cortex, anterior cingulate cortex, thalamus, insula and prefrontal cortex (1,3-5). Moreover patients suffering from irritable bowel syndrome (IBS), displayed different response patterns (4). Relatively few animal studies have investigated the brain-gut associations in an integrative (bio-obehavioral) way. The data that have been collected, however, are very intriguing. For example, traumatic brain injury caused intestinal dysfunction and affected concentrations of several brain/intestinal peptides such as vasoactive intestinal peptide (VIP) and cholecystokinin (CCK) in blood and intestinal tissue (jejunum) (6). Further, secretin, a secretory gut hormone, which has also been suggested to ameliorate autistic symptoms, activated a number of brain areas as measured by *c-fos* gene expression in rat brain. These brain structures included not only the dorsal vagal complex structures which are part of the brain-gut axis, but also the prefrontal cortex, which is thought to play a role in autism (7).

The brain-gut axis has been the subject of a number of recent reviews (3,5,8,9). Adipose tissue is now recognized as an endo- and paracrine secretory organ regulating a number of functions beyond lipid and energy homeostasis (reviewed in 10,11). Recently, the ECBR system has been shown to be fully represented and functional in adipocytes (12,13). In this review, we will examine the role of the relatively recently discovered endocannabinoids and their cannabinoid CB receptors (the 'ECBR system') (14,15) in alimentary control and adipocyte-regulated energy balance *via* the brain-gut-adipose system. Thus first, the major aspects of the alimentary control system will be briefly outlined; next, the ECBR system will be reviewed, after which its multiple role in alimentary control will be discussed. Finally, a brief discussion of developmental aspects of the ECBR system in alimentary control will be offered followed by a comparison with the hormone ghrelin.

THE BRAIN-GUT-ADIPOSE AXIS AND THE ALIMENTARY CONTROL SYSTEM

Ingestion

Ingestion is controlled by a large number of interacting hormones originating from the brain, GIT and adipose tissue.

Hormones from adipose tissue

Leptin is a major adipocyte-derived hormone, its plasma concentrations being proportionate to body fat content and it enter the brain according to its plasma levels. Inside the arcuate nucleus of the hypothalamus, it regulates a number of peptides involved in energy control and food intake (11,16,17) (see below). Recently more than 100 adipose-secreted signaling proteins, collectively named adipokines, have been isolated and reviewed; many of these are pleiotropic (10,11). The present review will concern itself only with those which are pimarily associated with energy balance, including leptin and adiponectin. Additional adipokines, like nerve growth factor (NGF), which have many additional functions and locations, will be discussed in the appropriate sections.

Hormones from the GIT

Cholecystokinins (CCK-8, CCK-33, CCK-39) activate CCK_1 receptors on vagal afferents to the nucleus of the solitary tract (NTS). Cholecystokinins also enters the bloodstream, reducing appetite directly in the hypothalamus (see below) (2,14,18,19). It is interesting to note that CCK has the ability to stimulate the synthesis of nerve growth factor (NGF), an adopokine (10) which is also found in additional peripheral tissue and in the brain, thus enhancing NGF-induced anti-inflammatory activity (20).

Peptide tyrosine tyrosine (PYY*)* activates vagal Y2 receptors, but also penetrates the brain through the median

eminence, reaching the hypothalamus, where it activates hypothalamic Y2 receptors. Plasma PYY levels rise almost immediately after the onset of a meal (2,21), thus suggesting a tight control mechanism on meal size.

Ghrelin is produced primarily by the stomach, but minor amounts are also made in the small intestine and hypothalamus (22). Endogenous ghrelin levels increase before a meal and fall almost immediately upon food intake, while exogenous ghrelin enhances food intake (2,21). Conversely, a ghrelin antagonist reduced food intake and body weight in mice (23). In addition to appetite and weight control, ghrelin enhances intestinal motility, acid secretion and conveys mucosal protection (2). It also fulfills other physiological roles such as cardiovascular and sleep regulation (22). In view of its rapid response to the commencement of food intake, ghrelin is thought to play a major role in the *initiation* of food intake (2) (see Table 2). Exogenous ghrelin almost tripled food intake and decreased plasma leptin by 30%. The suppression is mutual ('ghrelin-leptin tango') (2,24).

Orexin A and B are found in the gut and enhance food intake by inhibiting vagal afferent discharge, thus negatively interacting with the anorexic effects of CCK (2)

Hormones in the brain

Many of the GI hormones including those mentioned in the previous section are also synthesized in the CNS, where they directly influence appetite and also have additional functions (18,22,25,26). However, several hormones are particularly involved in the central neural network responsible for the regulation of food intake and these are briefly described below.

The adipocyte-secreted hormone leptin, entering the brain from the blood circulation, is a major regulator of a number of peptides located in the arcuate nucleus of the hypothalamus. There, leptin inhibits orexigenic peptides (neuropeptide tyrosine, NPY, and *agouti*-related potein, AgRP) and simultaneously, activates the anorexigenic precursor molecule proopiomelanocortin (POMC) (27). An increase in POMC in turn, produces enhanced levels of its cleavage product, α-melanocyte-stimulating hormone (α-MSH). Cocaine-and-amphetamine-regulated transcript (CART) which is colocalized with POMC in arcuate nucleus neurons, is also stimulated by leptin, eventually suppressing food intake (16,17,28). CCK, a satiety-inducing peptide previously found in the gut, directly signals satiety through its receptors in several feeding centers of the hypothalamus (arcuate nucleus, lateral hypothalamus and paraventricular nucleus (PVN) (29,30).

