UTRECHT UNIVERSITY

Pharmacological intervention in obesity

Utilizing the endocannabinoid and opioid system

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Abstract

Introduction: Obesity and its secondary chronic diseases are a massive burden on the Western healthcare system, therefore the pharmacological industry has taken an interest in the development of weight reducing therapeutics.

Objective: To provide an overview of the current literature concerning the endocannabinoid and opioid systems of the brain in relation to their pharmacological potential in the treatment of obesity.

Summary: Antagonists of both the endocannabinoid and opioid systems have proven their anorectic effect in laboratory animals but human trials have been plagued by side effects. Simultaneous administration of these compounds has shown to create a supra-additive anorectic effect, if this is the case dosage could be significantly lowered and side effects possibly abolished.

Conclusion: Since Rimonabant (a full inverse agonists of the CB1 receptor) has been taken of the market, the scientific interest has diverted away from cannabinoid antagonists in the treatment of obesity. Recent efforts have uncovered various possibilities to eradicate the negative side effects associated with antagonising the endocannabinoid system; this could possibly rekindle the scientific interest in this field.

Keywords: Obesity, endocannabinoids, opioids, polytherapy, food intake

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1 Introduction

This thesis provides an overview of the current literature concerning the possibilities of pharmacological therapy in the treatment of obesity, utilizing the endocannabinoid and opioid systems of the brain.

1.1 Obesity

Obesity is one of the biggest health threats, for Western society, in the twenty-first century. Obesity is defined as having a body mass index (BMI; weight, in kilograms, divided by square of height, in meters) greater then 30, whereas overweight is defined as having a BMI over 25. Obesity is a massive burden on the Western healthcare system, because of the increased risk of secondary chronic diseases such as; type 2 diabetes, stroke, cardiovascular diseases, hypertension, gallbladder disease, respiratory problems, sleep apnoea, osteoarthritis and certain types of cancer (Cooke and Bloom 2006). Furthermore, obesity also has social and psychological consequences such as depression and low self-esteem.

The abundance of 'high calorie food' and sedentary life style in the industrialized countries is perceived as largely responsible for the current epidemic.

1.2 Epidemiology

Currently 65% of the US population is overweight and 25% is obese (Flegal, Carroll et al. 2002). The prevalence of obesity has increased radically over the previous decade (Mokdad, Ford et al. 2003) and unfortunately, this development is also seen in various industrialized countries of the world (Kopelman 2000). According to the World Health Organization (WHO), there were 1.6 billion overweight adults and more then 400 million obese adults in 2005 worldwide. The WHO further predicts that by 2015 this number will increase to approximately 2.3 billion overweight adults and at least 700 million will be obese (WHO 2005).

1.3 Scientific background

Body weight is controlled through a balance between energy expenditure and intake. Energy homeostasis is an intricate process that consists of various interacting homeostatic and non-homeostatic pathways (Chakrabarti 2009). Homeostatic mechanisms primarily involve the brain, receiving peripheral feedback signals such as hormones, neurons and metabolites, and sending signals to higher brain centres and the autonomic nervous system.

It is important to realize that non-homeostatic mechanisms also play a significant role in controlling feeding behaviour. The hedonic aspect of food can initiate feeding, and is unresponsive to the body's homeostatic feedback, therefore uncontrolled feeding can occur. Uncontrolled overfeeding may lead to obesity, therefore anti-obesity drugs are an attractive option. Especially because dieting and exercise to lose weight require a great deal of effort, persistence and willpower, resulting in low compliance in such regimes. The pharmacological industry has therefore taken an interest in the development of weight reducing therapeutics.

It has been discovered that the endocannabinoid and opioid system play a crucial role in the hedonic experience of palatable foods. Since over-consumption of palatable foods is deeply associated with obesity, it is hypothesized that; blocking the function on food intake of the endocannabinoid and opioid systems might be essential for the pharmacological intervention of obesity.

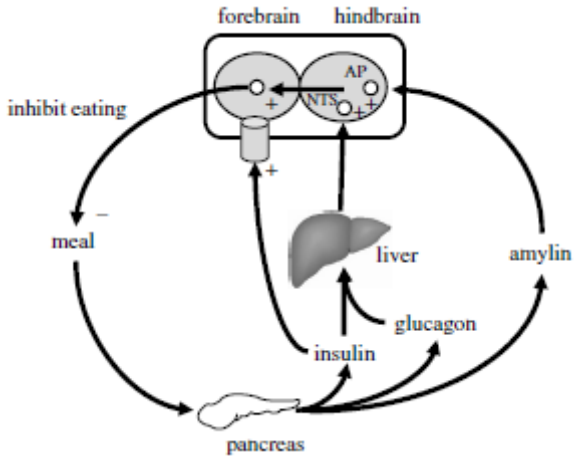
This report will discuss possible pharmacological obesity therapies that are currently in development, taking special interest in the endocannabinoid and opioid system. First the peripheral and central mechanism of energy homeostasis will be discussed, followed by an analysis of the endocannabinoid and opioid system. Finally, the possibilities of pharmacological intervention of these two systems for the treatment of obesity will be considered.

