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Review

Orphan G protein-coupled receptors and obesity

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Abstract

The use of orphan G protein-coupled receptors (GPCRs) as targets to identify new transmitters has led over the last decade to the discovery of 12 novel neuropeptide families. Each one of these new neuropeptides has opened its own field of research, has brought new insights in distinct pathophysiological conditions and has offered new potentials for therapeutic applications. Interestingly, several of these novel peptides have seen their roles converge on one physiological response: the regulation of food intake and energy expenditure. In this manuscript, we discuss four deorphanized GPCR systems, the ghrelin, orexins/hypocretins, melanin-concentrating hormone (MCH) and neuropeptide B/neuropeptide W (NPB/NPW) systems, and review our knowledge of their role in the regulation of energy balance and of their potential use in therapies directed at feeding disorders.

Keywords: Feeding; Ghrelin; Orexin/hypocretin; Melanin-concentrating hormone; Neuropeptide B; Neuropeptide W

Contents

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1. Introduction

G protein-coupled receptors (GPCRs) form the largest family of membrane proteins that recognize extracellular messengers. They share a common topography composed of seven hydrophobic segments expected to be transmembrane domains. GPCRs are activated by small transmitters such as odorants, biogenic amines and other amino acid derivatives, but also by bigger molecules such as neuropeptides, chemokines and glycoprotein hormones (Bockaert and Pin, 1999; Civelli et al., 2001). GPCR activation mediates a variety of intracellular responses leading to the regulation of numerous physiological functions. GPCRs offer enormous potential for new drug developments, as they already are the targets of nearly 50% of all prescription drugs including antihistamines, neuroleptics and antihypertensives (Drews, 2000; Howard et al., 2001).

Most new GPCRs have been sought for by using homology screening approaches, such as low-stringency hybridization, degenerate polymerase chain reaction (PCR) or more recently by bioinformatic analyses of the genomes. GPCRs found using these approaches are discovered on the basis of their sequences, so their natural ligands are unknown. They are called "orphan" GPCRs, i.e. GPCRs with no known ligand (Marchese et al., 1999; Civelli et al., 2001; Howard et al., 2001). While over the years, numerous orphan GPCRs have been matched to specific ligands, about half of all the GPCRs are still orphan GPCRs.

2. Searching for the natural ligands of orphan GPCRs

Because GPCRs are targets of neurotransmitters, peptides, hormones and other transmitters that direct intercellular interactions, orphan GPCRs are also expected to be activated by transmitter molecules. Finding the natural ligand of an orphan GPCR therefore equals finding a novel transmitter, a result that can yield new insight on our understanding of basic biological responses and that opens the door to new drug discovery ventures. However, finding the natural ligands of an orphan GPCR is a challenge, since one knows neither the biochemical properties of the ligand nor the response that receptor activation will induce. Yet two related approaches have been adopted and have been successful at identifying the natural ligands of orphan GPCRs: one called reverse pharmacology (Libert et al., 1991) and the other referred to as the orphan receptor strategy (Civelli, 1998).

2.1. Reverse pharmacology

Historically, the first approach used to identify the natural ligands of orphan GPCRs took advantage of the fact that only a handful of GPCRs had been cloned and has consisted of testing orphan GPCRs against a range of potential transmitters. This approach led in 1988 to the first "deorphanizations" of two orphan GPCRs, the serotonin 5-HT_{1A} (Fargin et al., 1988) and dopamine D_2 receptors (Bunzow et al., 1988). It has over the next years led to numerous deorphanizations, in particular it can be credited to the discovery of most GPCR subtypes. More recently, it has been adapted to large-scale ventures that include a battery of orphan GPCRs tested against hundreds of potential ligands by using high-throughput screening techniques. This has led to the matching of several dozen of orphan GPCRs to their ligands (Civelli et al., 2001; Howard et al., 2001). But all these ligands had been discovered previously.

2.2. Orphan receptor strategy

On the other hand, it strikes us by the end of the 1980s that the number of GPCRs discovered by cloning would outnumber the natural ligands that we knew could be potentially matched to GPCRs. There was therefore a need for a different strategy aimed at discovering novel transmitters. In this strategy, an orphan GPCR is expressed in cell lines that are challenged with extracts from tissues expected to contain the orphan receptor ligand. Activation of the orphan GPCR is monitored by measuring second messenger

Table 1
Novel peptides identified by the orphan receptor strategy

Ligand	Year	Major functions[a]
Nociceptin/Orphanin FQ[b]	1995	Stress, pain
Orexins/Hypocretins[c,d]	1998	Feeding, sleep–wakefulness
Prolactin-releasing peptide[b,e]	1998	Sleep, absence seizure
Apelin[b]	1998	Unknown
Ghrelin[f,g]	1999	Feeding, GH secretion
MCH[h,i]	1999	Feeding
Urotensin II[b]	1999	Vasoconstriction
Neuromedin U[b]	2000	Unknown
Metastin[j,k]	2001	Cell proliferation, development
Prokineticin 1/2[l,m]	2002	Angiogenesis, circadian rhythm
NPB and NPW[n,o]	2002	Feeding, unknown
Relaxin-3[p,q]	2003	Unknown

[a] As presently viewed by the authors.
[b] Civelli et al., 2001.
[c] Sutcliffe and de Lecea, 2002.
[d] Smart et al., 2002.
[e] Lin et al., 2002.
[f] Hosoda et al., 2002.
[g] Muccioli et al., 2002.
[h] Saito et al., 1999.
[i] Shimada et al., 1998.
[j] Funes et al., 2003.
[k] Ohtaki et al., 2001.
[l] LeCouter et al., 2002.
[m] Cheng et al., 2002.
[n] Shimomura et al., 2002.
[o] Tanaka et al., 2003.
[p] Liu et al., 2003a.
[q] Liu et al., 2003b.

responses. Positive extracts are fractionated biochemically until the active component is isolated and characterized (Civelli, 1998; Civelli et al., 2001).

This strategy was successfully reported for the first time in 1995 in the identification of the novel neuropeptide Nociceptin/Orphanin FQ (Reinscheid et al., 1995). Since then the orphan receptor strategy has led to the discovery and isolation of twelve novel bioactive peptide families (Table 1). These novel peptides have been and continue to be actively studied. A variety of physiological responses are modulated differently by these new peptides. Interestingly, six of these novel peptides have been shown to have a profound impact on our understanding of the mechanisms that regulate energy balance and the pathophysiology of obesity. These six peptides are the focus of this review.

3. Orphan receptors and obesity

Obesity is characterized by a chronic imbalance between energy expenditure and energy intake. While the mechanisms underlying obesity are far from being fully understood, it has become clear in the last years that obesity is in part centrally regulated and that several neuropeptides play an important role in this regulation. In particular, six recently discovered neuropeptides that are important in the pathophysiology of obesity are ligands of deorphanized GPCRs; they are ghrelin, the hypocretins/orexins (hypocretin 1/orexin A, hypocretin 2/orexin B), melanin-concentrating hormone (MCH) and neuropeptide B (NPB)/neuropeptide W (NPW).

3.1. The ghrelin system

Ghrelin is a 28-amino-acid peptide and was isolated from human and rat stomach. Ghrelin binds and activates a particular GPCR called the growth hormone secretagogue (GHS) receptor that stimulates the secretion of growth hormone (Kojima et al., 1999). Growth hormone secretagogues (GHSs) are a family of small synthetic molecules that increase the secretion of growth hormone. The GHS receptor was originally identified as a GPCR by expression cloning using one of the synthetic GHSs as a ligand (Howard et al., 1996; McKee et al., 1997). However, it remained an orphan GPCR until ghrelin was discovered as its endogenous ligand. Ghrelin possesses a unique structure in that the hydroxyl group of its third residue, a serine, is acylated by *n*-octanoic acid. This acylation is essential to the pharmacological and biological activity of the peptide (Kojima et al., 1999; Bednarek et al., 2000). The ghrelin system is involved in many physiological functions such as stimulation of growth hormone secretion and regulation of reproductive and endocrine systems (Kojima et al., 1999; Date et al., 2000b; Takaya et al., 2000; Arvat et al., 2001). Most of all, the ghrelin system is one of the central systems that regulate energy balances (Hosoda et al., 2002; Muccioli et al., 2002). So, it is no surprise that the ghrelin system has become one of the hottest topics in obesity research.