Additional CNS molecules which have been implicated in alimentary control include the neurotransmitters serotonin (5), GABA (31), and the opioid neuropeptides (32). However, an exhaustive discussion of such molecules is beyond the scope of this review.

Digestion

The gastrointestinal tract has an independent nerve center (the enteric nervous system, ENS, or "gut brain") which maintains bilateral neural communication with the CNS along the nerves of the sympathetic and parasympathetic systems (2,5). Under normal conditions CNS control is minor. However, when homeostasis is perturbed such as in digestive disorders or during psychological stress, CNS control plays a major role (5).

Gastrointestinal tract activity (mainly secretory and motor functions) is regulated *via* a 3-tier loop system (see ref. 2,5): *(i)* short (local ENS) loop, relying on independent ENS activity, *(ii)* spinal cord-reflexes, via the autonomic (sympathetic and parasympathetic systems), and *(iii) via* the brain: a number of brain regions play a role and/or are influenced by the GIT: the first target region is the brainstem, where vagal afferents target the nucleus of the solitary tract (NTS), adjacent to the dorsal motor nucleus of the vagus (DMV). *Via* interneurons connecting the NTS with the DMV, a long vago-vagal neural reflex loop is established. In addition, part of the intestinal neural input to the NTS is transmitted further to the hypothalamus (the paraventricular nucleus [PVN] and the arcuate nucleus), and further to the amygdala, the bed nucleus of the stria terminalis (BNST) and ventral thalamus (2). Cortical areas, including the anterior cingulate cortex, somatosendory cortex, and prefrontal cortex, have been shown to represent GI sensations (1,3,5). Notably, several if these structures (amygdala, hypothalamus, cingulate and prefrontal cortices) are part of the limbic system for emotional processing (28).

EMOTIONAL STRESS AND THE ALIMENTARY CONTROL SYSTEM

As stated above, gastrointestinal functions are regulated by both the local ENS as well as by the CNS, the latter becoming more dominant when homeostasis is perturbed (5). It is well

known that emotional processes including stress and depression influence food intake (33,34) as well as GI functioning, by for example, inhibiting gastric emptying and stimulating GI motility (35,36). Interestingly, it has now been shown with brain imaging methods, that the limbic (emotional) system structures, the anterior cingulate cortex and prefrontal cortex, are activated during visceral pain, while patients with irritable bowel disease display a different pattern of activation (4,37).

Corticotrophic releasing hormone (CRH), which is the hypothalamic hormone first in line in the series of hormones released by the hypothalamic-pituitary-adrenal (HPA) axis upon stress exposure, is an important mediator of stress-induced regulation of GI functioning (35). Thus the HPA and brain-gut-adipose axes are closely interrelated; it even has been suggested that the HPA axis is the hormonal arm of the brain-gut system (34). Indeed, traditionally, and still in various regions of the globe, food deprivation is a primary threat to survival and logically, food searching and the motivation for food ingestion, should be driven by hunger- and food deprivation-induced stress. Such integrative approach to feeding mechanisms (34) is supported by the neuranatomical and functional associations and overlap between the brain areas regulating stress responsivity and the alimentary system. Thus the limbic (emotional) system, including the hypothalamus, amygdala, anterior cingulate cortex and the prefrontal cortex, also contains the focal CNS structures which direct GIT activities such as GI motility and gastric emptying (1). Functionally, CRH induces an anxiogenic response, but also participates in the stress-induced alterations in upper GIT and gastric motility (35). Conversely, the gastrin-releasing peptide (GRP) and its receptors, appear to be necessary for the memory of traumatic memories through their location in the amygdala (38). Additionally, a number of molecules including unicortin, CCK, ghrelin and the melanocortin product of POMC, which all regulate appetite and/or GI function, also influence emotional processes (22,39,40).