2 Mechanisms of energy homeostasis

Since obesity is caused by an energy intake that exceeds the energy expenditure of the body, this chapter will discuss the peripheral and central signalling mechanisms of energy homeostasis.

2.1 Peripheral mechanisms of energy homeostasis

A variety of gastrointestinal and pancreatic hormones such as cholecystokinin (CCK), insulin, glucagon and amylin are released in anticipation of, and during meals to limit meal size. These hormones provide information about gastrointestinal filling through afferents of the vagal nerve to the nucleus of the of the solitary tract (NTS) and area postrema (AP) of the brainstem in order to control meal size (Grill 2006) (Depicted in Figure 1). The NTS is connected to other brainstem nuclei, hypothalamic and corticolimbic areas implicated in feeding. The parabrachial nucleus (PBN) is an important relay between the NTS-AP and higher brain centers (Moran 2006).



Peripheral signals of satiety (Figure 2) The hormones insulin, glucagons and amylin affect the NTS and AP to limit meal size. (Woods, Lutz et al. 2006)

In addition to their effects on meal size through function on the brainstem, oxyntomodulin, GLP-1 and PYY_{3-36} strongly activate the hypothalamus. The plasma levels of these hormones are at their peak after meal termination, therefore they are predominantly involved in satiety (postponing the next meal) and not satiation (meal termination) (Adan, Vanderschuren et al. 2008).

Another peripheral hormone involved in energy-balance is leptin. Leptin is an adipocytokin which is hypothesized to signal long-term adiposity. Therefore, leptin plasma levels do not fluctuate with the ingestion of meals. Low levels of leptin strongly trigger food intake and activate the hypothalamus (Ahima,

Saper et al. 2000). It is possible that leptin activates pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus (ARC), which then project towards the NTS-dorsal motor nucleus of the vagus nerve (DMV) to modulate its response to CCK (Sutton, Duos et al. 2005). Therefore, even though leptin is a long term signal, it could influence satiety signalling.

Ghrelin is the only known peripheral hormone that stimulates food intake. Ghrelin is released from the stomach, and acts at the level of the vagal nerve and hypothalamus. It has been demonstrated that, in the hypothalamus, the neuropeptides agouti-related protein (AgRP) and neuropeptide Y (NPY), situated in the ARC, mediate the effects of ghrelin on food intake (Chen, Trumbauer et al. 2004).

2.2 Central mechanisms of energy homeostasis

During food intake, food enters the stomach and intestines, followed by nutrient delivery to the liver; this produces neural signals, via the vagal nerve, to the brainstem, that play a role in satiation (ending a meal). This was discovered by Yang and colleagues who disrupted vagal inputs in rats and observed that; meal size and frequency is increased, resulting in hyperphagia (Yang, Ratto et al. 1992). Alternatively, rats with lesions between the brainstem and the midbrain do not increase meal size when food is restricted, as normal hungry rats do (Seeley, Grill et al. 1994). This implicates the involvement of the brainstem in satiation, but not hunger.

Several electrical lesioning and stimulation studies on the hypothalamus of rats have found multiple nuclei that are involved in the regulation of feeding. Destruction of the ventromedial hypothalamus increased feeding and led to obesity, by increasing meal frequency. Furthermore, lesions of the hypothalamic paraventricular nucleus (PVN) and ARC, two intensively connected nuclei, also caused hyperphagia and obesity. Finally, electrical stimulation of the lateral hypothalamus (LH) activated a motor program of feeding and hoarding in satiated rats (Herberg and Blundell 1967). Together, these data suggest a main role for the hypothalamus in the initiation of feeding (Adan, Vanderschuren et al. 2008).

The arcuate nucleus of the hypothalamus (ARC) has been recognized as a node for both the integration and propagation of energy balance signals in the

brain. There are two distinct populations of neurons in the ARC that are responsive to gastrointestinal and pancreatic hormones, glucose and fatty acids namely the NPY-AgRP and POMC neurons (Wang, Cruciani-Guglielmacci et al. 2006; Adan, Vanderschuren et al. 2008).

When the efferent signals from these two types of neurons from the ARC reach the 'second-order' neurons in the PVN, they modulate the secretion of anorexigenic compounds as corticotrophin- releasing hormone (CRH), thyrotrophin-releasing hormone (TRH) and oxytocin (Valassi, Scacchi et al. 2008).