Ghrelin is synthesized predominantly in the stomach (Kojima et al., 1999; Date et al., 2000a), while its receptor is mainly expressed in the somatotroph cells of the pituitary and in the hypothalamus, in particular in the arcuate nucleus (Howard et al., 1996; Guan et al., 1997; Muccioli et al., 1998; Mitchell et al., 2001). Central administration of ghrelin induces expression of the immediate early gene c-fos in multiple nuclei of the hypothalamus that are involved in regulating energy balance such as the arcuate nucleus, the dorsomedial hypothalamic nucleus and the ventromedial hypothalamic nucleus (Nakazato et al., 2001). Moreover, peripheral systemic administration of ghrelin also induces c-fos expression in neurons in the arcuate nucleus that coexpress neuropeptide Y (NPY), agouti gene-related protein (AGRP) and the GHS receptor (Hewson and Dickson, 2000). The anatomical distribution of ghrelin and the GHS receptor indicated that ghrelin might be involved in feeding behavior.

Many studies have shown that acute central administration of ghrelin stimulates food intake in rats (Wren et al., 2000; Nakazato et al., 2001; Wren et al., 2001b; Lawrence et al., 2002). This stimulatory effect was also observed after peripheral administration of ghrelin (Tschöp et al., 2000; Wren et al., 2000, 2001b). Because GHSs are agonists to the ghrelin receptor, a number of studies investigated the effects of the ghrelin system on food intake in humans using GHSs. Activation of the ghrelin receptor by GHSs has been reported to induce sensations of hunger and to increase appetite and food intake in humans (Arvat et al., 2000; Wren et al., 2001a).

In rodents, chronic administration of ghrelin has also been shown to decrease energy expenditure and to increase adipogenesis, which leads to increased fat mass and weight gain (Tschöp et al., 2000; Kamegai et al., 2001; Wren et al., 2001b). Weight gain was significant within 48 h after chronic ghrelin administration and continued to increase becoming obvious after 2 weeks. Increased fat mass but not lean mass and longitudinal skeletal growth were also observed, suggesting that ghrelin induces a positive energy balance (Tschöp et al., 2000). Several groups have suggested that this weight gain induction is independent of growth hormones because the orexigenic stimulating effects of ghrelin are not altered in growth hormone deficient dwarf rats (Shintani et al., 2001) or by using growth hormone releasing hormone (GHRH) antagonists (Bagnasco et al., 2003).

Although data from chronic ghrelin and GHS administration in rodents demonstrate consistent orexigenic effects, ghrelin knockout (ghrelin−/−) mice (Sun et al., 2003; Wortley et al., 2004) and GHS receptor knockout (Ghsr-null) mice (Sun et al., 2004) exhibit surprisingly normal appetite, feeding behavior, body composition, normal growth rate and normal size (Table 2). Acute injection of ghrelin to ghrelin−/− mice increases food

intake in a manner comparable to that found in wild type mice (Sun et al., 2003). This indicates that ghrelin−/− mice retain a functional ghrelin signaling pathway. These data seem to question the central role of the ghrelin system in regulating energy balance. However, these data must be interpreted cautiously as alternative pathways may compensate for the loss of ghrelin or its receptor.

Ghrelin regulates energy balance by serving as a link between the periphery and the central nervous system. Circulating ghrelin levels and ghrelin mRNA expression levels are raised during fasting and chronic food deprivation and decreased during food intake. In humans, plasma ghrelin levels are increased before a meal and decreased after a meal (Cummings et al., 2001; Tschöp et al., 2001a), suggesting ghrelin is involved in meal initiation.

Similar to ghrelin, leptin is also a peripheral hormone that is synthesized in adipose tissues and is involved in feeding regulation (Crowley et al., 2002). The ghrelin and leptin systems however seem to have opposite effects in regulating feeding behavior. Both systems target the arcuate nucleus, which encompasses both the orexigenic NPY/AGRP and the anorexigenic proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART) expressing neurons. Acute and chronic ghrelin administration upregulates NPY-AGRP levels (Kamegai et al., 2001; Nakazato et al., 2001; Shintani et al., 2001), while leptin decreases them (Baskin et al., 1999a,b). Leptin inhibits ghrelin-stimulated feeding, while ghrelin abolishes the anorexic effects of leptin (Nakazato et al., 2001). Interestingly, NPY or AGRP receptor antagonists or antibodies also reduce the effects of ghrelin on food intake (Nakazato et al., 2001; Lawrence et al., 2002; Bagnasco et al., 2003). Recently, it has been reported that the orexigenic effects of ghrelin are abolished in NPY and AGRP double knockout mice (Chen et al., 2004). These results suggest that the activation of the NPY-AGRP system is necessary to mediate the orexigenic effects of ghrelin (Fig. 1).

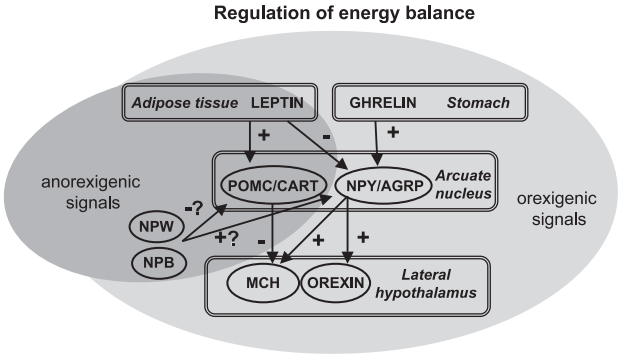

Fig. 1. Schematic representation of the interactions between the ghrelin, MCH, orexin and NPW/NPB systems. Ghrelin upregulates (+) NPY, MCH and Orexin, while, leptin downregulates them. NPW/NPB may downregulate (−) POMC/CART and upregulate (+) NPY/AGRP but this needs to be confirmed (?). ⬭ represents a separated neuronal nucleus.

The discovery of ghrelin and its profound effect on energy balance has led to numerous studies on the association of ghrelin with metabolic disorders. Circulating levels of active ghrelin are significantly reduced in obese patients (Tschöp et al., 2001b; Shiiya et al., 2002). These levels recover but do not normalize after dietary intervention and weight loss (Cummings et al., 2002; Soriano-Guillen et al., 2004). In states of negative energy balance such as anorexia nervosa and cachexia, ghrelin levels are significantly increased. They however normalize in anorexia nervosa patients after weight recovery (Otto et al., 2001; Shiiya et al., 2002; Tanaka et al., 2002; Soriano-Guillen et al., 2004). Thus, ghrelin levels are elevated in negative energy situations such as fasting and anorexia nervosa, and reduced during positive energy situations such as obesity. Recent screenings of the ghrelin gene in a large number of Italian obese children and adolescents found no specific mutations (Miraglia del Giudice et al., 2004). Yet two polymorphisms, Arg51Gln and Leu72Met, have been reported and the Leu72Met polymorphism is thought to be associated with the onset of obesity among children (Ukkola et al., 2001; Korbonits et al., 2002; Miraglia del Giudice et al., 2004). When the ghrelin receptor gene was screened in extremely obese children and adolescents and compared to that of underweight subjects and normal weight controls, several sequence variations were detected. However, no significant correlation between these variations and body weight was found (Wang et al., 2004). These screening studies indicate that obesity is not caused by a mutation in the ghrelin precursor or its receptor genes.

Based on the evidence provided so far, the ghrelin system could be a promising therapeutic target in fighting energy imbalances such as obesity (Fig. 1). Indeed the ghrelin receptor antagonist [D-Lys-3]-GHRP-6 has been found to reduce food intake and to lower body weight in mice (Asakawa et al., 2003). These data point at ghrelin receptor antagonists as interesting candidates in the treatment of obesity.

3.2. The orexins/hypocretins system

The orexin receptors were deorphanized in 1998 using the orphan receptor strategy (Sakurai et al., 1998) and have subsequently been shown to play an important role in energy homeostasis (Sakurai et al., 1998; Smart et al., 2002). The natural ligands of these receptors had been first discovered as new hypothalamic peptides named hypocretins (de Lecea et al., 1998). Orexin A (hypocretin-1) and B (hypocretin-2) are 33- and 28-amino-acid neuropeptides, respectively, cleaved from a common 130-amino-acid precursor peptide, prepro-orexin (de Lecea et al., 1998; Sakurai et al., 1998). Two GPCRs, orexin-1 receptor and orexin-2 receptor, were found to be activated by both orexin A and B. The orexin-1 receptor has a higher affinity for orexin A than for orexin B, while the orexin-2 receptor has the same affinity for both orexin A and orexin B (Sakurai et al., 1998).