REWARD AND THE ALIMENTARY CONTROL SYSTEM

As noted above, increased levels of the anorexigenic precursor molecule POMC (27) results in enhanced production of α-MSH thus contributing to the suppression of food intake (16,17,28). Additional products of POMC are the opioids, which are intimately involved in the regulation of reward in the dopaminergic mesolimbic system (a neural pathway projecting from the ventral tegmental area in the midbrain to the forebrain *nucleus accumbens* (28,34)). The dopamine system, in turn, has been associated with food-related behaviors in humans (41). In rats, POMC neurons were inhibited after the *nucleus accumbens* shell was manipulated to elicit feeding behavior in rats (42). Thus, POMC's involvement in both food intake (43) and reward may be part of the rewarding qualities of food ingestion. An additional important observation was made in a self-stimulation paradigm, where the stimulating electrode was placed in the lateral hypothalamus of food-restricted rats. Thus whereas, replicating previous data (44), food restriction enhanced self stimulation, administering leptin to such rats, decreased self stimulation. Together with the multitude of orexigenic and anorexigenic peptides which activate and/or reside in the nuclei of the lateral hypothalamus (including NPY, α-MSH, AgRP, CART, orexin, MCH) these observations strongly suggest that the reward/incentive value of food plays a major role in feeding and body weight regulation and has far reaching implications for addictive patterns of food intake (45). As outlined above, higher brain centers including the prefrontal cortex (PFC), have also been implicated in the neural circuits comprising the brain-gut axis, while the PFC also comprises an important component of the reward/addiction system (46,47). This anatomical overlap between alimentary control and incentive/reward processing further supports common ground for feeding behavior, metabolism, and emotional processing of reward and addiction. Thus all of these processes need to be taken into account when designing strategies to combat abnormal feeding behaviors such as seen in obesity and anorexia nervosa.

THE ENDOCANNABINOID-CB RECEPTOR SYSTEM

Cannabinoid CB receptors

Thus far two CB receptors, CB_1 and CB_2, have been identified and cloned (48-50). In addition, 2 splice variants to the "classical" CB_1 receptor have been identified (51-53). Widely different lines of evidence suggest the existence of an additional (or several additional) CB ("CB_x", or "CB_3") receptors (54-56). The CB_1 and CB_2 receptors both belong to the superfamily of G-protein-coupled receptors (57). The CNS

concentrations of the CB_1 receptor in general, reach levels similar to those of GABA- and glutamate-gated ion channel receptors (57). At the single cell resolution, high CB_1 receptor mRNA expressing neurons in mouse forebrain were found to be mostly GABAergic, while most low CB_1 expressing cells were not (58). In the same study, very high co-expression with the neuromodulator CCK was found in limbic as well as non-limbic cortical neurons. Since brain CCK and CBs act usually in opposite directions (59), the ECBR and CCK systems may conduct cross talk in their regulation of a number of physiological or pathophysiological functions including learning and memory (60), food intake (2) and schizophrenia (58,61). CB_1 receptor mRNA is generally found at about 10-fold lower levels in peripheral tissues than in the CNS (53). The CB_2 receptor is generally found in non-neural, mostly immune tissue (57,62,63) but also in the GIT (53,64,65) (also see below). Recently, CB_2 receptors have been discovered in neural cells throughout the brain. Perhaps the reason for their belated detection on neurons of the CNS, is their appearance in relatively low concentrations (66,67).

Endocannabinoids and their enzymes

Several endocannabinoids (ECs) have been discovered thus far, all derivatives of arachidonic acid (14). The apparently major and most thoroughly studied ECs are anandamide (AEA) (68) and 2-arachidonyl glycerol (2-AG) (69). Additional ECs include noladin ether (64), N-arachidonoylglycerol dopamine (NADA) (70,71); virodhamine is thought to be a partial agonist/antagonist on CB_1 receptors (72). Noladin ether, NADA and virodhamine have not been systematically studied for their presence in the alimentary control system except for our observation that noladin ether inhibits intestinal motility in mice (64).

Up-to-date information on the synthesis, release, reuptake and degradation mechanisms for the ECs has been reviewed (73). In short, ECs are synthesized "on demand". This concept is strongly supported by a positive relationship between EC production and intracellular calcium concentrations. Anandamide is synthesized mainly from N-arachidonylphosphatidyl-ethanolamine using the enzyme phospholipase-D. 2-AG is enzymatically formed with diacylglycerol lipases α and β. The degradation of AEA is catalyzed by fatty acid amide hydrolase (FAAH). On the other hand, 2-AG can be broken down by several pathways: primarily by monoacylglycerol lipase (MAGL) but also by FAAH, by an esterification pathway

Table 1. *Levels of alimentary control by the ECBR system*

Location	Effect	Selected references
CNS-forebrain including hypothalamus	Rewarding/Stress effect on food intake and digestion; Orexigenic effect	74,76-78
CNS-hindbrain	Gastric secretion and Intestinal motility/secretion reduced; Antiemetic effect	99,101
Mouth	Salivation decreased	91
Lower esophageal sphincter	Relaxation	85,92
Stomach	Decreased gastric secretion	93
Intestinal tract	Inhibit motility Inhibit of secretion	100-102 104

and by Cox-2 together with PGE synthase, transforming it to PGE2-glyceryl ester. Uptake and release of ECs is probably regulated by a common transport system, although these mechanisms remain to be fully elucidated (73).

THE ECBR SYSTEM AND THE ALIMENTARY CONTROL SYSTEM

The multifaceted involvement of the ECBR system in alimentary control will be discussed in the present section. Thus it appears that the ECBR system plays a role at every level of alimentary control including reward/addiction and stress. Therefore, the remainder of this article will be devoted to ECBR involvement in alimentary control in health and disease and in the neonatal organism. Finally, the extent of the ECBR involvement in *in*-and *di*gestion will be compared to functions of the ghrelin peptide, another recently discovered and apparently multileveled player in alimentary control and other physiological systems.