2.3.1 The nucleus accumbens

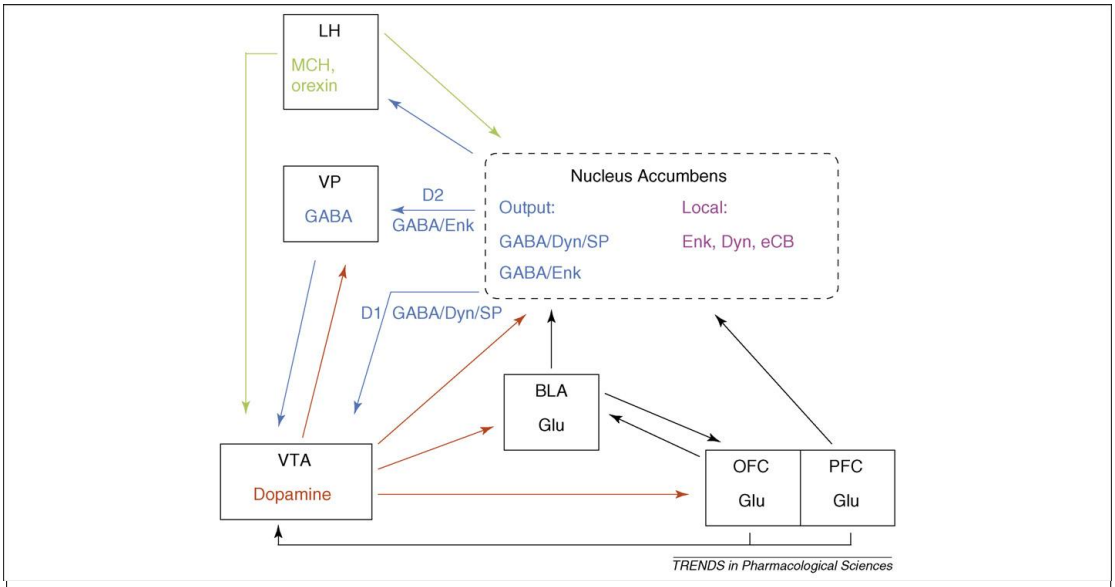
The NAcc sends output signals to brain regions that mediate the motivational, homeostatic and motor control of feeding (Adan, Vanderschuren et al. 2008). And therefore, makes an excellent target for pharmacological control of feeding behaviour.

The NAcc plays a key role in integrating four aspects of feeding namely; motivational, homeostatic, cognitive and hedonic (Kelley, Baldo et al. 2005). The motivational property of food (the effort and time an animal is willing to invest in obtaining food) is mediated by dopaminergic projections originating from the ventral tagmental area (VTA) (Kelley, Baldo et al. 2005; Berridge 2007; Narayanan, Guarnieri et al. 2009). The hedonic property of food is mediated by μ-opioid, in addition to CB_1 cannabinoid receptors in the NAcc (Barbano and Cador 2007; Mahler, Smith et al. 2007).

Internal and external food and appetite related information can gain access to the NAcc by several pathways (as depicted in figure 2). The NAcc receives information on taste and visceral function through a direct input from the nucleus of the solitary tract (NTS) of the brainstem (Kelley, Baldo et al. 2005). Furthermore, the NAcc receives indirect inputs from the gustatory cortex via parabrachial (PB) projections to the thalamus (Kelley, Baldo et al. 2005). Also, gustatory and viscerosensory regions of the agranular insular cortex display major projections towards infra- and prelimbic regions of the medial prefrontal cortex, which then densely project towards the NAcc (Kelley, Baldo et al. 2005). Taste and visceral information can alternatively influence the NAcc through two amygdaloid pathways; the NTS-PB-VTA connection, and the

gustatory orbito frontal cortex (OFC)-basolateral amygdala (BLA) –NAcc pathway (McDonald and Jackson 1987). Projections concerning internal homeostasis that reach the NAcc constitute mainly of orexin and MCH positive projections from the LH, which is directly associated to the ARC. The VTA presents dopaminergic innervations towards the NAcc, via the basolateral amygdala (BLA), prefrontal cortex (PFC), orbitofrontal cortex (OFC) and ventral pallidum (VP) (Adan, Vanderschuren et al. 2008).