The anatomical distributions of the orexins and their receptors suggest involvement of this system in the regulation of food intake. Orexin expressing neurons are found exclusively in the lateral hypothalamus area and the posterior hypothalamus, in particular the perifornical nucleus, a region that is known to regulate feeding behavior (de Lecea et al., 1998; Peyron et al., 1998; Sakurai et al., 1998). The orexin neurons project to a wide range of brain regions involved in the regulation of energy homeostasis including the paraventricular hypothalamic, ventromedial hypothalamic and arcuate nuclei (Peyron et al., 1998; Date et al., 1999; Nambu et al., 1999). Correspondingly, the orexin-1 receptor and/or the orexin-2 receptor are expressed in the paraventricular hypothalamic, ventromedial hypothalamic and arcuate nuclei (Marcus et al., 2001; Trivedi et al., 1998). These localizations indicate a modulatory role of the orexin system on food intake.

Acute central administration of orexin A stimulates food intake as do targeted site injections in the paraventricular hypothalamic nucleus, dorsomedial hypothalamic nucleus, lateral hypothalamus or the perifornical area (Sakurai et al., 1998; Dube et al., 1999; Sweet et al., 1999). Interestingly, injections of orexin A into the arcuate nucleus or ventromedial hypothalamic nucleus do not increase food intake (Dube et al., 1999). It has been reported that the orexin effects are circadian specific (Haynes et al., 1999; Thorpe et al., 2003). Central administration of orexin A simulates food intake only when it is administered during the resting (daytime) phase of rodents (Yamanaka et al., 1999; Thorpe et al., 2003). Similarly, chronic infusion increases daytime food intake, and suppresses it during the active (night) phase, which may be a secondary effect due to the extra food ingested during daytime. Chronic orexin A administration however does not significantly change food intake over a 24-h period nor body weight (Haynes et al., 1999; Yamanaka et al., 1999).

The effects of orexin B on food intake have been less consistent. I.c.v. administration of orexin B has been reported to either have no effect (Haynes et al., 1999) or to stimulate food intake (Sakurai et al., 1998). Site-specific microinjections of orexin B in discrete areas of the hypothalamus have failed to demonstrate significant effects on food intake (Dube et al., 1999).

Consistent with the hyperphagic effects of orexin A, prepro-orexin knockout mice exhibit hypophagia while maintaining normal body weight (Willie et al., 2001) (Table 2). Similarly, the selective deletion of orexin neurons using ataxin-3 genetic constructs induced hypophagia in mice. These mice develop late-onset obesity in contrast to the prepro-orexin knockouts (Hara et al., 2001). This phenotype occurs in spite of hypophagia and may be due to a lower level of energy expenditure that could override the hypophagia and lead to an increase in body weight. This is in agreement with other reports that central administration of orexin A increases oxygen consumption and body temperature, important indexes of energy expenditure (Wang et al., 2001, 2003).

Table 2
Orphan GPCR system knockouts

	Ghrelin system	Orexin system	MCH system	NPB/NPW system
Precursor KO	orexigenic normal	orexigenic hypophagic normal body weight narcoleptic	orexigenic hypophagic increased metabolic rate lean	anorexigenic[a] N/A
Receptor KO	normal	sleep–wake cycle fragmentation (orexin-1 receptor) narcoleptic (orexin-2 receptor) dimorphic (male only)	hyperphagic increased metabolic rate hyperactive	hyperphagic decreased energy expenditure adult-onset obesity

N/A not available.
[a] Refers to anorexigenic effect in the dark phase.

Normal body weight after chronic administration of orexin and late-onset of obesity in mice with ablated orexin neurons indicate that the orexin system may not be the cause of obesity. Recent studies have reported that prepro-orexin mRNA expression is downregulated in two different obesity animal models with dysfunctional leptin systems (ob/ob and db/db mice) (Yamamoto et al., 1999), as well as in obese Zucker rats (Cai et al., 1999; Beck et al., 2001). Furthermore, fasting increases prepro-orexin mRNA expression (Sakurai et al., 1998). This suggests that the orexin system may be a feedback regulatory system in response to obesity.

Among the two orexin receptors, the orexin-1 receptor is clearly involved in the regulation of food intake and energy expenditure. The selective orexin-1 receptor antagonist, SB-334867, reduces food intake when given at the start of the dark phase and decreases stimulated feeding induced by overnight fasting in rats. It also blocks the orexin-induced hyperphagia (Haynes et al., 2000). In ob/ob mice, SB-334867 administration leads to the reduction of weight gain with decreased total fat mass gain but no changes in fat free mass. The reduced weight gain is attributed to cumulative food intake and possible stimulation of thermogenesis as indicated by decreased brown adipose tissue (Haynes et al., 2002). The lack of a selective antagonist to the orexin-2 receptor has impeded the study of the specific involvement of the orexin-2 receptor in feeding.

In addition to its modulatory role on food intake, the orexin system is a major regulator of sleep and arousal (Sutcliffe and de Lecea, 2002). Central administration of orexin A increases locomotor activity and promotes wakefulness (Hagan et al., 1999; Bourgin et al., 2000). Orexin knockout and orexin/ataxin-3 transgenic mice are narcoleptic (Chemelli et al., 1999; Hara et al., 2001). Moreover, in humans, significant decreases in the number of orexin

containing neurons have been found in postmortem brains of narcoleptic individuals (Thannickal et al., 2000). Among the two receptors, the orexin-2 receptor is the predominant one that is involved in mediating the arousal effects of orexin. Narcolepsy results from mutations that abrogate the activity of the orexin-2 receptor in a canine model (Lin et al., 1999). Orexin-2 receptor knockout mice also are narcoleptic. On the other hand, the orexin-1 receptor knockout mice exhibit only mild fragmentation of the sleep–wake cycle but no obvious narcolepsy (Willie et al., 2003) (Table 2). Together, these data demonstrate the importance of the orexin system in the pathophysiology of narcolepsy and the relative preference of the orexin-2 receptor in mediating the arousal effects of orexin when compared to the orexin-1 receptor.

Consequently, the important issue in evaluating the effects of the orexin system on food intake and energy expenditure is whether the stimulating effects of orexin could be due to increased activity or wakefulness. Several data have suggested that the effects of the orexin system on energy balance are not simply the secondary effects on arousal induced by orexin. In anesthetized rats, orexin A still had the ability to increase oxygen consumption, skin and body temperature, which are signs of increased energy expenditure, in spite of the anesthetized condition (Wang et al., 2001). Moreover, orexin-1 receptor antagonists reduce food intake and decrease body weight while the orexin-1 receptor knockout mice induce only a slight fragmentation of the sleep–wake cycle without obvious behavioral abnormality. Interestingly, recent evidence demonstrates that the orexin system plays a role in linking the regulation of energy balance with the regulation of arousal. The orexin system has been shown to modulate food seeking behavior in response to energy balance. Signals involved in metabolic and energy balance such as glucose, leptin and ghrelin regulate the activity of orexin neurons and expression levels of orexin. Orexin neurons are able to induce or suppress food seeking behavior by integrating energy-related signals coming from peripheral or hypothalamic pathways. It has been shown that mice that lack orexin expressing neurons (orexin/ataxin-3 transgenic mice) fail to increase their wakefulness state and their motor activity in response to fasting (Yamanaka et al., 2003). Therefore, the orexin system appears to link adaptive food seeking behavior in response to energy homeostasis to the regulation of arousal (Sakurai, 2003).

3.3. The MCH system

Melanin-concentrating hormone (MCH) is a cyclic 19-amino-acid polypeptide that acts on two receptors, MCH-1 receptor and MCH-2 receptor (Saito et al., 1999; Sailer et al., 2001). The MCH-1 receptor is present in both rodents and humans and its activation is coupled to the Gi/o and Gq pathways (Saito et al., 1999; Tan et al., 2002). The MCH-2 receptor is expressed in humans but not in rodents and its activation is coupled only to Gq (Sailer et al., 2001). Most of the studies related to the MCH system have been carried out in rodents and therefore deal with the MCH-1 receptor only. The distributions of the MCH precursor and of the MCH-1 receptor in mammalian brains suggest involvement of the MCH-1 receptor system in regulating energy balance. MCH expression is confined to the lateral hypothalamus and zona incerta, brain regions that are at the center of feeding behavior (Bittencourt et al., 1998; Bittencourt and Elias, 1998). The MCH-1 receptor on the other hand is widely distributed in the central nervous system and is found in particular in regions that are involved in rewarding behavior, feeding behavior and metabolic regulation such as the arcuate nucleus, the ventromedial hypothalamic nucleus and the nucleus accumbens (Saito et al., 2001). The distribution of the MCH-2 receptor is less clear. It has been reported to be mainly in the brain including the arcuate nucleus and the ventromedial hypothalamic nucleus (Sailer et al., 2001) but others report that it is not in the human hypothalamus (Rodriguez et al., 2001).