Cannabinoid CB_1 receptors in alimentary control

Forebrain, reward and stress

Components of the ECBR system are present in most structures throughout the brain (14,57), including the nucleus accumbens, the target area of the dopaminergic mesolimbic "reward" system (74). Importantly, CB_1 receptors are co-localized with dopamine receptors in the mouse forebrain (75). Not surprisingly then, 2-AG directly administered to the nucleus accumbens of the mesolimbic system, induced profound hyperphagia (76). These findings strongly suggest a role for the ECBR system in regulating food intake through the incentive value of food.

A role for the ECBR system in the ability to cope with stress has been studied using behavioral and biochemical (77-79) techniques. Further, endocrinologically, ECs influence the HPA stress axis at the pituitary and hypothalamic levels (77,79-81). As outlined above, the HPA stress axis strongly influences both feeding and digestion. Therefore, the ECBR system may be expected to exert part of its influence on alimentary control through the stress-regulating HPA axis.

The forebrain is also the interactive site for the ECBR system and other alimentary control hormones. Thus the orexigenic effect of local infusion of ghrelin into the hypothalamic PVN of rats, was abolished by a peripheral injection with rimonabant (82). These data suggest that hypothalamic ghrelin and CB_1 receptors interact as part of a finely tuned system aimed at maintaining balanced food intake. As mentioned above, the gut and brain peptide CCK induces a feeling of satiety mainly after its release in the gut by protein and fat digests (2). However, CCK is also highly coexpressed with CB_1 receptors in brain structures including the cerebral cortex, hippocampus (58) and the amygdala (83). As noted above, the limbic brain plays a role in emotional aspects of food intake and appetite. It has also been noted that CCK and the ECBR system often signal in opposite directions (59). Therefore, it is plausible to expect that the ECBR and CCK systems interact with respect to food intake, appetite and satiety through emotional regulation in the limbic system. Coexpression of CCK (29,30) and CB_1 receptors in feeding-related nuclei of the hypothalamus has not specifically been studied. Therefore, cross-talk between the CCK and ECBR systems at the hypothalamic level should also be examined.

Hindbrain

Evidence has accumulated that CB_1 receptors are located in the dorsal vagal complex in the brainstem, comprising the area postrema, nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus (DMV). From these locations it is thought now that the CBs mediate their antiemetic effects and relaxation of the lower oesophagal sphincter (LOS) (84-86) and inhibition of gastric motility (87) and gastric acid secretion (84).

It was also suggested that CBs may enhance appetite by suppression of inhibitory synapses in the DMV (88). In support of this assertion, CB_1 receptors are coexpressed with CRH and CART in a significant proportion of neurons of the PVN and/or DMV/lateral hypothalamus of mice (89). Likewise, neuronal CB_2 receptors in the CNS have now been demonstrated in number of structures including cerebellum, cerebral cortex, hippocampus, brain stem and more (66,67). Their functional presence with respect to the brainstem, notably in the DMV, was investigated functionally. Thus evidence was found that CB_2 receptors participate together with CB_1, in the suppression of the emetic response (66). Such cooperation with CB_1 receptors would explain how much lower levels of CB_2 receptors (1.5% of those present in spleen ref. 66,67), can be of

functional importance.

Vagus

CB_1 receptors are present in the peripheral vagal afferent nerve endings in the nodose ganglion to influence visceral perception (84,85). Also at this location CB_1 receptors are co-expressed with CCK receptors, thus suggesting that, in addition to central interactions, the ECBR system interacts with the CCK system to regulate food intake and satiety in the periphery (90).

Mouth

Very recently, functional CB_1 and CB_2 receptors have been identified in rat saliva. Thus activation of these CB receptors reduced saliva secretion (91). Therefore it is possible that CB receptors play a role in digestion from the first stage of the process.

Lower oesophageal sphincter

CB_1 receptors are located in the lower oesophageal sphincter (LOS) muscle (84). Cannabinoid ligands (Δ9-THC, WIN 55,212-2) inhibited its relaxation in ferrets and dogs, thereby counteracting gastroesophageal acid reflux (85,92). These studies also suggested however, that this effect is mediated by CB_1 receptors in the dorsal vagal complex of the hindbrain.

Stomach

CB_1 receptors are also present in gastric muscle and mucosa where they are localized in pre- and postganglionic cholinergic neurons (93). Izzo's group (94), investigating CB_1 receptor concentrations throughout the GIT, confirmed that concentrations are highest in the stomach and in the colon. CB_2 receptor mRNA has also been demonstrated in rat gastric tissue (95).