There are three output pathways of the NAcc that are associated with feeding. Two GABA-ergic outputs, named the direct and indirect pathways because of their direct and indirect projections to the ventral midbrain (including VTA). Of these two outputs only the indirect pathway plays a major role in feeding. The neurons of this indirect pathway express enkephalin (an endogenous opioid) and dopamine D_2 receptors and project towards the VP. Food reward is associated by enkephalin release of these neurons (Maldonado-Irizarry, Swanson et al. 1995; Kelley, Baldo et al. 2005). The third output originates



Main accumbal neuro-cicuits. (Figure 2)
The VTA projects dopamine (red) towards: NAcc, PFC, OFC, BLA and VP. The PFC projects glutamate (black) towards the NAcc. The LH innervates the NAcc with orexigenic peptides MCH and orexin (green). Local opioid (enkhepalin) and endocannabinoid in the NAcc are reported to mediate the hedonic properties of food. The NAcc sends direct GABA-ergic outputs to the VTA, with cells expressing Dopamine D_1 receptors and the opioid dynorphin (blue), the indirect pathway is more directly associated with feeding behaviour, projects GABA-ergic towards the VP, and expresses D_2 receptors and enkephalin (blue). Finally, GABA-ergic projections originating from the NAcc shell project towards the LH (blue).
(Adan, Vanderschuren et al. 2008).

from the shell subregion of the NAcc and is a projection to the LH. Inhibition of this output by stimulation with a GABA agonist or AMPA antagonist disinhibits feeding circuits, resulting in a dramatic short-latency feeding response in rats. (Kelley, Baldo et al. 2005). From the LH, projections containing MCH and orexin run backwards to the NAcc, where these peptides stimulate feeding. In addition, LH orexin projections to the VTA also contribute to feeding.
So the NAcc is heavily involved in feeding, and both the endocannabinoids and opioids in this brain area are involved in the hedonic experience of feeding.

3 The endocannabinoids

The endocannabinoid system has been implicated in the hedonic experience of palatable foods. Since the hedonic aspect of food is a crucial reason for the uncontrolled feeding seen in obesity, the current chapter will discuss the endocannabinoid system in more detail, taking a special interest in their role in feeding.

3.1 The endogenous cannabinoid system

The appetite inducing effects of marijuana (and its main active component Δ^9-THC) have long been known, more recently it has been discovered that an endogenous cannabinoid system provides the neurological basis for these effects. Besides the appetite inducing effects the cannabinoid system is also involved in other physiological processes namely; motor activity, pain, memory and feeding (Di Marzo 1998; Piomelli 2003).

3.2 Cannabinoid receptors

Two distinct subtypes of cannabinoid receptors (CB1 and CB2) have been identified and cloned thus far (Matsuda, Lolait et al. 1990; Munro, Thomas et al. 1993). Studies have shown that CB1 receptors are widely expressed in the CNS and are also present in the periphery (Cota and Woods 2005). CB2 receptors are primarily expressed in the periphery, but it has been demonstrated that they are also localized in brain-derived immune cells (Porter and Felder 2001).

CB1 and CB2 are seven trans-membrane G-protein coupled receptors (GPCRs). Interestingly the CB1 has been considered the most abundant GPCR in the mammalian brain. It has been localized pre-synaptically on the axon terminals of multiple cell types, in various brain regions. (Freund, Katona et al. 2003).

In the neocortex, hippocampus and amygdala CB1 is highly expressed in GABA-ergic interneurons expressing CCK. Alternatively, in the basal ganglia CB1 transcript is found in other interneurons and prodynorphin and preproenkephalin expressing neurons. And in the hypothalamus, CB1 is expressed on glutamatergic neurons. (For review see (Freund, Katona et al. 2003)) Since the pre-synaptic localization and function of CB1 in the CNS, the endocannabinoid system is able to modulate the release of a myriad of neurotransmitters, including noradrenaline, glutamate, serotonin, GABA and dopamine (Schlicker and Kathmann 2001).

3.3 Endocannabinoids

The endocannabinoids are a group of amides, ethers and amides of long-chain polyunsaturated fatty acids (Di Marzo 1998). Endocannabinoids are not stored in vesicles but synthesized upon stimuli following membrane depolarization and heightened $[Ca^{2+}]_i$. Following CB receptor binding cannabinoids are swiftly inactivated by an elusive reuptake mechanism (Di Marzo and Matias 2005); (Piomelli 2003). The biochemical characteristics of the endocannabinoids, together with their unique 'on demand' production and presynaptic localization of receptors, emphasize the idea that endocannabinoids function mainly as retrograde neuromodulators.

As of now, five endocannabinoids have been identified: Arachidonoylethanolamide (anandamide), 2-arachidonoylglycerol (2-AG), noladin ether, virhodamine and N-arachidonoyldopamine (NADA) (Cota, Tschop et al. 2006).

3.4 Synthetic cannabinoid receptor ligands

Over the years several plant-derived and synthetic cannabinoid receptor ligands have been discovered. These are now widely used in experimental models. HU-210 is a potent synthetic CB1 agonist and has a 3-ring structure similar to Δ^9-THC (Howlett, Barth et al. 2002). Another group of CB1 agonists

utilized like CP-55,940; include bi- and tricyclic analogs of Δ^9-THC. Aminoalkylindols like WIN-55,212 constitute a final group of compounds with CB1 agonism (Howlett, Barth et al. 2002).