Behavior studies have demonstrated a physiological role for the MCH system in regulating food intake. Acute central administration of MCH leads to a rapid and significant increase in food intake as do MCH-1 receptor agonists in rodents (Qu et al., 1996; Suply et al., 2001). The potency at increasing food consumption is correlated with the affinity of the agonist for the receptor, suggesting that the MCH-1 receptor mediates directly the orexigenic effects of MCH (Suply et al., 2001). Chronic i.c.v. infusion of MCH or of synthetic MCH-1 receptor agonists induces obesity in rodents. The weight gain is accompanied by hyperphagia, reduced core temperature and increased lipogenesis (Della-Zuana et al., 2002; Ito et al., 2003; Shearman et al., 2003). This suggests that MCH may be an important player in the development of obesity by both increasing energy intake and reducing energy expenditure.

Genetic manipulation of the MCH precursor or the MCH-1 receptor further confirmed the central role of the MCH system in regulating energy homeostasis (Table 2). MCH precursor knockout mice are lean due to hypophagia and increased metabolic rate (Shimada et al., 1998). In contrast, MCH overexpression leads to mild obesity and hyperphagia (Ludwig et al., 2001). MCH-1 receptor deficient (MCHR1−/−) mice are lean and exhibit increased metabolic rates that are secondary to a hyperactive phenotype. These mice are surprisingly hyperphagic, suggesting that the leanness observed in MCHR1−/− mice is due to stimulated energy expenditure (Marsh et al., 2002). The discrepancy in the phenotypes of the MCH precursor knockout mice and the MCH-1 receptor-deficient mice may be due to the deletion of two additional functional unknown peptides which are encoded by the same precursor in MCH precursor knockout mice.

The MCH system is also regulated by energy homeostasis and interacts with other central modulators such as leptin. Prepro-MCH mRNA is upregulated in leptin-deficient ob/ob mice (Segal-Lieberman et al., 2003). It has been shown that the MCH system is a downstream

mediator of the leptin pathway and is responsible, at least partially, for the obesity induced by leptin deficiency. Deletion of prepro-MCH in leptin-deficient mice (MCH−/−ob/ob mice, double null mice) causes the obesity phenotype normally seen in leptin deficient mice to be dramatically reduced. The weight loss in these double null mice is mainly attributed to a significant reduction in body fat. These mice display significantly increased metabolic rates as compared to ob/ob mice, but are surprisingly more hyperphagic than ob/ob mice. This indicates that the weight loss induced by the absence of MCH in ob/ob mice is secondary to the increased energy expenditure (Segal-Lieberman et al., 2003).

Compelling evidence has suggested that the MCH/MCH-1 receptor system is a central regulatory system in energy homeostasis (Fig. 1) and that the MCH-1 receptor is one of the more promising therapeutic targets in the treatment of obesity. Various MCH-1 receptor antagonists have been developed. Chronic administration of MCH-1 receptor antagonists in rodents led to significantly reduced food intake and sustained reduction of body weight gain and body fat gain during the whole treatment period (Borowsky et al., 2002; Shearman et al., 2003).

3.4. The NPB/NPW system

In 2002, neuropeptide B (NPB) and neuropeptide W (NPW) were identified as the ligands for two orphan GPCRs, GPR7 and GPR8 (Fujii et al., 2002; Shimomura et al., 2002; Brezillon et al., 2003; Tanaka et al., 2003). Both NPB and NPW peptides come in two forms, 23 and 29, 23 and 30 amino acids in length, respectively. The two forms of NPB like NPW are produced from a single precursor gene by differential proteolytic processing at two arginine residues. Unlike NPW, NPB is unique by containing a brominated tryptophan residue at its N-terminus.

While both NPB and NPW can activate GPR7 and GPR8 potently, GPR7 shows a slightly higher affinity for NPB and GPR8 confers a slightly higher affinity for NPW (Fujii et al., 2002; Shimomura et al., 2002; Brezillon et al., 2003; Tanaka et al., 2003). While NPB, NPW and GPR7 are expressed in both humans and rodents, GPR8 is absent in rodents. GPR8 is however expressed in many other species including human, flying lemur, tree shrew, bovine, and rabbit (Lee et al., 1999).

In rodents, NPB mRNA was found to be widely distributed in the central nervous system including the paraventricular hypothalamic nucleus. Conversely, in mice, NPW mRNA was detected only in several discrete regions: the periaqueductal gray matter, ventral tegmental area, Edinger-Westphal nucleus and the dorsal raphe nucleus (Tanaka et al., 2003). GPR7 mRNA is robustly expressed in several discrete nuclei in the hypothalamus that are important regions for energy regulation such as the arcuate nucleus as well as the ventromedial, paraventricular and dorsomedial hypothalamic nuclei. GPR7 mRNA was also found in the suprachiasmatic nucleus (Lee et al., 1999). The distributions of GPR7 and its ligand in the hypothalamus suggest a possible role of this system in energy balance.

Behavioral studies have been carried out to investigate the effects of NPB and NPW on energy balance. Acute central administrations of NPB or NPW demonstrate divergent diurnal effects on feeding behavior. In the light phase, these peptides have been reported to have either no effects (Tanaka et al., 2003) or to stimulate food intake (Shimomura et al., 2002; Baker et al., 2003). In the dark phase, these peptides produce a major suppression of food intake (Mondal et al., 2003; Tanaka et al., 2003). The effect of these peptides during the dark phase has been reported to be biphasic. The first 2 h following i.c.v. injections are characterized by either a hyperphagic state (Tanaka et al., 2003) or no changes in food intake (Mondal et al., 2003) that is followed by a longer hypophagic phase. However, the overall effect is a major suppression of food intake during the dark phase. The divergent diurnal effects suggest a possible involvement of this system in circadian rhythms and could be related to the expression of GPR7 mRNA in the suprachiasmatic nucleus.

Chronic infusion of NPW reduces body weight gain, suppresses food intake and increases body temperature, oxygen consumption and heat production. These results point at this system as being anorexigenic, both increasing energy expenditure and decreasing energy intake (Mondal et al., 2003). In agreement with the anorexigenic effects of chronic NPW administration, male GPR7 knockout (GPR7−/−) mice are hyperphagic and show decreased energy expenditure (Table 2). These mice develop age-related progressive adult-onset obesity. In contrast, female GPR7 knockout mice did not show any significant differences in body weight gain or fat mass. These different phenotypes suggest that the effects of the NPB/NPW system on energy balance may be sexually dimorphic (Ishii et al., 2003).

This system has also been shown to interact with other molecules involved in energy balance regulation such as NPY, POMC and leptin. Selective lesion of the ventromedial hypothalamic nucleus using gold-thioglucose (GTG) causes obesity. GPR7 mRNA was found to be down-regulated in GTG-treated animals. Deletion of the GPR7 gene leads to the upregulation of the anorexigenic POMC products, and the downregulation of the orexigenic signal NPY in male mice. These effects are opposite to those seen in ob/ob mice. Male GPR7−/−ob/ob double null mice demonstrate increased weight gain when compared to ob/ob mice. No significant differences were seen in female mice (Ishii et al., 2003). All these data suggest that the NPB/NPW system and leptin mediate energy balance via different pathways (Fig. 1).

Many gaps still need to be filled to study the effects of this system in regulating energy balance, such as the function of GPR8, and possible differential regulatory effects of NPB and NPW. Studies so far suggest that GPR7 agonists would be promising therapeutic agents in the treatment of obesity. No GPR7 agonists are available at this

time, but it has been shown that the NPB/NPW system is a promising target that still waits to be further explored.

4. Conclusions and perspectives

The orphan receptor strategy has been successful over the past 10 years in identifying a dozen novel neuropeptide families. Interestingly, while these novel neuropeptides have been shown to modulate distinct physiological responses, several have been demonstrated to be important regulators of food intake and energy balance. Some such as ghrelin, hypocretins/orexins and MCH are orexigenic, while some such as NPB and NPW are anorexigenic. Why is food intake such a preferential target for newly described peptides over other physiological responses? One reason may be that these peptides have been discovered at the time when our understanding of food intake took a new turn with the discovery of leptin and thus testing of novel natural entities for their effects on food intake was common. This would mean that these novel neuropeptides may have several other physiological effects, some that may even be more pronounced than their effects on obesity, as indeed already demonstrated by the effect of orexins on wakefulness. But it remains true that these peptides have greatly enhanced our understanding of the pathophysiology of obesity. Moreover, being ligands of GPCRs they represent promising targets for developing drugs to treat obesity, a disorder that affects the lives of millions of people.