Intestines

CB_1 receptors are widely present in the GIT (96). The colon (together with the stomach as mentioned above) has the highest CB_1 receptor density in the GIT (94). CB_1 receptors are widely present along the GIT as studied in rats, mice and guinea pigs (see (97)). Thus CB_1 receptors were found on cell bodies and fibers in myenteric plexus of cholinergic sensory, interneuronal and motor neurons. In the submucosal plexus CB_1 receptors are colocalized with VIP and NPY (98). These findings are consistent with the influential role of the CBs on both secretory activity and motility of the gut (97). CB_1 receptors have also been shown to mediate the inhibition gastric emptying (99) and intestinal transit/motility (100-102). Moreover, from the data collected thus far it seems that to a great extent intestinal CB_1 receptors located on extrinsic neurons, by inhibiting the release of acetylcholine (Ach), are responsible for CB-directed regulation of secretory patterns and motility under normal conditions (103). However, it has been suggested that in pathophysiological states, CB_1 receptors located on intrinsic submucosal neurons are mobilized (84). Thus in an *in vivo* study, Izzo and colleagues demonstrated that cholera toxin-induced intestinal fluid accumulation, resulting in diarhoea, was inhibited by the CB_1 receptor agonist CP 55,940. This effect was not prevented by ganglionic blockade with chlorisondamine (104). The importance of CB_1 receptors in protecting the organism against inflammation of the GI system was demonstrated in a study where CB_1 receptor knockout mice were found to be more susceptible to experimentally induced colitis compared to controls; conversely, mice lacking the FAAH enzyme (thus presumably having higher levels of ECs), had greater protection against inflammatory agents (105).

A role for CB_2 receptors in gastrointestinal activity was only recently fully accepted. An earlier report on CB_2-mediated effects (by the CB_2-selective agonist HU-308) on defecation in mice (64), was followed by many negative reports on a role for CB_2 receptors in GI functions (96,97,106). For example croton oil-stimulated gastrointestinal transit was inhibited by WIN 55,212-2; this effect was reversed by rimonabant but not by the CB_2 receptor antagonist SR144528, indicating a CB_1 receptor-mediated inhibition of gastrointestinal transit (101). However, more recent reports indicated that while during resting conditions, CB_2 receptor agonists did not affect gastrointestinal transit in rats, acceleration of intestinal motility was reduced by CB_2, and not by CB_1 receptor activation after stimulation with lipopolysaccharide (LPS) (65). Although the issue needs further clarification, the accompanying commentary to this report was aptly entitled "*Cannabinoids and intestinal motility: welcome to CB_2 receptor*" (106).

Adipose tissue

As indicated above, leptin is one of the first adipokine to be discovered. Several years ago, Di Marzo, Kunos and colleagues (107) showed for the first time a link between leptin and the ECBR system. Thus they reported that leptin injection to rats reduced concentrations of AEA and 2-AG. In the same study, elevated levels of ECs were found in mice (*ob/ob and db/db*)

and rats (Zucker) which display inherent impairments of the leptin system (107). Subsequently, CB_1 receptors have been detected in animal adipose tissue (89,108,109), while the full set of ECBR system components (CB_1 receptors, AEA and 2-AG, synthesizing and degrading enzymes) has recently been described in human adipose tissue (12). In a different study, CB_2 receptors were also detected on human adipose cells (110). Importantly, CB1 receptor knockout mice have less adipose tissue than wild type controls (89) and CB_1 receptors are dysregulated in human abdominal obesity (109).

The "classical", Rita Levi-Montalcini's neurotrophin NGF was recently introduced into the growing list of adipokines (10,11). Biology of NGF may be regulated by CB receptors, although thus far evidence for an interaction between NGF and the ECBR system has been shown only in nonadipose tissue. Thus, NGF receptor tropomyosin-related kinase A (TrkA) activation-induced inflammatory hyperalgesia was shown to be mediated by EC stimulation of CB_1 and CB_2 receptors on peripheral nerves (111). Further, interactions between (endo)CBs and NGF and/or brain-derived nerve growth factor (BDNF) have been proposed for the regulation of reward/addiction (112), pain processing (113) and cancer control (114). Recent evidence suggest that NGF/BDNF and the ECBR system interact in their modulation of brain plasticity, and emotional processes (112,115). Interestingly, NGF/BDNF have been associated with autism (116), while plasma NGF level were significantly raised in subjects classified as being in the first stage of romantic love (117). Thus it will be interesting to further investigate reciprocal relationships between these adipokines in the regulation of emotions/addiction, stress and social interaction. Furthermore, interactions between between NGF/BDNF and the ECBR system at the level of the adipose tissue should be addressed.

Adiponectin is another example of multifunctional adipokines (10,11,108,118-120). Plasma adiponectin levels, like those of NGF/BDNF (10), and differently from many other adipokines such as leptin, tumor necrosis factor-α and resistin (119), are reduced in patients with obesity and related cardiometabolic diseases (118,119). Interestingly, weight reducing effects of the CB_1 receptor antagonist/inverse agonist rimonabant was accompanied by enhanced levels of adiponectin in animal (108) as well as human (120) studies.

Endocannabinoids in alimentary control

AEA-induced inhibition of defecation in mice was the first report that ECs influence intestinal function (121). The ECs AEA and 2-AG have been detected in the small intestine and colon (97,100,122). Overall, the ECs are present in the GIT at several-fold higher concentrations than in the brain (100,123) and are physiologically active (122). Additionally, the degrading enzymes and uptake transporters for the ECs have been located in the GIT (100,122,124).

Two aspects of EC functioning in the alimentary system await final clarification.

