Dysregulation of the Endocannabinoid System in Obesity

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Endocannabinoids are lipid derivatives that activate cannabinoid receptors. Well studied endocannabinoids are arachidonoyl ethanolamine (AEA or anandamide) and 2-arachidonoylglycerol (2-AG). Endocannabinoid biosynthesis involves an acute rise of intracellular Ca\(^{2+}\) caused by extracellular triggers (e.g. neurotransmitters, cellular stress from mechanical damage, or metabolic challenges), which activates enzymes involved in endocannabinoid synthesis. Endocannabinoids are not stored in cells, but are rapidly synthesised, released and degraded by intracellular enzymes. Among endocannabinoid degrading enzymes are fatty acid amide hydrolase (FAAH), and monoglyceride lipase (MGL) (1). The two cannabinoid receptors (CB\(_1\) and CB\(_2\)) are seven-transmembrane-domain G\(_i\)-protein-coupled receptors. Typical endocannabinoid signalling involves inhibition of cAMP synthesis and Ca\(^{2+}\) mobilisation, as well as activation of K\(^{+}\) efflux in neuronal cells. CB\(_1\) is highly expressed in several brain regions; lower expression levels are found in peripheral tissues. CB\(_2\) is predominantly located on immune cells (2).

Pathophysiology and pharmacology of the endocannabinoid system (ECS) have extensively been exploited during past years (1). The successful clinical development of selective inverse agonists of CB\(_1\), rimonabant and tarenanabant for weight loss and the treatment of obesity-associated metabolic disorders might well be through blocking this overstimulation of cannabinoid receptors. At present, no single mechanism has been identified that explains the increased bioavailability of endocannabinoids in obesity. Both increased synthesis and decreased degradation appear to operate in a species- and tissue-dependent manner, but many pieces of the puzzle still need to be collected. For example, most data show decreased fatty acid amide hydrolase (FAAH) expression and/or activity as a result of obesity or high-fat intake, but the endocannabinoid predominantly increased in tissues is 2-arachidonoylglycerol (2-AG), which is not degraded by FAAH \textit{in vivo}. Furthermore, the influence of dietary fatty acids on the synthesis of endocannabinoids needs to be studied in much more detail. Although weight loss does not seem to influence activation of the endocannabinoid system (ECS) in human obesity, suggesting an underlying mechanisms independent of body weight, no such mechanism at the genetic level has yet been identified either. Thus, activation of the ECS is a hallmark of abdominal obesity, and explains the success of pharmacological CB\(_1\) blockade, but serious attempts have to be made to clarify the underlying mechanisms of this activation.

Key words: diet-induced obesity, weight reduction, 2-arachidonoyl glycerol, CB\(_1\)-receptors, fatty acid amide hydrolase, adipose tissue.

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Leptin deficiency and activation of the endocannabinoid system

The pathophysiological role and regulation of the ECS has been studied in several animal models of obesity. Some of them develop obesity as a result of mutations in either the leptin or the leptin receptor gene (for example, ob/ob mice, db/db mice, fa/fa rats). Thus, obesity and leptin deficiency must both be considered as possible confounders of the results obtained. In normal rats, leptin injection decreased hypothalamic levels of anandamide and 2-AG, and endocannabinoid levels were normal rats, leptin injection decreased hypothalamic levels of anandamide and 2-AG, and endocannabinoid levels were increased in all three models of leptin deficiency (ob/ob mice) or defective leptin receptors signalling (db/db mouse, fa/fa rats). Leptin treatment of ob/ob mice again decreased hypothalamic endocannabinoid levels (5). These findings were specific for endocannabinoids in the hypothalamus, because neither palmitoyl ethanolamine (PEA), which does not act on CB, nor cerebellar levels of endocannabinoids, were affected by genotype. These early findings clearly demonstrated negative regulation of endocannabinoids by leptin in the hypothalamus, but no enzymatic mechanisms were identified.