Finally, it should be reminded that there are still numerous orphan GPCRs that await to be deorphanized. Among those, some probably will be involved in the pathophysiology of obesity, but finding the natural ligands of these orphan GPCRs is a long and difficult quest. If our understanding of the pathophysiology of obesity serves as an example, then the search for the natural ligands of orphan GPCRs will undoubtedly broaden our knowledge of many aspects of human physiology and will have far reaching impact on the treatment of numerous disorders.

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References

Arvat, E., Di Vito, L., Broglio, F., Papotti, M., Muccioli, G., Dieguez, C., Casanueva, F.F., Deghenghi, R., Camanni, F., Ghigo, E., 2000. Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. J. Endocrinol. Invest. 23, 493–495.

Arvat, E., Maccario, M., Di Vito, L., Broglio, F., Benso, A., Gottero, C., Papotti, M., Muccioli, G., Dieguez, C., Casanueva, F.F., Deghenghi, R., Camanni, F., Ghigo, E., 2001. Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. J. Clin. Endocrinol. Metab. 86, 1169–1174.

Asakawa, A., Inui, A., Kaga, T., Katsuura, G., Fujimiya, M., Fujino, M.A., Kasuga, M., 2003. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. Gut 52, 947–952.

Bagnasco, M., Tulipano, G., Melis, M.R., Argiolas, A., Cocchi, D., Muller, E.E., 2003. Endogenous ghrelin is an orexigenic peptide acting in the arcuate nucleus in response to fasting. Regul. Pept. 111, 161–167.

Baker, J.R., Cardinal, K., Bober, C., Taylor, M.M., Samson, W.K., 2003. Neuropeptide W acts in brain to control prolactin, corticosterone, and growth hormone release. Endocrinology 144, 2816–2821.

Baskin, D.G., Breininger, J.F., Schwartz, M.W., 1999a. Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. Diabetes 48, 828–833.

Baskin, D.G., Hahn, T.M., Schwartz, M.W., 1999b. Leptin sensitive neurons in the hypothalamus. Horm. Metab. Res. 31, 345–350.

Beck, B., Richy, S., Dimitrov, T., Stricker-Krongrad, A., 2001. Opposite regulation of hypothalamic orexin and neuropeptide Y receptors and peptide expressions in obese Zucker rats. Biochem. Biophys. Res. Commun. 286, 518–523.

Bednarek, M.A., Feighner, S.D., Pong, S.S., McKee, K.K., Hreniuk, D.L., Silva, M.V., Warren, V.A., Howard, A.D., Van Der Ploeg, L.H., Heck, J.V., 2000. Structure–function studies on the new growth hormone-releasing peptide, ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretagogue receptor 1a. J. Med. Chem. 43, 4370–4376.

Bittencourt, J.C., Elias, C.F., 1998. Melanin-concentrating hormone and neuropeptide EI projections from the lateral hypothalamic area and zona incerta to the medial septal nucleus and spinal cord: a study using multiple neuronal tracers. Brain Res. 805, 1–19.

Bittencourt, J.C., Frigo, L., Rissman, R.A., Casatti, C.A., Nahon, J.L., Bauer, J.A., 1998. The distribution of melanin-concentrating hormone in the monkey brain (*Cebus apella*). Brain Res. 804, 140–143.

Bockaert, J., Pin, J.P., 1999. Molecular tinkering of G protein-coupled receptors: an evolutionary success. EMBO J. 18, 1723–1729.

Borowsky, B., Durkin, M.M., Ogozalek, K., Marzabadi, M.R., DeLeon, J., Lagu, B., Heurich, R., Lichtblau, H., Shaposhnik, Z., Daniewska, I., Blackburn, T.P., Branchek, T.A., Gerald, C., Vaysse, P.J., Forray, C., 2002. Antidepressant, anxiolytic and anorectic effects of a melanin-concentrating hormone-1 receptor antagonist. Nat. Med. 8, 825–830.

Bourgin, P., Huitron-Resendiz, S., Spier, A.D., Fabre, V., Morte, B., Criado, J.R., Sutcliffe, J.G., Henriksen, S.J., de Lecea, L., 2000. Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. J. Neurosci. 20, 7760–7765.

Brezillon, S., Lannoy, V., Franssen, J.D., Le Poul, E., Dupriez, V., Lucchetti, J., Detheux, M., Parmentier, M., 2003. Identification of natural ligands for the orphan G protein-coupled receptors GPR7 and GPR8. J. Biol. Chem. 278, 776–783.

Bunzow, J.R., Van Tol, H.H., Grandy, D.K., Albert, P., Salon, J., Christie, M., Machida, C.A., Neve, K.A., Civelli, O., 1988. Cloning and expression of a rat D2 dopamine receptor cDNA. Nature 336, 783–787.

Cai, X.J., Widdowson, P.S., Harrold, J., Wilson, S., Buckingham, R.E., Arch, J.R., Tadayyon, M., Clapham, J.C., Wilding, J., Williams, G., 1999. Hypothalamic orexin expression: modulation by blood glucose and feeding. Diabetes 48, 2132–2137.

Chemelli, R.M., Willie, J.T., Sinton, C.M., Elmquist, J.K., Scammell, T., Lee, C., Richardson, J.A., Williams, S.C., Xiong, Y., Kisanuki, Y., Fitch, T.E., Nakazato, M., Hammer, R.E., Saper, C.B., Yanagisawa, M., 1999. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98, 437–451.

Chen, H.Y., Trumbauer, M.E., Chen, A.S., Weingarth, D.T., Adams, J.R., Frazier, E.G., Shen, Z., Marsh, D.J., Feighner, S.D., Guan, X.M., Ye, Z., Nargund, R.P., Smith, R.G., Van Der Ploeg, L.H., Howard, A.D., MacNeil, D.J., Qian, S., 2004. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y (NPY) and agouti-related protein (AgRP). Endocrinology 145 (6), 2607–2612.

Cheng, M.Y., Bullock, C.M., Li, C., Lee, A.G., Bermak, J.C., Belluzzi, J., Weaver, D.R., Leslie, F.M., Zhou, Q.Y., 2002. Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. Nature 417, 405–410.

Civelli, O., 1998. Functional genomics: the search for novel neurotransmitters and neuropeptides. FEBS Lett. 430, 55–58.

Civelli, O., Nothacker, H.P., Saito, Y., Wang, Z., Lin, S.H., Reinscheid, R.K., 2001. Novel neurotransmitters as natural ligands of orphan G-protein-coupled receptors. Trends Neurosci. 24, 230–237.

Crowley, V.E., Yeo, G.S., O'Rahilly, S., 2002. Obesity therapy: altering the energy intake-and-expenditure balance sheet. Nat. Rev., Drug Discov. 1, 276–286.

Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E., Weigle, D.S., 2001. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50, 1714–1719.

Cummings, D.E., Weigle, D.S., Frayo, R.S., Breen, P.A., Ma, M.K., Dellinger, E.P., Purnell, J.Q., 2002. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N. Engl. J. Med. 346, 1623–1630.

Date, Y., Ueta, Y., Yamashita, H., Yamaguchi, H., Matsukura, S., Kangawa, K., Sakurai, T., Yanagisawa, M., Nakazato, M., 1999. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. Proc. Natl. Acad. Sci. U. S. A. 96, 748–753.

Date, Y., Kojima, M., Hosoda, H., Sawaguchi, A., Mondal, M.S., Suganuma, T., Matsukura, S., Kangawa, K., Nakazato, M., 2000a. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology 141, 4255–4261.

Date, Y., Murakami, N., Kojima, M., Kuroiwa, T., Matsukura, S., Kangawa, K., Nakazato, M., 2000b. Central effects of a novel acylated peptide, ghrelin, on growth hormone release in rats. Biochem. Biophys. Res. Commun. 275, 477–480.

de Lecea, L., Kilduff, T.S., Peyron, C., Gao, X., Foye, P.E., Danielson, P.E., Fukuhara, C., Battenberg, E.L., Gautvik, V.T., Bartlett 2nd, F.S., Frankel, W.N., van den Pol, A.N., Bloom, F.E., Gautvik, K.M., Sutcliffe, J.G., 1998. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc. Natl. Acad. Sci. U. S. A. 95, 322–327.