1. Are the EC's constitutively active, or only when stimulated (such as in pathophysiological conditions)? It appears that both situations exist, depending on factors including genetic and species differences. For example, rimonabant induced emesis by itself in the shrew but not in the ferret. This species difference maybe explained by a higher constitutive level of ECs in shrews than in ferrets, in which the emetic stimulus may have to enhance EC concentrations before an emetic effect of rimonabant can be revealed (125,126). Further, cholera-toxin-induced fluid accumulation in the mural intestine, was accompanied by upregulated CB_1 receptor mRNA and elevated levels of AEA (104).
2. At least some of AEA's effects are mediated by non-CB receptors, some of which are known and some are not yet defined (55). For example, AEA activates TRPV1 vanilloid receptors (127). Thus, although exogenous CBs, acting *via* CB receptors, have anti-inflammatory effects in the GIT similarly to their effect in other systems and organs, application of the ECs AEA and 2-AG resulted in pro-inflammatory effects *via* TRPV1 receptors (128). Thus the 'duplicity' of the ECs as endovannilloids/ECs (129) suggests a complex role of the ECs in inflammatory states of the GIT. Furthermore, AEA inhibits, probably by allosteric modulation, serotonin $5\text{-}HT_3$ receptor activation (130,131) and activates at least one nonCB_1-nonCB_2 cannabinoid receptor ("CB3") (54-56).

Concluding this section, it clear that every level of the alimentary tract is affected by the ECBR system. Strikingly, ECBR influence on ingestion, digestion and emesis is always, at the organismic level, in harmony (see also Table 1). Thus (endo)CBs reduce intestinal and gastric motility, gastric acid secretion, emesis and nausea, are anti-diarrheal and enhance appetite. Conversely, CB_1 receptor antagonists cause emesis, anorexia and enhanced motility.

Future studies will have to address the relative importance of the various levels of ECBR involvement in alimentary

control, such as CNS-directed influence, compared to direct modulation by gastrointestinal/ENS sites. Izzo and colleagues have started to address this issue when they compared ED_{50}-values of the CB receptor agonist WIN55,212- and cannabinol-induced inhibition of intestinal transit after central (intracerebroventricular, *icv*) *versus* peripheral (intraperitoneal, *ip*) injections. ED_{50} values were significantly, but not dramatically lower when injected *icv*. This prompted the authors to suggest that CB inhibit GI transit by both routes. However, after croton-oil-induced diarrhea, there were no differences in ED_{50} values of *icv* or *ip* injected CBs. Moreover, ganglionic blockade with hexamethonium did not affect the inhibitory effect of *ip* injected CBs (101). In a subsequent study, the same group showed that after croton oil, intestinal CB_1 receptors were upregulated and that EC levels were not changed while the FAAH enzyme appeared at higher concentrations. These observations suggested that during inflammatory/diarhoea states, the local gut ECBR system is upregulated (100), apparently to reduce diarhoea and inflammation. Thus a dominantly peripheral response to pathological states by the ECBR system contrasts with the impression that in general in the brain-gut axis, CNS control plays a more major role when homeostasis is perturbed such as in digestive disorders or during psychological stress (5). Future studies will hopefully address the brain *versus* gut division of ECBR system labor in more detail.

Overall, the unified activity makes the ECBR system in the alimentary control system exquisitely suitable for multi-leveled treatment of syndromes such as irritable bowel syndrome and cachexia/anorexia, using ECBR-activating agents (84) or obesity/metabolic syndrome, using ECBR-inhibitors (132). A discussion of CB-based treatment of the metabolic syndrome/obesity is beyond the scope of this review and the reader is referred to previous publications (132-134). However, some of the promising approaches to treating GIT discomfort and disease, are presented in the next section.

BRAIN-GUT BIDIRECTIONAL CONTROL VIA THE ECBR SYSTEM IN PATHOPHYSIOLOGICAL AND PSYCHOSOMATIC CONDITIONS

The ECBR system, as described above, is strongly represented in both brain and gut, or, more precisely in the CNS, in the dorsal vagal complex and at almost all levels of the gastrointestinal tract. Especially its involvement in stress and emotional processing make the ECBR system an excellent candidate to study both as an underlying cause for pathological and psychosomatic problems of the digestive system as well as, as a target for new therapeutic approaches. Only conditions with a stress/psychosomatic component are considered here.

Irritable bowel syndrome. There is little doubt that stress and the HPA axis play a major role in IBS symptomatology. Experimentally, upregulation of CRH receptors in the PVN and locus ceroeleus have been shown after colonic stimulation (135). The role of cortisol in IBS has also been demonstrated although its levels may be up- or down-regulated depending on perhaps, the type of patients and conditions (36,136). This is in agreement with our own findings that low levels of corticosterone may signify decreased stress coping capability in mice (77). Solid evidence exists for an (endo)CB-regulated reduction of intestinal motility (121,122,137,138) through CB_1 (100) and CB_2 (65,121,139) receptors. This property of ECBR has been proposed to be beneficial for IBS which is often characterized by enhanced intestinal motility and contractility (122,124).