All the above mentioned compounds also exert some CB2 agonistic activity, ACEA (arachidonoyl-2'-chloroethanolamide), a structural analogue of anandamide, has been recognized as a potent and selective CB1 agonist lacking the CB2 activity (Hillard, Manna et al. 1999).

Synthetic antagonists of the CB receptors have also been discovered, AM251 (Gatley, Gifford et al. 1996), and AM281 (Lan, Gatley et al. 1999) are both specific antagonists for CB1. SR141716 (Rimonabant; (Rinaldi-Carmona, Barth et al. 1994), and MK-0364 (Taranabant; (Lin, Lanza et al. 2006; Fong, Guan et al. 2007)) have been characterized as full inverse agonists of the CB1 receptor.

3.5 Cannabinoids in feeding

Studies conducted in humans have shown that Δ^9-THC stimulates appetite with a parallel effect on food selection (Cota, Marsicano et al. 2003).It is postulated that the increase in feeding subsequent to cannabinoid treatment is mediated by an increase in the hedonic value of palatable foods. If this is the case, then it is conceivable to hypothesize that the endocannabinoid system might affect eating behaviour towards the modulation of reward circuits (Cota and Woods 2005).

In 1977 Brown et al. discovered that low oral doses of Δ^9-THC (0.25 and 0.40 mg/kg) in rats, increased preference for palatable food and sucrose intake following a dose dependent fashion (Brown, Kassouny et al. 1977). More recently, it has been shown that even pre-satiated rats, still over consumed comparable with fasted animals when orally administered Δ^9-THC (Williams, Rogers et al. 1998). The effect of the endocannabinoid anandamide (1mg/kg,s.c.) on food intake was greatly reduced in pre-fed rats, when the CB1 antagonist SR141716 was pre-administered. This supports the notion that endocannabinoid induced hyperphagia is mediated by central CB1 receptors (Williams and Kirkham 1999).

Injection of the endocannabinoids 2-AG or anandamide into the shell subregion of the NAcc, rapidly causes hyperphagia of palatable foods

(Kirkham, Williams et al. 2002; Shinohara, Inui et al. 2009). Suggesting that the endocannabinoid system in this brain area is responsible for the increasing the hedonic value of palatable foods, thereby causing hyperphagia. Recently, Bellocchio and colleagues used conditional mutant mice, to find that the hyperphagic effect of Δ^9-THC is mediated by CB1 receptors that inhibit glutamatergic neurotransmission in the ventral striatum. Conversely, they showed that higher doses of Δ^9-THC inhibit GABA-ergic neurotransmission in the ventral striatum, causing hypophagia. The authors postulate that the pharmacology of the CB1 receptor might differ according to the neuronal population that they are expressed in. This could be mediated by cell type-specific heterodimerization of the CB1 (Bellocchio, Lafenetre et al. 2010).

4 The opioids

Similar to the endocannabinoid system, the opioid system has also been implicated in the hedonic experience of palatable foods. This is an interesting parallel that could provide pharmacological possibilities in the treatment of obesity. Therefore, this chapter will discuss the endogenous opioid system and their role in feeding in more depth.

4.1 The opioid system

Opium, derived from the poppy (*Papaver somniferum*), has been utilized as analgesics for centuries. In the 70s, multiple laboratories described animals also synthesize opiate-like substances. These compounds are now known as endogenous opioids (Bodnar and Klein 2004). Opioids are synthesized in both the CNS and the periphery and affect a wide range of functions, including: nociception, gastric motility, immune status, cell death, memory hormone secretion, and food intake. (Bodnar 2009)

4.2 Opioid receptors

Endogenous opioids bind to a set of receptors that are members of the super-family of G-coupled protein receptors (GCPRs)(Waldhoer, Bartlett et al. 2004). The distinct receptors are called the μ, Δ and ϰ - opioid receptor, or MOR, DOR and KOR, respectively. The opioid receptors are mostly distributed

within the CNS, and are particularly well represented in brain structures associated with food intake and reward (Pert, Kuhar et al. 1976).

Similar to the cannabinoid receptors, MOR, DOR and KOR employ their G-protein subunit to inhibit adenylate cyclase and inhibit Ca^{2+} channels (Law and Loh 1999). It should be noted that opioid receptors form dimers and heterodimers with other opioid receptors additionally to other GPCRs (Law and Loh 1999; Rios, Jordan et al. 2001). Recently, the likely existence of μ-opioid/ CB1- cannabinoid receptor heterodimers has been reported (Christie 2006; Rios, Gomes et al. 2006).

