Peripheral tissue–brain interactions in the regulation of food intake

Miguel López1, Sulay Tovar1, María J. Vázquez1, Lynda M. Williams2 and Carlos Diéguez1*

1Department of Physiology, School of Medicine, University of Santiago de Compostela, C/ San Francisco s/n 15782, Santiago de Compostela, A Coruña, Spain

2Obesity and Metabolic Health Division, Rowett Research Institute, Aberdeen, UK

More than 70 years ago the glucostatic, lipostatic and aminostatic hypotheses proposed that the central nervous system sensed circulating levels of different metabolites, changing feeding behaviour in response to the levels of those molecules. In the last 20 years the rapid increase in obesity and associated pathologies in developed countries has involved a substantial increase in the knowledge of the physiological and molecular mechanisms regulating body mass. This effort has resulted in the recent discovery of new peripheral signals, such as leptin and ghrelin, as well as new neuropeptides, such as orexins, involved in body-weight homeostasis. The present review summarises research into energy balance, starting from the original classical hypotheses proposing metabolite sensing, through peripheral tissue–brain interactions and coming full circle to the recently-discovered role of hypothalamic fatty acid synthase in feeding regulation. Understanding these molecular mechanisms will provide new pharmacological targets for the treatment of obesity and appetite disorders.

Food intake regulation: Gastrointestinal signals: Adipose and pancreatic hormones: Neural control

The prevalence of overweight and obesity in most developed countries has increased strikingly during the last 30 years (Friedman, 2000, 2003; Flier, 2004; Farooqi & O’Rahilly, 2005). Body weight depends on the balance between energy intake and energy consumption. Despite wide daily variation in food intake and energy expenditure, for most individuals body weight remains extremely stable over long periods of time. For this stability to occur, feeding and energy expenditure must be constantly modulated and balanced. Obesity results when the former exceeds the latter and there is an accumulation of an excess of fat in peripheral tissues such as white adipose tissue, which is specifically adapted for this function, liver and muscle, which results in metabolic disease (Friedman, 2000, 2003; Flier, 2004; Farooqi & O’Rahilly, 2005). Obesity has a profound impact on human health and lifespan. Being obese correlates not just with associated metabolic dysfunction such as type 2 diabetes and CVD, but is also associated with the occurrence of certain cancers (Calle & Kaaks, 2004).

The first hypotheses proposed to explain the peripheral–brain interaction in the regulation of food intake were the glucostatic, lipostatic and aminostatic hypotheses. These models proposed that circulating factors, e.g. lipids (lipostatic hypothesis), glucose (glucostatic hypothesis) or protein products (aminostatic hypothesis), that are generated in proportion to body fat stores and/or nutritional status act as signals to the brain, eliciting changes in energy intake and expenditure (Campfield et al. 1996). The current ‘obesity epidemic’ has driven forward research efforts in the investigation of body-weight homeostasis. For this reason, in the last decade there has been a major increase in the knowledge of the physiological and molecular mechanism regulating body mass. Animals are now known to regulate body weight by a complex homeostatic mechanism involving interactions between peripheral organs and the central nervous system (CNS). Peripheral organs, such as white adipose tissue, gut, thyroid, muscle and gonads produce signals that inform brain centres of the nutritional, as well as metabolic, status of the animal.

Abbreviations: AgRP, agouti-related peptide; ARC, arcuate nucleus of the hypothalamus; BBB, blood–brain barrier; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin; CB, cannabinoids; CNS, central nervous system; DMH, dorsomedial nucleus of the hypothalamus; EC, endocannabinoids; FAS, fatty acid synthase; GHS-R, growth hormone secretagogue receptor; GLP, glucagon-like peptide; NPY, neuropeptide Y; LHA, lateral hypothalamic area; MCH, melanin-concentrating hormone; MCnR, melanocortin receptor (n 1–5); NTS, nucleus of the solitary tract; OB-Ra–f, isoforms of leptin receptor; OX, orexin; OXM, oxyntomodulin; POMC, pro-opiomelanocortin; PP, pancreatic polypeptide; PVH, paraventricular nucleus of the hypothalamus; PYY, peptide YY; VMH, ventromedial nucleus of the hypothalamus.

*Corresponding author: Professor Carlos Diéguez, fax +34 981574145, email fscadigo@usc.es
(Flier, 2004; Horvath et al., 2004; Abizaid et al., 2006; Morton et al., 2006). The CNS receives and integrates this entire signalling system, adjusting energy intake (food intake) and energy expenditure, according to the demands of the organism.