Inflammatory bowel disease (IBD). Inflammatory bowel diseases, including Crohn's disease and ulcerative colitis, result from inflammatory processes in the intestine and is characterized by ulcers, rectal bleeding diarrhea, nausea and lack of appetite (124). Major life stresses are thought to influence disease activity in IBS, while a role for minor daily stressors in IBD expression needs further investigation (36). In an animal model for Crohn's disease (orally applied croton oil to mice which causes inflammation in the small intestine), a beneficial, slowing effect of CBs on intestinal motility has been shown (100,101). This effect was apparently peripherally mediated (in the gut). Therefore such treatment is promising when central/psychological treatment is not likely to be helpful. As stated above, the importance of CB_1 receptors in protecting the organism against inflammation of the GI system was demonstrated in a study where CB_1 receptor-deficient mice were more vulnerable, while FAAH-deficient mice were less vulnerable to experimentally-induced colitis (105).

Interestingly, adiponectin levels were increased in mesenteric adipose tissue of patients with Crohn's disease, but not in patients with ulcerative colitis (140). Thus similarly to obesity, at least in Crohn's pathology too, ECs and adiponectin are counterbalanced. The putative significance of this finding needs further investigation.

Gastroesophageal reflux disease (GORD). This disorder results from a weak lower oesophagal sphincter causing gastric content to flow upward, hence symptoms including heartburn and acid regurgitation appear (124,141). The influence of psychological stress, anxiety and depression on this condition has been shown (141-143). An CB-centered treatment may be developed either through central effects or through peripheral muscle relaxation. Indeed, cannabinoid CB_1 receptor agonists have been shown to relax the oesophageal sphincter in dogs and ferrets. This effects was mediated by vagal neural loops at peripheral and central (dorsal vagal complex) sites (85,92,97).

Secretory diarrhea. Fluid-induced increase in stool volume, that is, secretory diarrhea, is caused by abnormal secretion and/or absorption of water and electrolytes as well as dysregulated intestinal motility (144,145). Psychological stress may precipitate secretory diarhoea, probably mediated by corticotrophin releasing hormone (CRH1) receptors (145). Activation of the HPA stress axis results in stress-induced secretory diarrhea (144). Stimulation of the ECBR system by the uptake inhibitor VDM11 reduced intestinal fluid accumulation; conversely, the CB_1 receptor antagonist rimonabant increased fluid accumulation (97). These observations suggest that the ECs exert a tonic regulation of intestinal secretory activity which can be up-or down-regulated according to the (psycho)physiological environment. Further, cannabinoid CB_1 receptor activation resulted in decreased fluid accumulation in the small intestine of the rat, suggesting that cannabinoid-based treatment may be beneficial for diarhoea (146).

Gastric ulcer. Summarizing a vast body of literature on the association between psychosocial factors (such as stress and the perception of stress) and peptic ulcers (36), Jones conservatively concludes that psychosocial factors may play a role in ulcer formation (147). Cannabinoid-induced reduction in gastric secretions and in ulcer formation has been observed many years ago and more recently, several reports have replicated and extended such observations to include the mediation by CB_1 receptors in stomach and/or central locations (93,124,148). More specifically, Win 55,212 reduced stress-induced ulcers in rats (148). From this study it is not clear whether the anti-stress effect was mediated centrally or peripherally. Possibly either or both routes are used under such conditions, since central stress-reducing effects of CBs have been shown (149), while the presence of CB_1 receptors in (pre- and postganglionic) vagal pathways leading to the stomach (93) may have been activated as well.

Emesis. Antiemetic effects of (endo)cannabinod have been extensively demonstrated and several Δ9-THC-like cannabinoid drugs (the synthetic nabilone and dronabinol) are selectively available for oral clinical use (97,124,150,151). The antiemetic effects are mediated by CB_1 receptor-mediated responses on gastric vagal nerves or centrally via the area postrema and dorsal vagal complex (97). Taken together, it is widely agreed that CB-based drugs especially those which do not have central side effects, should be developed as antiemetic drugs for cancer and AIDS patients who receive chemotherapeutic treatment. Cannabinoids may be especially effective in combating anticipatory nausea and vomiting (152). Since the area postrema has receptors located outside the CNS, CB_1 receptor-specific cannabinoids which are not active within the CNS, should be particularly suitable (153,154).

DEVELOPMENT

It is evident from the data summarized thus far, that the ECBR system is abundantly present and functionally important at all stages of ingestion and digestion in the adult organism. Although perhaps one of the very major systems (see also Table 1), the ECBR system and its CB_1 and CB_2 receptors do not seem critical for survival, as most CB_1 and/or CB_2 knockout mice reach adulthood, be it with reduced body weights (155-157). Similarly, very long term treatment (4 months) with the CB_1 receptor antagonist rimonabant did not cause mortality or (overt) detrimental effects, except a robust reduction in body weight (about 8%) (158). In contrast, we have shown that a single injection with rimonabant administered within 24 h of birth in mice permanently interferes with milk ingestion, resulting in growth failure and death in many cases. The effect was dose dependent and was observed in several strains of mice (ICR, SABRA, C57BL/6). The growth stunting effect was not seen in CB_1 receptor knockout pups but mortality was induced by rimonabant even in these knockouts, suggesting that these pups had developed a compensatory CB_1-like ("CB_3") receptor (56,159,160). Further psychobiological analyses of rimonabant-treated neonates suggested that CB_1 receptor activation in neonates is required for oral-motor development required for sucking (161). Since the behavioral and physiological deficiencies of CB_1 receptor-blocked mouse pups resemble infants suffering from "non-organic failure-to-thrive" (NOFTT), we have suggested that CB_1 receptor-blocked neonates may be used as a model for NOFTT and form the basis for the devel-