Della-Zuana, O., Presse, F., Ortola, C., Duhault, J., Nahon, J.L., Levens, N., 2002. Acute and chronic administration of melanin-concentrating hormone enhances food intake and body weight in Wistar and Sprague–Dawley rats. Int. J. Obes. Relat. Metab. Disord. 26, 1289–1295.

Drews, J., 2000. Drug discovery: a historical perspective. Science 287, 1960–1964.

Dube, M.G., Kalra, S.P., Kalra, P.S., 1999. Food intake elicited by central administration of orexins/hypocretins: identification of hypothalamic sites of action. Brain Res. 842, 473–477.

Fargin, A., Raymond, J.R., Lohse, M.J., Kobilka, B.K., Caron, M.G., Lefkowitz, R.J., 1988. The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT1A receptor. Nature 335, 358–360.

Fujii, R., Yoshida, H., Fukusumi, S., Habata, Y., Hosoya, M., Kawamata, Y., Yano, T., Hinuma, S., Kitada, C., Asami, T., Mori, M., Fujisawa, Y., Fujino, M., 2002. Identification of a neuropeptide modified with bromine as an endogenous ligand for GPR7. J. Biol. Chem. 277, 34010–34016.

Funes, S., Hedrick, J.A., Vassileva, G., Markowitz, L., Abbondanzo, S., Golovko, A., Yang, S., Monsma, F.J., Gustafson, E.L., 2003. The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. Biochem. Biophys. Res. Commun. 312, 1357–1363.

Guan, X.M., Yu, H., Palyha, O.C., McKee, K.K., Feighner, S.D., Sirinathsinghji, D.J., Smith, R.G., Van der Ploeg, L.H., Howard, A.D., 1997. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Brain Res. Mol. Brain Res. 48, 23–29.

Hagan, J.J., Leslie, R.A., Patel, S., Evans, M.L., Wattam, T.A., Holmes, S., Benham, C.D., Taylor, S.G., Routledge, C., Hemmati, P., Munton, R.P., Ashmeade, T.E., Shah, A.S., Hatcher, J.P., Hatcher, P.D., Jones, D.N., Smith, M.I., Piper, D.C., Hunter, A.J., Porter, R.A., Upton, N., 1999. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc. Natl. Acad. Sci. U. S. A. 96, 10911–10916.

Hara, J., Beuckmann, C.T., Nambu, T., Willie, J.T., Chemelli, R.M., Sinton, C.M., Sugiyama, F., Yagami, K., Goto, K., Yanagisawa, M., Sakurai, T., 2001. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 30, 345–354.

Haynes, A.C., Jackson, B., Overend, P., Buckingham, R.E., Wilson, S., Tadayyon, M., Arch, J.R., 1999. Effects of single and chronic intracerebroventricular administration of the orexins on feeding in the rat. Peptides 20, 1099–1105.

Haynes, A.C., Jackson, B., Chapman, H., Tadayyon, M., Johns, A., Porter, R.A., Arch, J.R., 2000. A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. Regul. Pept. 96, 45–51.

Haynes, A.C., Chapman, H., Taylor, C., Moore, G.B., Cawthorne, M.A., Tadayyon, M., Clapham, J.C., Arch, J.R., 2002. Anorectic, thermogenic and anti-obesity activity of a selective orexin-1 receptor antagonist in ob/ob mice. Regul. Pept. 104, 153–159.

Hewson, A.K., Dickson, S.L., 2000. Systemic administration of ghrelin induces Fos and Egr-1 proteins in the hypothalamic arcuate nucleus of fasted and fed rats. J. Neuroendocrinol. 12, 1047–1049.

Hosoda, H., Kojima, M., Kangawa, K., 2002. Ghrelin and the regulation of food intake and energy balance. Mol. Interv. 2, 494–503.

Howard, A.D., Feighner, S.D., Cully, D.F., Arena, J.P., Liberator, P.A., Rosenblum, C.I., Hamelin, M., Hreniuk, D.L., Palyha, O.C., Anderson, J., Paress, P.S., Diaz, C., Chou, M., Liu, K.K., McKee, K.K., Pong, S.S., Chaung, L.Y., Elbrecht, A., Dashkevicz, M., Heavens, R., Rigby, M., Sirinathsinghji, D.J., Dean, D.C., Melillo, D.G., Patchett, A.A., Nargund, R., Griffin, P.R., DeMartino, J.A., Gupta, S.K., Schaeffer, J.M., Smith, R.G., Van der Ploeg, L.H.T., 1996. A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273, 974–977.

Howard, A.D., McAllister, G., Feighner, S.D., Liu, Q., Nargund, R.P., Van der Ploeg, L.H., Patchett, A.A., 2001. Orphan G-protein-coupled receptors and natural ligand discovery. Trends Pharmacol. Sci. 22, 132–140.

Ishii, M., Fei, H., Friedman, J.M., 2003. Targeted disruption of GPR7 the endogenous receptor for neuropeptides B and W, leads to metabolic defects and adult-onset obesity. Proc. Natl. Acad. Sci. U. S. A. 100, 10540–10545.

Ito, M., Gomori, A., Ishihara, A., Oda, Z., Mashiko, S., Matsushita, H., Yumoto, M., Sano, H., Tokita, S., Moriya, M., Iwaasa, H., Kanatani, A., 2003. Characterization of MCH-mediated obesity in mice. Am. J. Physiol: Endocrinol. Metab. 284, E940–E945.

Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H., Wakabayashi, I., 2001. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. Diabetes 50, 2438–2443.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., Kangawa, K., 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402, 656–660.

Korbonits, M., Gueorguiev, M., O'Grady, E., Lecoeur, C., Swan, D.C., Mein, C.A., Weill, J., Grossman, A.B., Froguel, P., 2002. A variation in the ghrelin gene increases weight and decreases insulin secretion in tall, obese children. J. Clin. Endocrinol. Metab. 87, 4005–4008.

Lawrence, C.B., Snape, A.C., Baudoin, F.M., Luckman, S.M., 2002. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. Endocrinology 143, 155–162.

LeCouter, J., Lin, R., Ferrara, N., 2002. Endocrine gland-derived VEGF and the emerging hypothesis of organ-specific regulation of angiogenesis. Nat. Med. 8, 913–917.

Lee, D.K., Nguyen, T., Porter, C.A., Cheng, R., George, S.R., O'Dowd, B.F., 1999. Two related G protein-coupled receptors: the distribution of GPR7 in rat brain and the absence of GPR8 in rodents. Brain Res. Mol. Brain Res. 71, 96–103.

Libert, F., Vassart, G., Parmentier, M., 1991. Current developments in G-protein-coupled receptors. Curr. Opin. Cell Biol. 3, 218–223.

Lin, L., Faraco, J., Li, R., Kadotani, H., Rogers, W., Lin, X., Qiu, X., de Jong, P.J., Nishino, S., Mignot, E., 1999. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 98, 365–376.

Lin, S.H., Arai, A.C., Espana, R.A., Berridge, C.W., Leslie, F.M., Huguenard, J.R., Vergnes, M., Civelli, O., 2002. Prolactin-releasing peptide (PrRP) promotes awakening and suppresses absence seizures. Neuroscience 114, 229–238.

Liu, C., Chen, J., Sutton, S., Roland, B., Kuei, C., Farmer, N., Sillard, R., Lovenberg, T.W., 2003. Identification of relaxin-3/INSL7 as a ligand for GPCR142. J. Biol. Chem. 278, 50765–50770.

Liu, C., Eriste, E., Sutton, S., Chen, J., Roland, B., Kuei, C., Farmer, N., Jornvall, H., Sillard, R., Lovenberg, T.W., 2003. Identification of relaxin-3/INSL7 as an endogenous ligand for the orphan G-protein-coupled receptor GPCR135. J. Biol. Chem. 278, 50754–50764.

Ludwig, D.S., Tritos, N.A., Mastaitis, J.W., Kulkarni, R., Kokkotou, E., Elmquist, J., Lowell, B., Flier, J.S., Maratos-Flier, E., 2001. Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. J. Clin. Invest. 107, 379–386.

Marchese, A., George, S.R., Kolakowski Jr., L.F., Lynch, K.R., O'Dowd, B.F., 1999. Novel GPCRs and their endogenous ligands: expanding the boundaries of physiology and pharmacology. Trends Pharmacol. Sci. 20, 370–375.