The present review summarises the current knowledge about periphery–brain interactions in the regulation of feeding. A full understanding of these mechanisms will allow the establishment of effective therapies to counter eating disorders and obesity.

**Gastrointestinal signals regulating food intake**

In addition to its evident function in the digestion and absorption of nutrients, the gut and associated organs (liver, pancreas and visceral white adipose tissue depots) play an important role in the control of energy homeostasis, particularly in the short-term regulation of food intake. Both the enteric nervous system and gut hormones are known to control the initiation and termination of individual meals (Halford & Blundell, 2000a; Badman & Flier, 2005; Perez-Tilve et al., 2006).

**Enteric nervous system**

The gastrointestinal tract receives a dual extrinsic innervation from the autonomic nervous system via its parasympathetic (cholinergic) division, which includes vagal and pelvic nerves, and its sympathetic (nor-adrenergic) division, which comprises splanchnic nerves. Parasympathetic innervation is mainly inhibitory, and sympathetic innervation is mainly excitatory. In addition to this autonomic innervation, the gastrointestinal tract also has its own nervous system, i.e. the enteric nervous system (Konturek et al., 2004; Badman & Flier, 2005), which is involved in every aspect of gut function, from mastication to defaecation. Besides these roles, the enteric nervous system is also implicated in gastric and pancreatic exocrine secretion, gut motility, blood supply and hormone release (Konturek et al., 2004; Badman & Flier, 2005).

The enteric nervous system projects to the CNS through vagal and sympathetic (spinal) nerves. These projections transmit a variety of information to several CNS areas, including mechanical stimuli (distension, contraction), chemical stimuli (presence of nutrients in the gut lumen) and neurohumoral stimuli (gut hormones, neurotransmitters and neuromodulators; Langley, 1994; Konturek et al., 2004). Most of these afferent vagal fibres terminate in the nucleus of the solitary tract (NTS) in the brainstem, and in lamina 1 and V of the dorsal horn of spinal cord (Maggi, 1991; Konturek et al., 2004). Some signals from the gut are transmitted from the NTS to higher neural centres, such as the paraventricular (PVH) and arcuate (ARC) nuclei of the hypothalamus (Berthoud et al., 1990), the bed nucleus of the stria terminalis and the ventral thalamus. The integration of all these afferent signals related to food presence in the gut regulates the size of individual meals (Flier, 2004; Konturek et al., 2004; Badman & Flier, 2005).

**Gut hormones**

**Cholecystokinin.** Despite the profuse development of the enteric nervous system, the main route of communication between the brain and the gut in relation to energy homeostasis is via the circulation. One of the hormones first identified in regulating energy homeostasis was the gastrointestinal hormone cholecystokinin (CCK), which is secreted by I cells in the duodenum and the jejunum into the circulation in response to nutrient ingestion (protein and fatty acids; Larsson & Rehfeld, 1978; Bray, 2000; Haldor & Blundell, 2000a; Badman & Fliser, 2005; Stanley et al., 2005; Perez-Tilve et al., 2006). CCK exists in several molecular forms, the major forms in the plasma being CCK-8, -33 and -39 (Haldor & Blundell, 2000a; Konturek et al., 2004; Stanley et al., 2005). Once secreted, CCK reduces meal size and duration in both man and animals (Gibbs et al., 1973; Kissileff et al., 1981; Smith et al., 1981a; Pt-Sunyer et al., 1982; Muurahainen et al., 1988) and infusion of a CCK antagonist increases energy intake in human subjects (Beglinger et al., 2001). However, despite its anorectic actions, repeated administration of CCK does not influence body weight because although meal frequency is increased, there is no overall change in feeding (West et al., 1984; Wei & Mozov, 1995). Thus, CCK is mostly involved in the short-term control of food intake, together with distension of the upper gastrointestinal tract (Konturek et al., 2004; Badman & Flier, 2005).

CCK signals via two distinct G-protein-coupled receptors termed CCK_A and CCK_B (Wank et al., 1992a; Halford & Blundell, 2000a). Both receptors are widely expressed in the CNS and in the periphery (Moran et al., 1986, 1990; Wank et al., 1992a,b). The effect of CCK on food intake is mediated via CCK_A (Asin et al., 1992; Halford & Blundell, 2000a). CCK crosses the brain–blood barrier (BBB; Reidelberger et al., 2004) and acts on neuropeptide Y (NPY) neurons in the dorsomedial nucleus of the hypothalamus (DMH), as well as the NTS in the brainstem (Moran et al., 1997; Bi et al., 2001). The effects of CCK on feeding are also mediated through paracrine and neuroendocrine activation of vagal fibres (Reidelberger & Solomon, 1986; Schwartz & Moran, 1994; Moran et al., 1997).