The different opioid receptors differ from each other in relation to their contribution to specific opioid effects. μ-opioid receptors have shown to be involved in the rewarding features of feeding, but kappa ligands are known to cause dysphoria. Also the potency of opioid ligands on feeding behaviour is not only mediated by which receptor subtype they activate, but also the site of injection.

4.3 Endogenous opioids

As of now three endogenous opioids have been discovered and cloned. β-endorphin, dynorphin and enkephalin are derivates of the prohormones pro-opiomelanocortin (POMC), prodynorphin and proenkephalin respectively. Also there are μ- selective agonists endomorphins 1 and 2. The precursor for the endomorphins is not known (Cota, Tschop et al. 2006). The endogenous opioids for the μ-opioid receptor include: endomorphin-1 and -2, β-endorphin, β-neoendorphin and dermorphin.

4.4 Synthetic opioid receptor ligands

[$_{D}$Ala2, N-MePHe4, Gly-ol]-enkephalin (DAMGO) is a synthetic μ-opioid antagonist, often used in experimental setups. The primary synthetic opioid antagonists used in animal studies are: naltrexone, naloxone and nalmefene. These have also been approved for human use for other afflictions. They are all unselective opioid antagonists, blocking all three receptors, and nalmefene and naltrexone have a significantly longer half-life than naloxone (Cota, Tschop et al. 2006).

4.5 Opioids in feeding

Opioids have also been implicated in stimulating highly palatable food intake. Several lines of evidence point towards opioid induced potentiation of palatability of food items. To start, opioid induced hyperphagia is more vigorous when presented with reinforcing foods, like sweet and fatty food (Cooper and Turkish 1989). Furthermore, opioid agonists increase (Pecina and Berridge 1995) and antagonists decrease measurements of hedonic taste reactivity (Ferraro, Hill et al. 2002). To continue, opioid induced hyperphagia is independent of post-ingestive feeding, since opioid effects are both seen in sham feeding animals (Kirkham and Cooper 1988) as in animals fed non-caloric substances (Beczkowska, Bowen et al. 1992). This data support the notion that opioids manipulate the neuronal representation of palatability, rather then acting through effects of satiety. Finally, pharmacological blocking of opioid receptors in human test subjects, attenuated hedonic response of palatable meal tests (Yeomans and Wright 1991; Yeomans and Gray 1996). Although opioid manipulation attenuated palatability of sweet and fatty foods, taste discrimination was not affected.

Opioid agonists are particularly potent when administered directly into the NAcc (Bakshi and Kelley 1993; Taha, Katsuura et al. 2009). Opioid signalling in the NAcc induces feeding behaviour similar to that after systemically administered opioids. Also, direct naltrexone administration (a non-specific opioid antagonist) into the NAcc, attenuated the consumption of sucrose, implicating that this brain structure has an endogenous signalling system in promoting palatable food intake (Kelley, Bless et al. 1996).

In the NAcc, stimulation of the μ-opioid receptor (MOR) is the most potent activator of feeding behaviour. Intra NAcc administration of the MOR-specific agonist, DAMGO results in hours of uninterrupted hyperphagia in rats (Zhang, Gosnell et al. 1998). Signalling of the DOR has also been reported to increase feeding (Zhang and Kelley 1997). But the function of the DOR is under debate since administration of DOR antagonists also result in an increase in feeding (Kelley, Bless et al. 1996). Studies on food intake after NAcc infusion with KOR agonists, demonstrated limited effects (Bakshi and Kelley 1993). Whereas KOR antagonists, have been reported to have no effect (Kelley, Bless et al. 1996). These studies suggest MOR as a critical site of action for

opioid induced hyperphagia. However, the role of endogenous opioids in feeding is still not clear. Enkephalin signalling in a subset of NAcc neurons has been implicated in modulating food intake by activating MOR and DOR (Katsuura and Taha 2009).

5 Interactions of the endocannabinoid and opioid systems

The various similar physiological properties of opioids and endocannabinoids suggest that these two systems interact (Manzanares, Corchero et al. 1999). And indeed, multiple groups have found indications that the opioid and endocannabinoid systems interact in their regulation of feeding.