Endocannabinoids have also been studied in the uterus of ob/ob mice. Again, anandamide and 2-AG concentrations were increased in obese mice and could be reversed by leptin treatment, whereas PEA concentrations in the uterus were not affected by genotype (6). In these experiments both pathways of endocannabinoid synthesis and degradation were affected in the ob/ob mice and leptin treatment restored all enzyme activities to wild-type levels. Of interest, cannabinoid binding to cell membranes isolated from the uterus of ob/ob mice was similar to that seen in wild-type mice, and was not changed by leptin treatment. By contrast, mRNA expression of CB1 was decreased in the soleus muscle of ob/ob mice (7), unchanged in the kidney (8), and increased in abdominal subcutaneous adipose tissue of fa/fa rats (9) when compared with lean control mice.

Influence of diet and obesity on the endocannabinoid system in animal models

Dietary long-chain polyunsaturated fatty acids and ECS activity

Diet-induced obesity (DIO) by high-fat feeding is a common animal model that resembles human obesity in more aspects than the rather specific obesity models with naturally occurring or experimentally engineered gene knockouts. Several data on the influence of DIO on ECS regulation will be summarised below. It is intriguing to speculate that specific ingredients of a high-fat diet have a direct effect on endocannabinoid synthesis because then modulation of ECS activity by dietary supplements could be a possible treatment. Unfortunately, such data are rare at present. Increased brain concentrations of anandamide were found after feeding piglets a diet enriched in long-chain polyunsaturated fatty acids (LC-PUFA) such as arachidonic acid (20:4n-6) and docosahexaenoic acid (DHA, 22:3n-3) (10). In contrast, a fish-oil diet with high levels of DHA decreased arachidonic-phospholipids in the mouse brain and increased the proportion of DHA-phospholipids. These changes were associated with reduced 2-AG concentrations in the brain (11). More investigations in this field are needed, particularly in peripheral tissues, but increased dietary intake of arachidonic acid under the condition of high-fat feeding, and the possibly enhancing effect on anandamide synthesis must be considered.

Obesity and the endocannabinoid system in the brain

Aside from the findings, described above, on endocannabinoid concentrations in animal models of leptin deficiency (5), only one report is currently available on CB1 levels in the brain of DIO rats. After 10 weeks of high-fat feeding, rats weighed 84 g more than rats on the standard chow diet. Autoradiography of several brain regions revealed that DIO was associated with lower CB1 density in several extrahypothalamic regions, for example the hippocampus, layers I and VII of the cortex, the entopeduncular nucleus, and the nucleus accumbens. Specifically, lower CB1 density was correlated with cumulative energy intake from the palatable, fat-enriched diet. Consistent with earlier studies, the hypothalamus presented with low CB1 density, but DIO was not associated with any changes in expression in the hypothalamus (12). The authors suggest that CB1 downregulation of CB1 upon high-fat feeding is the result of increased endocannabinoid availability in extra-hypothalamic regions that are partly involved in the preference for palatable foods. Unfortunately, endocannabinoid concentrations were not measured in this study, and thus the mechanisms of CB1 downregulation in DIO remain uncertain.

Obesity and the endocannabinoid system in adipose tissue

All essential components of the ECS can be found in adipose tissue of mammalian species (13). High-fat feeding was associated with increased concentrations of 2-AG in epididymal adipose tissue in mice, but no experimental mechanism was revealed in this study (14). Some ideas on the regulation of CB1 in adipocytes of DIO rats were reported recently. In this study, high-fat feeding over 30 weeks increased body weight by 115 g and was associated with a significant increase in adipocyte size. Both changes were mostly prevented by concomitant regular exercise training of the rats on the high-fat diet (15). CB1 protein expression increased significantly with increasing adiposity and adipocyte size, but exercise training significantly inhibited that DIO-related effect on CB1 in the subcutaneous and the visceral adipose tissue depot. The authors found that high-fat feeding decreased, and exercise enhanced, peroxisome proliferator-activated receptor-δ (PPARδ) protein expression, and that PPARδ tonically inhibits CB1 expression in a mouse clonal adipocyte cell line (15). These results suggest a new regulatory pathway for CB1 expression in adipocytes that has implications for obesity and needs to be confirmed in further studies. Following the experiments on the influence of dietary LC-PUFA on endocannabinoid synthesis (10, 11), a recent paper examined endocannabinoid...
synthesis in mouse 3T3-F442A adipocytes under high concentrations of exogenous LC-PUFAs (16). In these experiments, arachidonic acid increased the synthesis of 2-AG, of triglycerides containing arachidonic acid, and of phospholipids with arachidonic acid in sn-2 position. Incubation with DHA decreased 2-AG and anandamide synthesis and the concentration of phospholipids with arachidonic acid esterified in sn-2 and sn-1. These findings are in agreement with the data on the influence of dietary arachidonic acid and DHA on endocannabinoid concentrations in mouse brain (11) and suggest a role for exogenous LC-PUFAs in the regulation of ECS activity.