**Glucagon-like peptide-1 and oxyntomodulin.** The preproglucagon gene product yields two important satiety peptides, glucagon-like peptide (GLP)-1 and oxyntomodulin (OXM; Tang-Christensen et al., 2001; Stanley et al., 2005). The preproglucagon gene is widely expressed in the gut, the pancreas and the NTS in the brainstem. Tissue-specific processing of preproglucagon by prohormone convertases 1 and 2 produces different products: glucagon is the main product in the pancreas; GLP-1 and -2 and OXM are the major products in CNS and gut (Tang-Christensen et al., 2001; Badman & Flier, 2005; Stanley et al., 2005).

GLP-1 and OXM are released from L cells in response to NEFA and carbohydrates (Ghatei et al., 1983; Le Quellec et al., 1992; Herrmann et al., 1995; Hirata et al., 2005). Both peptides inhibit feeding when they are centrally or peripherally administered (Turton et al., 1996;
Dakin et al. 2004), and chronic administration of GLP-1 and OXM decreases weight gain and adiposity in rodents (Meeran et al. 1999; Dakin et al. 2004). The actions of both GLP-1 and OXM on feeding may be mediated via the GLP-1 receptor, which is expressed in the hypothalamus, brainstem and periphery (Uttenthal et al. 1992; Wei & Mojsov, 1995; Shughrue et al. 1996; Bullock et al. 1996). The anorectic effect of GLP-1 and OXM is also present in man (Schmidt et al. 2005). No effect of GLP-2 on feeding has not been fully established; however, central administration of both PYY 1–36 and PYY 3–36 has also been reported that peripheral administration of these peptides together with obesity (Wynne et al. 2004; Badman & Flier, 2005), whereas mice lacking the gastric inhibitory polypeptide receptor are protected against obesity induced by both a high-fat diet and leptin deficiency (ob/ob mice; Miyawaki et al. 2002). Thus, gastric inhibitory polypeptide may be involved in the development of obesity in response to high fat intake.

Ghrelin. Ghrelin is a twenty-eight-amino acid acylated hormone mainly synthesised and secreted by the gut in the gastric oxyntic cells (A/X cells) at the fundus of the stomach, as well as the duodenum, ileum, caecum and colon (Kojima et al. 1999; Date et al. 2000; Gualillo et al. 2001; Sakata et al. 2002). Ghrelin expression has also been detected in other tissues, such as the hypothalamus (Kojima et al. 1999; Horvath et al. 2001; Cowley et al. 2003) testis (Barreiro et al. 2002; Tena-Sempere et al. 2002), pituitary (Caminos et al. 2003a), ovary (Caminos et al. 2003b; Gaytan et al. 2003), heart (Iglesias et al. 2004) and placenta (Gualillo et al. 2001).

Ghrelin, which was initially identified as the endogenous ligand of the growth hormone secretagogue receptor (GHS-R), exerts a potent and specific growth hormone-releasing activity in vitro (Kojima et al. 1999) and in vivo (Arvat et al. 2000; Seoane et al. 2000), as well as increasing the transcription rate of the Pit-1 gene (Garcia et al. 2001). Further studies have led to the recognition that ghrelin also plays an important role in energy homeostasis. Ghrelin administration induces positive energy balance in rodents by decreasing fat utilisation without markedly changing energy expenditure or locomotor activity (Wren et al. 2000; Nakazato et al. 2001). Furthermore, peripheral and central administration of ghrelin to rodents increases feeding, as well as fat mass, and reduces fat utilisation (Tschop et al. 2000; Nakazato et al. 2001; Wren et al. 2001b; Seoane et al. 2003). Plasma levels of ghrelin are regulated by food intake, rising during fasting and immediately before meals, and falling after food intake (Ariyasu et al. 2001; Cummings et al. 2001; Tschop et al. 2001a). These changes in ghrelin expression are directly modulated by energy intake and nutritional signals such as blood glucose and ingestion of fat or carbohydrate (Tschop et al. 2000; Sakata et al. 2002). For this reason a physiological role of ghrelin in meal initiation has been proposed (Cummings et al. 2001; Cummings & Shannon, 2003). This suggestion is supported by experiments (Nakazato et al. 2001) that use anti-ghrelin antibodies to block the actions of ghrelin, which results in an attenuation of fasting-induced refeeding.