Kirkham and colleagues describe that when rats are administered sub-anorectic doses (0.1, 0.5 and 1 mg/kg, s.c.) of CB1 inverse agonist SR141716 (Rimonabant) or opioid antagonist naloxone alone, no effect on feeding behaviour is observed, however they effectively suppress feeding when administered together (Kirkham and Williams 2001). Correspondingly, while oral administration of just the opioid antagonist nalmefene does not influence feeding behaviour in mice, the combined administration with sub-anorectic doses of CB1 antagonist AM251 does reduce feeding behaviour (Chen, Huang et al. 2004). Interestingly, the same group has reported that mice lacking the MOR display unaltered effects of AM251 on food intake and body weight. This suggests that the endogenous opioid and cannabinoid system are also able to exert their effects on appetite independently (Chen, Frassetto et al. 2006). Also, combined sub-anorectic doses of CB1 and opioid receptor antagonists can attenuate the hyperphagia induced by oral administration of Δ^9-THC in rats (Williams and Kirkham 2002). The most convincing literature describing a synergistic interaction between CB1 and opioid antagonists has come from Rowland and colleagues (Rowland, Mukherjee et al. 2001). In their paper they used isobolograms (a visual graph to analyse if drug interactions result in a synergistic effect ;Berenbaum 1989)) to demonstrate that Rimonabant and naloxone produce a supra-additive anorectic effect.

More recently, Tallet and colleagues have tried to reproduce this data in rats by administering the CB1 inverse antagonists Rimonabant and AM251 simultaneously with naloxone, however, they found an additive effect, but no supra-additive anorectic effect. Interestingly, it is reported that the administration of naloxone was able to effectively attenuate the scratching behaviour (pruritus) reported after CB1 inverse agonist administration in rats (Tallett, Blundell et al. 2008; Tallett, Blundell et al. 2009).

Next to pharmacological evidence suggesting an interaction between the endocannabinoid and opioid system, c-Fos expression studies have indicated that the VMD, DMN, VTA, PVN and NAcc are key brain structures to cannabinoid- opioid interactions in the regulation of feeding (Allen, McGregor et al. 2003; Singh, Verty et al. 2004). Electron microscopy localization studies by Pickel and colleagues also confirm CB1 and MOR compartmentalization in both the NAcc shell and core (Pickel, Chan et al. 2004). Using the taste reactivity paradigm (a measure for the effect of brain manipulations on orofacial responses to pleasurable and unpleasurable flavours), Mahler and colleagues found a small cannabinoid hedonic hotspot in the NAcc dorsal medial shell. Selective microinjections of the endocannabinoid anandamide to this anatomical hotspot produced intense hedonic enhancement and stimulated feeding behaviour (Mahler, Smith et al. 2007). This hotspot is reported to overlap with an opioid hedonic hotspot found in another taste reactivity study. Here Pecina and Berridge, found that MOR agonist DAMGO administered to this specific region also amplified the hedonic value of food (Pecina and Berridge 2005).

Although several studies have demonstrated interactions between the two systems, the mechanism(s) of endogenous cannabinoid-opioid crosstalk have not been well investigated: One possible mechanism is the heterodimerization of μ-opioid with the CB1- cannabinoid receptor (Christie 2006; Rios, Gomes et al. 2006).

Because of their anatomical overlap and synergistic effect on feeding behaviour, it seems likely that the endocannabinoid and opioid system interact on local circuits in the NAcc medial shell to amplify hedonic impact of palatable foods. Therefore, these systems seem to be an excellent

pharmacological target for treating obesity, specifically their actions on the NAcc could be of great interest.

6 Cannabinoids and opioids in the treatment of obesity

Both cannabinoid- and opioid-antagonists are associated with attenuated feeding behaviour in laboratory animals. These compounds have also been tested for their anorectic characteristics in humans. This chapter will discuss the cannabinoids and opioids in the treatment of obesity.

6.1 Cannabinoids in the treatment of obesity

Following the promising results with cannabinoid antagonists in animals, Sanofi-Aventis started human clinical trials with Rimonabant in the treatment of obesity. The Rimonabant in obesity (RIO) randomised, double-blind, placebo controlled trials included 6600 participants with a BMI > 30kg/m^2 or a BMI > 27kg/m^2 with obesity-induced disease. At 1-year follow-up, the results of the trials showed a placebo corrected weight loss of 4.7 kg. (Butler and Korbonits 2009)

These results suggested that Rimonabant was a promising anorectic agent; therefore the European Union licensed it as anti-obesity drug and the National institute for health and clinical excellence approved use in the UK. Sadly, the RIO trials also reported a high incidence of adverse effects; the most common of which were nausea, headache, depression and anxiety. As a result of the psychiatric side effects reported, the US Food and Drug Administration (FDA) refused permission for Rimonabant. In October 2008, Rimonabant was also suspended in the EU. The European Medicines Agency (EMEA) disputed the psychiatric safety due to increased suicide risk and depression (Butler and Korbonits 2009).

Rimonabant and Taranabant have a high affinity for the CB1 receptor and function as a full inverse agonist. Therefore, it might be possible to negate some of the negative side effects of Rimonabant using a partial agonist of the CB1 receptor. It has been reported that partial agonists generally have a lower

prevalence of adverse side effects then either inverse agonists or antagonists, with a limited reduction of drug efficacy (Ohlsen and Pilowsky 2005).