Obesity and the endocannabinoid system in the pancreas

Pancreatic cells do also synthesise endocannabinoids, but some obscurities exist regarding the exact cellular profile of cannabinoid receptor expression on pancreatic cells (17, 18). CB1 and CB2 were found in human islets, and biosynthesis of endocannabinoids was modulated by glucose. Whereas CB1 was densely located in glucagon-secreting α-cells and less so in insulin-secreting β-cells, CB2 was present in somatostatin-secreting δ-cells, but absent from all other cells. In vitro experiments revealed that CB1 stimulation enhanced insulin and glucagon secretion, whereas CB2 agonism lowered glucose-dependent insulin secretion (19). High-fat feeding was associated with increased concentrations of anandamide and 2-AG in whole pancreas preparations in mice. Loss of islets, in contrast, did not influence pancreatic concentrations of endocannabinoids in diabetic mice (14). No experimental data were obtained in these studies that explained the mechanisms of increased pancreatic endocannabinoid bioavailability upon high-fat feeding. In obese Zucker rats, the decrease of islet number and total pancreatic weight was prevented by rimonabant treatment. Together with improved renal function and histopathology, these findings might explain the significantly decreased mortality of rimonabant-treated animals compared with untreated and pair-fed animals (8).

Obesity and the endocannabinoid system in the liver

Important experimental data have been recently reported regarding the positive effects of rimonabant treatment on fatty liver associated with obesity in obese diabetic rats (20). These data were preceded by a report that described increased concentrations of anandamide in the liver of mice fed a high-fat diet. The most likely mechanism was a concomitant decrease of FAAH activity. Of importance is the observation that both decreased FAAH activity and increased anandamide concentrations were already present after some days on the high-fat diet, before the animals had developed obesity. High-fat feeding also increased the protein expression of CB1 in the liver (21). These results suggest a predominant role of fat content over caloric intake in the regulation of the ECS in the liver, and indicate that increased synthesis — as well as decreased degradation — should always be considered in the context of ECS regulation in obesity.

Activation of the peripheral endocannabinoid system in human obesity

Increased circulating 2-arachidonoyl glycerol in abdominal obesity

The first study that determined circulating endocannabinoid concentrations in obese humans reported increased anandamide concentrations in plasma of young obese women with Binge Eating Disorder (22). In this study, blood levels of anandamide were also increased in patients with Anorexia Nervosa; thus, it was unclear whether obesity per se, or the underlying psychiatric disorder, or both, contributed to the finding (22). We found increased anandamide and 2-AG concentrations in the plasma of menopausal obese women when compared with lean women of the same age. Increased anandamide levels were correlated to decreased FAAH mRNA expression in the subcutaneous adipose tissue depot (23). Anandamide and 2-AG concentrations were also increased in plasma of obese diabetic subjects (14). In this study, an acute decrease of circulating anandamide was observed following a test meal in healthy subjects when compared with the fasting state. This finding resembled animal data on hypothalamic endocannabinoid levels that were changed in the same manner by fasting and food intake (24).