Marcus, J.N., Aschkenasi, C.J., Lee, C.E., Chemelli, R.M., Saper, C.B., Yanagisawa, M., Elmquist, J.K., 2001. Differential expression of orexin receptors 1 and 2 in the rat brain. J. Comp. Neurol. 435, 6–25.

Marsh, D.J., Weingarth, D.T., Novi, D.E., Chen, H.Y., Trumbauer, M.E., Chen, A.S., Guan, X.M., Jiang, M.M., Feng, Y., Camacho, R.E., Shen, Z., Frazier, E.G., Yu, H., Metzger, J.M., Kuca, S.J., Shearman, L.P., Gopal-Truter, S., MacNeil, D.J., Strack, A.M., MacIntyre, D.E., Van der Ploeg, L.H., Qian, S., 2002. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. Proc. Natl. Acad. Sci. U. S. A. 99, 3240–3245.

McKee, K.K., Palyha, O.C., Feighner, S.D., Hreniuk, D.L., Tan, C.P., Phillips, M.S., Smith, R.G., Van der Ploeg, L.H., Howard, A.D., 1997. Molecular analysis of rat pituitary and hypothalamic growth hormone secretagogue receptors. Mol. Endocrinol. 11, 415–423.

Miraglia del Giudice, E., Santoro, N., Cirillo, G., Raimondo, P., Grandone, A., D'Aniello, A., Di Nardo, M., Perrone, L., 2004. Molecular screening of the ghrelin gene in Italian obese children: the Leu72Met variant is associated with an earlier onset of obesity. Int. J. Obes. Relat. Metab. Disord. 28, 447–450.

Mitchell, V., Bouret, S., Beauvillain, J.C., Schilling, A., Perret, M., Kordon, C., Epelbaum, J., 2001. Comparative distribution of mRNA encoding the growth hormone secretagogue-receptor (GHS-R) in *Microcebus murinus* (Primate, lemurian) and rat forebrain and pituitary. J. Comp. Neurol. 429, 469–489.

Mondal, M.S., Yamaguchi, H., Date, Y., Shimbara, T., Toshinai, K., Shimomura, Y., Mori, M., Nakazato, M., 2003. A role for neuropeptide W in the regulation of feeding behavior. Endocrinology 144, 4729–4733.

Muccioli, G., Ghe, C., Ghigo, M.C., Papotti, M., Arvat, E., Boghen, M.F., Nilsson, M.H., Deghenghi, R., Ong, H., Ghigo, E., 1998. Specific receptors for synthetic GH secretagogues in the human brain and pituitary gland. J. Endocrinol. 157, 99–106.

Muccioli, G., Tschöp, M., Papotti, M., Deghenghi, R., Heiman, M., Ghigo, E., 2002. Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity. Eur. J. Pharmacol. 440, 235–254.

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., Matsukura, S., 2001. A role for ghrelin in the central regulation of feeding. Nature 409, 194–198.

Nambu, T., Sakurai, T., Mizukami, K., Hosoya, Y., Yanagisawa, M., Goto, K., 1999. Distribution of orexin neurons in the adult rat brain. Brain Res. 827, 243–260.

Ohtaki, T., Shintani, Y., Honda, S., Matsumoto, H., Hori, A., Kanehashi, K., Terao, Y., Kumano, S., Takatsu, Y., Masuda, Y., Ishibashi, Y., Watanabe, T., Asada, M., Yamada, T., Suenaga, M., Kitada, C., Usuki, S., Kurokawa, T., Onda, H., Nishimura, O., Fujino, M., 2001. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. Nature 411, 613–617.

Otto, B., Cuntz, U., Fruehauf, E., Wawarta, R., Folwaczny, C., Riepl, R.L., Heiman, M.L., Lehnert, P., Fichter, M., Tschöp, M., 2001. Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. Eur. J. Endocrinol. 145, 669–673.

Peyron, C., Tighe, D.K., van den Pol, A.N., de Lecea, L., Heller, H.C., Sutcliffe, J.G., Kilduff, T.S., 1998. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J. Neurosci. 18, 9996–10015.

Qu, D., Ludwig, D.S., Gammeltoft, S., Piper, M., Pelleymounter, M.A., Cullen, M.J., Mathes, W.F., Przypek, R., Kanarek, R., Maratos-Flier, E., 1996. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. Nature 380, 243–247.

Reinscheid, R.K., Nothacker, H.P., Bourson, A., Ardati, A., Henningsen, R.A., Bunzow, J.R., Grandy, D.K., Langen, H., Monsma Jr., F.J., Civelli, O., 1995. Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. Science 270, 792–794.

Rodriguez, M., Beauverger, P., Naime, I., Rique, H., Ouvry, C., Souchaud, S., Dromaint, S., Nagel, N., Suply, T., Audinot, V., Boutin, J.A., Galizzi, J.P., 2001. Cloning and molecular characterization of the novel human melanin-concentrating hormone receptor MCH2. Mol. Pharmacol. 60, 632–639.

Sailer, A.W., Sano, H., Zeng, Z., McDonald, T.P., Pan, J., Pong, S.S., Feighner, S.D., Tan, C.P., Fukami, T., Iwaasa, H., Hreniuk, D.L., Morin, N.R., Sadowski, S.J., Ito, M., Bansal, A., Ky, B., Figueroa, D.J., Jiang, Q., Austin, C.P., MacNeil, D.J., Ishihara, A., Ihara, M., Kanatani, A., Van der Ploeg, L.H., Howard, A.D., Liu, Q., 2001. Identification and characterization of a second melanin-concentrating hormone receptor, MCH-2R. Proc. Natl. Acad. Sci. U. S. A. 98, 7564–7569.

Saito, Y., Nothacker, H.P., Wang, Z., Lin, S.H., Leslie, F., Civelli, O., 1999. Molecular characterization of the melanin-concentrating-hormone receptor. Nature 400, 265–269.

Saito, Y., Cheng, M., Leslie, F.M., Civelli, O., 2001. Expression of the melanin-concentrating hormone (MCH) receptor mRNA in the rat brain. J. Comp. Neurol. 435, 26–40.

Sakurai, T., 2003. Orexin: a link between energy homeostasis and adaptive behaviour. Curr. Opin. Clin. Nutr. Metab. Care 6, 353–360.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., Williams, S.C., Richardson, J.A., Kozlowski, G.P., Wilson, S., Arch, J.R., Buckingham, R.E., Haynes, A.C., Carr, S.A., Annan, R.S., McNulty, D.E., Liu, W.S., Terrett, J.A., Elshourbagy, N.A., Bergsma, D.J., Yanagisawa, M., 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92, 573–585.

Segal-Lieberman, G., Bradley, R.L., Kokkotou, E., Carlson, M., Trombly, D.J., Wang, X., Bates, S., Myers, M.G., Flier Jr., J.S., Maratos-Flier, E., 2003. Melanin-concentrating hormone is a critical mediator of the leptin-deficient phenotype. Proc. Natl. Acad. Sci. U. S. A. 100, 10085–10090.

Shearman, L.P., Camacho, R.E., Sloan Stribling, D., Zhou, D., Bednarek, M.A., Hreniuk, D.L., Feighner, S.D., Tan, C.P., Howard, A.D., Van der Ploeg, L.H., MacIntyre, D.E., Hickey, G.J., Strack, A.M., 2003. Chronic MCH-1 receptor modulation alters appetite, body weight and adiposity in rats. Eur. J. Pharmacol. 475, 37–47.

Shiiya, T., Nakazato, M., Mizuta, M., Date, Y., Mondal, M.S., Tanaka, M., Nozoe, S., Hosoda, H., Kangawa, K., Matsukura, S., 2002. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J. Clin. Endocrinol. Metab. 87, 240–244.

Shimada, M., Tritos, N.A., Lowell, B.B., Flier, J.S., Maratos-Flier, E., 1998. Mice lacking melanin-concentrating hormone are hypophagic and lean. Nature 396, 670–674.

Shimomura, Y., Harada, M., Goto, M., Sugo, T., Matsumoto, Y., Abe, M., Watanabe, T., Asami, T., Kitada, C., Mori, M., Onda, H., Fujino, M., 2002. Identification of neuropeptide W as the endogenous ligand for orphan G-protein-coupled receptors GPR7 and GPR8. J. Biol. Chem. 277, 35826–35832.

Shintani, M., Ogawa, Y., Ebihara, K., Aizawa-Abe, M., Miyanaga, F., Takaya, K., Hayashi, T., Inoue, G., Hosoda, K., Kojima, M., Kangawa, K., Nakao, K., 2001. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 50, 227–232.