Table 2. *Comparison between the physiological roles of endocannabinoids and ghrelin*

Physiological function/location	**ECBR system**	**Ghrelin**	**Selected references**
Localization in GIT	Stomach, small intestine, colon	Mainly stomach, much smaller amounts in small intestine	22,100,122,174,175
Appetite/food intake	Orexigenic	orexigenic	74,175
Central/peripheral mechanism of food intake	Central (DVC, PVN/lateral hypothalamus) and peripheral	Central (arcutae nucleus, PVN) and peripheral	82,124,175,176
Colon	Decreased secretion/motility	enhanced motility	177
Ileum	Relaxation/ Decreased secretion	No effect/decreased intestinal transit	177,178
Stomach	Decreased acid secretion/delayed emptying	Main source of ghrelin production; Enhanced gastric emptying/ Decreased acid secretion	22,84,97,178
Lower esophageal sphincter	Inhibition of relaxation	?	85,92
Dorsal vagal complex	Anti-emetic, GIT relaxation, GIT decreased secretion	Transmits peripheral ghrelin-induced signaling to hypothalamus	84,179,180
Interaction with leptin	Inverse	Inverse	17,24,107
HPA(stress) axis	Corticosterone increased/decreased	Corticosterone increased	77,149,181
Hypothalamus	Hypothermia	Hyperthermia/ hypothermia	14,181,182
CNS Motor areas(?)	Decreased locomotion	Increased/decreased locomotion	14,181,182
Bone and-cartilage homeostais	Bone (increased/decreased density)	Bone and cartilage homeostasis	22,162,183
Immune system/ inflammation	Immune regulation, anti-inflammatory	Immune regulation/ antiinflammatory	22,164,168
anxiety	Incease/decrease	Increase	165,167
sleep	Increase	Increase/decrease	166,167

opment of CB-based treatments.

CANNABINOID *VERSUS* GHRELIN

As stated above the ECBR system is ubiquitous and involved at many, if not all, levels of alimentary control and in many other functions. Interestingly, the recently discovered appetitive hormone ghrelin which binds to GHS receptors and is largely synthesized in the stomach (as described above), also participates in alimentary control at a number of levels. In addition to their appetite stimulating qualities, ghrelin and the CBs have a number of functions in common, including modulation of bone homeostasis, anxiety, sleep regulation, anti-cancer potential and immune modulation (22,162-168). Moreover, direct interaction between the two systems in the hypothalamus mediating food intake, has been demonstrated (82). Thus the remarkable functional and anatomical overlap between ghrelin and the ECs is examined in Table 2. Inspection of this information reveals that despite the similarities between CBs and ghrelin, these systems also have opposite effects in a number of spheres including intestinal motility and gastric secretion and perhaps temperature control and motor activity (see Table 2 for references).

In conclusion, ghrelin and CBs share a number of areas of influence in alimentary control and in other functions. However, the overlap is not complete. Further investigations into the interactions between the two systems seem warranted.

CONCLUSIONS AND FUTURE DIRECTIONS

Three major conclusions emanate from the material reviewed here: *(i)* the ECBR system is one of the major mediators between brain, the alimentary system and the adipose tissue, *(ii)* it is suggested that the role of the ECBR system in adult regulation of digestion and ingestion is a remnant of its critical role for the initiation of feeding and survival in the newborn, and *(iii)* the pervasive influence of the ECBR system in alimentary control make it a highly suitable target for therapeutic developments aimed at alleviating pathophysiological conditions such as IBD, IBS, gastric ulcers, nausea and anorexia.

Especially manipulation of CB_1 receptor activation will have therapeutic value in a number of alimentary pathophysiological conditions. However CB_1 receptor agonists often have undesirable psychoactive effects, such as confusion and anxiety. In view of the predominantly CB_1 receptor-mediated effects on the GIT, it is desirable that CB-based treatment of GI disorders be mediated by ligands which do not cross the blood brain barrier or are not psychoactive for other reasons. We have recently demonstrated that several (+)cannabidiol derivates [such as (+)cannabidiol-demethyl heptyl and (+)carboxy-cannabidiol-demethyl heptyl], displayed CB_1-receptor mediated inhibition of intestinal motility in the absence of central effects (153,154). Further studies on such compounds should address peripheral mechanisms of energy control including their effect on adipose tissue.

Upregulating the ECBR system by the inhibition of enzymatic breakdown or reuptake inhibition is expected to yield more selective therapeutic effects. However, preventing EC degradation with oleamide, a fatty acid amide which is considered by most (169-172) not to bind CB_1 receptors, resulted in psychoactive effects similar to those of AEA itself (169). On the other hand, a highly selective FAAH inhibitor URB597 is thought to have anxiolytic and antidepressant potential without sedative and addictive properties (173).

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