None of these studies, however, presented detailed data on the obesity phenotype of the subjects, and the metabolic changes associated with obesity were also not reported. Thus, more intriguing data came from two other studies (25, 26) that measured adipose tissue distribution by imaging techniques and insulin sensitivity or glucose tolerance by the euglycaemic hyperinsulinaemic clamp technique or oral glucose load. Both studies investigated obese subjects within a reasonable range of body weight and did not focus on morbidly obese subjects. In a comparison of lean and obese men and women with either subcutaneous or visceral adipose tissue accumulation (n = 60), 2-AG plasma concentrations were clearly correlated with visceral adipose tissue mass, whereas obese subjects with subcutaneous adipose tissue accumulation were not different from the lean control group with regard to 2-AG plasma concentration. Anandamide was higher in women than in men, but no relationship with obesity or body fat distribution was found in this study (25). These findings were supported by a study in 62 well-characterised men showing that subjects in the highest tertile of 2-AG levels presented with markedly increased visceral adipose tissue mass despite having almost the same body mass index as those in the lower two tertiles of plasma 2-AG. Anandamide, by contrast, was negatively correlated with visceral fat mass (26). Both studies showed that increased plasma 2-AG correlates with markers of metabolic syndrome (e.g. free fatty acids, triglycerides, HDL cholesterol, adiponectin), and is associated with increased insulin sensitivity and enhanced glucose and insulin responses during an oral glucose load.

Unfortunately, none of these studies was designed to offer a pathophysiological explanation of the origin of increased plasma endocannabinoid concentrations in obese subjects. Furthermore, no
Experimental data have been provided to clarify the pathophysiological role of circulating endocannabinoids. At present, the most likely explanation is that increased formation and/or decreased degradation lead to increased endocannabinoid tissue levels. The circulating endocannabinoids probably just reflect a partial overflow from the tissues with no self-contained biological function. Future studies will clarify mechanisms and the biological importance of increased endocannabinoid availability in the blood (27).

Dysregulation of the endocannabinoid system in adipose tissue of obese humans

Morbid obesity was associated with increased concentrations of 2-AG in visceral – but not subcutaneous – adipose tissue when compared with normal weight controls, whereas anandamide was not affected. Furthermore, 2-AG concentrations in general are slightly higher in visceral compared with subcutaneous adipose tissue, a finding that was not replicated for anandamide concentration (14). In this small study, gene expression of synthesising and degrading enzymes for 2-AG in adipose tissue was not changed in obese subjects; thus, the authors speculate on a possible role of dietary fatty acids for increased bioavailability of adipose 2-AG, as discussed above.

We have replicated gene expression findings in two independent studies that clearly demonstrated decreased mRNA levels for both CB1 and FAAH genes in subcutaneous and visceral adipose tissue depots of obese subjects independent of fat-distribution phenotype (23, 25). A tendency to reduced CB1 gene expression was also reported for subjects with visceral adipose tissue in a study using obese diabetic patients (see above), but results were not statistically significant, probably only because of the small sample size and a considerable variation in gene expression levels (14). Although some uncertainty remains with regard to the mechanisms leading to increased 2-AG concentrations in adipose tissue, downregulation of CB1 mRNA in adipose tissue might be explained by the increased availability of endogenous ligands, as described in other tissues (5, 6, 14, 27). This hypothesis, however, needs further experimental verification because CB1 desensitisation on chronic agonistic stimulation appears to be predominantly regulated on the protein level, at least under experimental in vitro conditions (28). In another study using visceral adipose tissue samples, downregulation of CB1 and FAAH mRNA was also seen in obese patients, and the authors identified the cytokine tumour necrosis factor α (TNF-α) as a an inhibitor of FAAH gene expression in adipose tissue explants in vitro (29). Given the local inflammation, especially in visceral adipose tissue (30), these findings might help to explain the commonly seen changes in ECS gene expression in obesity.

In clear contrast to the aforementioned studies, other authors have reported no change in gene expression of CB1 in subcutaneous and omental adipose tissue of obese subjects (31), or even increased mRNA levels of CB1 and FAAH in the visceral depot (32). The reasons for these contrasting findings are unclear as yet and appear not be because of apparent differences in patient selection, co-morbidities or concomittant drug treatment. Thus, the issue of adipose tissue ECS dysregulation in obesity needs further clarification.