Smart, D., Haynes, A.C., Williams, G., Arch, J.R., 2002. Orexins and the treatment of obesity. Eur. J. Pharmacol. 440, 199–212.

Soriano-Guillen, L., Barrios, V., Campos-Barros, A., Argente, J., 2004. Ghrelin levels in obesity and anorexia nervosa: effect of weight reduction or recuperation. J. Pediatr. 144, 36–42.

Sun, Y., Ahmed, S., Smith, R.G., 2003. Deletion of ghrelin impairs neither growth nor appetite. Mol. Cell. Biol. 23, 7973–7981.

Sun, Y., Wang, P., Zheng, H., Smith, R.G., 2004. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. Proc. Natl. Acad. Sci. U. S. A. 101, 4679–4684.

Suply, T., Della Zuana, O., Audinot, V., Rodriguez, M., Beauverger, P., Duhault, J., Canet, E., Galizzi, J.P., Nahon, J.L., Levens, N., Boutin, J.A., 2001. SLC-1 receptor mediates effect of melanin-concentrating hormone on feeding behavior in rat: a structure–activity study. J. Pharmacol. Exp. Ther. 299, 137–146.

Sutcliffe, J.G., de Lecea, L., 2002. The hypocretins: setting the arousal threshold. Nat. Rev., Neurosci. 3, 339–349.

Sweet, D.C., Levine, A.S., Billington, C.J., Kotz, C.M., 1999. Feeding response to central orexins. Brain Res. 821, 535–538.

Takaya, K., Ariyasu, H., Kanamoto, N., Iwakura, H., Yoshimoto, A., Harada, M., Mori, K., Komatsu, Y., Usui, T., Shimatsu, A., Ogawa, Y., Hosoda, K., Akamizu, T., Kojima, M., Kangawa, K., Nakao, K., 2000. Ghrelin strongly stimulates growth hormone release in humans. J. Clin. Endocrinol. Metab. 85, 4908–4911.

Tan, C.P., Sano, H., Iwaasa, H., Pan, J., Sailer, A.W., Hreniuk, D.L., Feighner, S.D., Palyha, O.C., Pong, S.S., Figueroa, D.J., Austin, C.P., Jiang, M.M., Yu, H., Ito, J., Ito, M., Guan, X.M., MacNeil, D.J., Kanatani, A., Van der Ploeg, L.H., Howard, A.D., 2002. Melanin-concentrating hormone receptor subtypes 1 and 2: species-specific gene expression. Genomics 79, 785–792.

Tanaka, M., Naruo, T., Muranaga, T., Yasuhara, D., Shiiya, T., Nakazato, M., Matsukura, S., Nozoe, S., 2002. Increased fasting plasma ghrelin levels in patients with bulimia nervosa. Eur. J. Endocrinol. 146, R1–R3.

Tanaka, H., Yoshida, T., Miyamoto, N., Motoike, T., Kurosu, H., Shibata, K., Yamanaka, A., Williams, S.C., Richardson, J.A., Tsujino, N., Garry, M.G., Lerner, M.R., King, D.S., O'Dowd, B.F., Sakurai, T., Yanagisawa, M., 2003. Characterization of a family of endogenous neuropeptide ligands for the G protein-coupled receptors GPR7 and GPR8. Proc. Natl. Acad. Sci. U. S. A. 100, 6251–6256.

Thannickal, T.C., Moore, R.Y., Nienhuis, R., Ramanathan, L., Gulyani, S., Aldrich, M., Cornford, M., Siegel, J.M., 2000. Reduced number of hypocretin neurons in human narcolepsy. Neuron 27, 469–474.

Thorpe, A.J., Mullett, M.A., Wang, C., Kotz, C.M., 2003. Peptides that regulate food intake: regional, metabolic, and circadian specificity of lateral hypothalamic orexin A feeding stimulation. Am. J. Physiol., Regul. Integr. Comp. Physiol. 284, R1409–R1417.

Trivedi, P., Yu, H., MacNeil, D.J., Van der Ploeg, L.H., Guan, X.M., 1998. Distribution of orexin receptor mRNA in the rat brain. FEBS Lett. 438, 71–75.

Tschöp, M., Smiley, D.L., Heiman, M.L., 2000. Ghrelin induces adiposity in rodents. Nature 407, 908–913.

Tschöp, M., Wawarta, R., Riepl, R.L., Friedrich, S., Bidlingmaier, M., Landgraf, R., Folwaczny, C., 2001a. Post-prandial decrease of circulating human ghrelin levels. J. Endocrinol. Invest. 24, RC19–RC21.

Tschöp, M., Weyer, C., Tataranni, P.A., Devanarayan, V., Ravussin, E., Heiman, M.L., 2001b. Circulating ghrelin levels are decreased in human obesity. Diabetes 50, 707–709.

Ukkola, O., Ravussin, E., Jacobson, P., Snyder, E.E., Chagnon, M., Sjostrom, L., Bouchard, C., 2001. Mutations in the preproghrelin/ghrelin gene associated with obesity in humans. J. Clin. Endocrinol. Metab. 86, 3996–3999.

Wang, J., Osaka, T., Inoue, S., 2001. Energy expenditure by intracerebroventricular administration of orexin to anesthetized rats. Neurosci. Lett. 315, 49–52.

Wang, J., Osaka, T., Inoue, S., 2003. Orexin-A-sensitive site for energy expenditure localized in the arcuate nucleus of the hypothalamus. Brain Res. 971, 128–134.

Wang, H.J., Geller, F., Dempfle, A., Schauble, N., Friedel, S., Lichtner, P., Fontenla-Horro, F., Wudy, S., Hagemann, S., Gortner, L., Huse, K., Remschmidt, H., Bettecken, T., Meitinger, T., Schafer, H., Hebebrand, J., Hinney, A., 2004. Ghrelin receptor gene: identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature. J. Clin. Endocrinol. Metab. 89, 157–162.

Willie, J.T., Chemelli, R.M., Sinton, C.M., Yanagisawa, M., 2001. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Annu. Rev. Neurosci. 24, 429–458.

Willie, J.T., Chemelli, R.M., Sinton, C.M., Tokita, S., Williams, S.C., Kisanuki, Y.Y., Marcus, J.N., Lee, C., Elmquist, J.K., Kohlmeier, K.A., Leonard, C.S., Richardson, J.A., Hammer, R.E., Yanagisawa, M., 2003. Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of Non-REM and REM sleep regulatory processes. Neuron 38, 715–730.

Wortley, K.E., Anderson, K., Garcia, K., Murray, J., Malinova, L., Liu, R., Moncrieffe, M., Thabet, K., Cox, H., Yancopoulos, G.D., Wiegand, S.J., Sleeman, M.W., 2004. Deletion of ghrelin reveals no effect on food intake, but a primary role in energy balance. Obes. Res. 12, 170.

Wren, A.M., Small, C.J., Ward, H.L., Murphy, K.G., Dakin, C.L., Taheri, S., Kennedy, A.R., Roberts, G.H., Morgan, D.G., Ghatei, M.A., Bloom, S.R., 2000. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141, 4325–4328.

Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., Dhillo, W.S., Ghatei, M.A., Bloom, S.R., 2001a. Ghrelin enhances appetite and increases food intake in humans. J. Clin. Endocrinol. Metab. 86, 5992.

Wren, A.M., Small, C.J., Abbott, C.R., Dhillo, W.S., Seal, L.J., Cohen, M.A., Batterham, R.L., Taheri, S., Stanley, S.A., Ghatei, M.A., Bloom, S.R., 2001b. Ghrelin causes hyperphagia and obesity in rats. Diabetes 50, 2540–2547.

Yamamoto, Y., Ueta, Y., Date, Y., Nakazato, M., Hara, Y., Serino, R., Nomura, M., Shibuya, I., Matsukura, S., Yamashita, H., 1999. Down regulation of the prepro-orexin gene expression in genetically obese mice. Brain Res. Mol. Brain Res. 65, 14–22.

Yamanaka, A., Sakurai, T., Katsumoto, T., Yanagisawa, M., Goto, K., 1999. Chronic intracerebroventricular administration of orexin-A to rats increases food intake in daytime, but has no effect on body weight. Brain Res. 849, 248–252.

Yamanaka, A., Beuckmann, C.T., Willie, J.T., Hara, J., Tsujino, N., Mieda, M., Tominaga, M., Yagami, K., Sugiyama, F., Goto, K., Yanagisawa, M., Sakurai, T., 2003. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. Neuron 38, 701–713.