6.2 Opioids in the treatment of obesity

Following the promising results of opioid antagonist on weight loss in animals, several controlled trials have been done using oral naltrexone, nalmefene and intravenous naloxone to evaluate their effect on short term food intake in healthy normal weight humans (For review see (Yeomans and Gray 2002). Sadly, the number of subjects in these tests was limited, ranging from 7 to 26. The results did not show any significant effect on hunger, but one test did show decreased short-term food intake, ranging from 11-29%. And decreased pleasantness of the food was reported in some, but not all of the trials (Yeomans and Gray 2002). Nausea is a known side effect of naltrexone, this could possibly be a bias for the decrease in food intake reported. However, around 19% of subjects in the two trials reported nausea after naltrexone treatment, compared to 9% in the placebo group, and the authors conclude that there does not seem to be a direct correlation of reduced feeding and nausea.

The effect of naltrexone and naloxone on feeding has also been studied in obese individuals. Again these opioid antagonists were able to decrease short-term feeding behaviour (by about 33%). In some of these trials, a decrease in hunger was reported by participants, and once more there were reports of nausea after opioid antagonist administration (Yeomans and Gray 2002).

The above mentioned results indicate the opioid antagonists as useful compounds in combating obesity, as they suppress short-term feeding behaviour and are reported to decrease the hedonic value of palatable foods, provided that the negative side-effects can be attenuated. However, there are no published trials demonstrating significant weight loss in obese individuals following opioid antagonist treatment (Cota, Tschop et al. 2006).

7 Potential combined treatment of cannabinoids and opioids in obesity

The physiological mechanisms that regulate body weight are highly complex integrated and redundant, therefore the combined therapy of multiple anorectic agents is postulated to have a greater efficacy than a single obesity agent. And indeed, Rowland and colleagues have convincingly shown that Rimonabant and naloxone together produce a supra-additive anorectic effect.
Co-administration with an opioid antagonist not only provides an enlarged efficacy for these two known substances, it also provides a possibility for negating the psychiatric side effects of Rimonabant and other cannabinoid anorectic compounds. Since these two systems seem to synergistically suppress feeding behaviour, lower doses of both opioid and cannabinoid antagonists could be used.
Recently, pruritus has also been confirmed as a significant side effect of CB1 receptor inverse agonists in humans (Addy, Wright et al. 2008; Kirkham 2008). Tallet and colleagues have shown that in rats this side effect can be attenuated with naloxone treatment. This demonstrates that polytherapy of a CB1 inverse agonist with an opioid antagonist might not only effectively suppress feeding, but also attenuate at least one of the CB1 inverse agonist induced side effects. (Tallett, Blundell et al. 2009)

8 Conclusion and Discussion

Obesity is a massive burden on the Western healthcare system, but as of now, an effective pharmacological treatment for this affliction is still missing. Since the opioid and cannabinoid systems are implicated in mediating the hedonic value of palatable foods, both opioid and cannabinoid antagonists have been explored for their possible use as anorectic compounds, and both have demonstrated promising results. Sadly, the scientific interest in cannabinoid antagonists in the treatment of obesity has dissipated because of adverse (psychiatric) side effects.

Multiple groups have indicated that when opioid and cannabinoid antagonists are administered together in animals, they produce a supra-additive anorectic effect. Although, this conclusion is still subject to discussion, it is confirmed that polytherapy of sub-anorectic doses of opioid and CB1 antagonists/ inverse agonists do suppress appetite. Also, recent work has shown that at least one side effect of CB1 inverse agonists can be attenuated by opioid antagonist treatment, thereby further supporting the positive effect of polytherapy.

Furthermore, the opioid antagonists used in the aforementioned studies, are all unselective opioid antagonists. Since MORs are primarily associated with feeding behaviour, a μ- selective antagonist could possibly negate some side effects associated with opioid antagonists without losing efficacy.

Recent work by Bellocchio and colleagues has shown that the hyperphagic effect of low doses of Δ^9-THC is mediated by inhibition of glutamatergic neurotransmission in the ventral striatum. This raises the possibility of cell type-specific heterodimerizations of the CB1, with specific pharmacological characteristics. Future efforts could possibly focus on the development of CB1 antagonists for specific cell types in the ventral striatum, thereby greatly reducing side-effects.

Polytherapy of cannabinoid antagonists/ inverse agonists with opioid antagonists could rekindle the scientific interest in the cannabinoid antagonists in the treatment of obesity, however large long-term studies with human test subjects should determine if such combination therapy is effective over a longer duration and if any side effects remain absent.

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