Influence of weight loss on the endocannabinoid system in human obesity

One possible explanation for increased endocannabinoid concentrations in the blood and dysregulation of ECS genes in adipose tissue of obese subjects is the influence of obesity-associated hormonal, metabolic or inflammatory mediators. This hypothesis could be supported if weight loss could reverse the observed changes. To date, two smaller studies investigated the influence of weight loss on the ECS in obese subjects. In the first study, 17 menopausal women lost 5% initial body weight during 12 weeks by a combination of caloric restriction and a modest exercise programme (23). In the second study, 13 young obese men and women lost 5% initial body weight through treatment with the serotonin und norepinephrine re-uptake inhibitor sibutramine for 12 weeks (33). In both studies, neither circulating anandamide and 2-AG, nor CB1 and FAAH gene expression in subcutaneous abdominal adipose tissue, were influenced by weight loss, although metabolic parameters such as fasting insulin and blood pressure were significantly decreased as surrogates of successful therapy. Conversely, signs of local and systemic inflammation were also not influenced by this modest amount of weight loss; thus, one possible regulatory mechanism of the ECS remained unchanged. Another way of explaining these negative weight loss data is that weight loss might not have been large or fast enough and that, with a more rigid protocol (e.g. very low calorie diet), an influence on the ECS might have been seen. If weight loss does not influence the ECS in obese subjects, an alternative hypothesis is that dysregulation of the ECS occurs as a result of genetic modifications associated with obesity.

Genetics of the endocannabinoid system in human obesity

The C385A missense mutation of the FAAH gene leads to a proline to threonine amino acid substitution at position 129, which is associated with decreased FAAH stability and activity, a phenotype that could be related to the dysregulation of the ECS in obesity. In a first study, this missense AA genotype was significantly more frequent in obese Caucasians (4.8%) than in lean controls (2.1%). The significant difference in the prevalence of the AA genotype in obese African–American subjects compared with lean controls was, however, confused by the fact that a high frequency of the AA genotype was seen in underweight African Americans, and no association was found between the AA genotype and obesity in Asians (34). A second study in a large and homogenous Danish population found no correlation between the AA genotype with body mass index (BMI) or waist circumference (35). In a third study, the AA genotype was associated with a stronger reduction of triglycerides during 6 weeks of weight reduction in German dyslipidemic obese patients (36).

The gene encoding CB1 (CNR1) has been examined in more detail, and several single nucleotide polymorphisms and mutations have been described (37). Four studies have been published: the...
1359G>A polymorphism in the coding region was associated with BMI (n = 210), but not with changes in lipid values after 6 weeks of weight reduction in obese Caucasians (n = 451); the 3813A>G polymorphism was associated with skinfold thickness and waist circumference (n = 1500); the 4895A>G polymorphisms in exon 4 was not associated with measures of obesity; and the 1422G>A polymorphism was associated with increased waist circumference only in obese men (n = 1064) [36, 38–40]. The only family-based genetic analysis (599 families with at least one obese child) did not find any significant transmission equilibrium for the eight CNR1 genotypes under investigation (41). Thus, a significant contribution of currently known polymorphisms of the CNR1 gene to obese phenotypes was excluded.

Conclusion

An activation of the ECS in obesity and increased concentrations of endocannabinoids in several tissues and the circulation have been described in the literature. This increased availability of endocannabinoids might lead to an enhanced stimulation of cannabinoid receptors. Both rimonabant, and very recently taranabant, have been shown to be effective for weight loss and the treatment of obesity-associated metabolic disorders, and these might act by blocking this pathophysiological stimulation of cannabinoid receptors. At present, no single mechanism has been identified that explains the increased bioavailability of endocannabinoids in obesity. Both increased synthesis and decreased degradation operate in a species- and tissue-dependent manner, but many more pieces of the puzzle need to be collected. For example, most data show decreased FAAH expression and/or activity as a result of obesity or high-fat intake, but the endocannabinoid predominantly increased in the tissues is 2-AG, which is not degraded by FAAH in vivo. Moreover, the influence of dietary fatty acids on the synthesis of endocannabinoids needs further clarification. The apparent lack of influence of weight loss on the activated ECS suggests an underlying mechanism that is independent of body weight, but no such mechanism has been identified on a genetic level. Thus, in conclusion, the activation of the ECS is a hallmark of obesity, and explains the success of pharmacological CB1 blockade, but serious attempts now have to be made to identify the underlying mechanisms.

Conflicts of interest

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