The effects of ghrelin on feeding and growth hormone secretion are mediated via type 1a GHS-R (Kojima et al. 1999; Tschop et al. 2000; Shen et al. 2004). However, the orexigenic effects of ghrelin are independent of growth hormone-releasing properties (Tschop et al. 2000; Shintani et al. 2001; Tamura et al. 2002). The expression of GHS-R in the hypothalamus is

Peptide YY. Peptide YY (PYY) is secreted post-prandially by the L cells of the gastrointestinal tract, especially in the most distal portions such as the ileum, colon and rectum; PYY secretion is correlated with energy intake (Stanley et al. 2005). There are two main forms of PYY in the circulation: PYY1–36 and PYY3–36 (Grandt et al. 1994; Batterham et al. 2002; Wynne et al. 2004; Stanley et al. 2005). Peripheral administration of PYY has several actions, including delaying gastric emptying and gastric secretion, and increasing ileum absorption. It has also been reported that peripheral administration of PYY3–36 inhibits food intake and reduces weight gain in rodents, primates and man (Batterham et al. 2002; Challis et al. 2003; Moran et al. 2005). PYY crosses the BBB and probably exerts its actions via the presynaptic Y2 receptor of NPY neurons in the ARC, releasing inhibition of pro-opiomelanocortin (POMC) neurons and consequently inhibiting feeding (Broberger et al. 1997; Batterham et al. 2002; Challis et al. 2003).

Despite this evidence, the anorectic effect of PYY3–36 is controversial and not easily duplicated (Tschop et al. 2004; Coll et al. 2004a). Indeed, in contrast to peripheral injection, central administration of both PYY1–36 and PYY3–36 stimulates feeding in rodents (Stanley et al. 1985; Clark et al. 1987; Hagan et al. 1998; Corpa et al. 2001). It has also been suggested that the anorexie effect of PYY may be partially mediated by an aversive response (Halatchev & Cone, 2005).

Bombesin. Bombesin is a peptide that is widely distributed in the mammalian gut. Plasma levels of bombesin increase markedly after food intake (Gibbs et al. 1979; Wynne et al. 2004), and peripheral and central administration of bombesin is anorectic (Gibbs et al. 1979; Smith et al. 1981b). Bombesin is structurally very similar to gastrin-releasing peptide and neuromedin B, and binds to their receptors. Additionally, a bombesin-3 receptor has been cloned (Ladenheim et al. 1997). Knocking out bombesin-5 receptor induces moderate hyperphagia, obesity and metabolic alterations in mice (Ohki-Hamazaki et al. 1997).
nutritionally regulated in a nucleus-specific manner, with fasting increasing the mRNA levels of GHS-R in the ARC but not in the ventromedial nucleus of the hypothalamus (VMH). Additionally, the level of GHS-R expression in the ARC, but not in the VMH, is reduced by leptin and increased by ghrelin in a growth hormone-dependent fashion (Fig. 1; Nogueiras et al. 2004).

Ghrelin is also important in the regulation of energy homeostasis in man. Intravenous administration of ghrelin to healthy volunteers increases food intake (Wren et al. 2001a). Moreover, the rise in preprandial ghrelin correlates with hunger scores in human subjects eating spontaneously (Cummings et al. 2004). Interestingly, the levels of ghrelin are correlated with adiposity in man, with an inverse relationship between plasma ghrelin levels and BMI (Tschop et al. 2001b). Obese human subjects show reduced levels of plasma ghrelin, which rise to normal after diet-induced weight loss (Hansen et al. 2002; Cummings et al. 2002b). Moreover, in obese individuals the postprandial regulation of ghrelin seems to be altered, which may be related to continuous food intake and/or obesity (English et al. 2002). Finally, the severe hyperphagia seen in patients with Prader-Willi syndrome is associated with elevated ghrelin levels, in contrast to other forms of obesity in which ghrelin levels are low (Cummings et al. 2002a).

In the CNS the action of ghrelin on feeding is mainly exerted via the ARC. GHS-R mRNA is expressed in neurons in the ARC co-expressing NPY and agouti-related peptide (AgRP; Guan et al. 1997; Tannenbaum et al. 1998; Willesen et al. 1999; Zigman et al. 2006), and the central administration of ghrelin increases the mRNA content of NPY and AgRP in the ARC in fed and fasting conditions (Kamegai et al. 2001; Nakazato et al. 2001; Seoane et al. 2003). There is also some evidence that orexin (also termed hypocretin; OX) neurons in the lateral hypothalamic area (LHA; Lawrence et al. 2002; Toshinai et al. 2003) and neurons in the NTS and the area postrema in the brainstem may mediate the orexigenic actions of ghrelin (Nakazato et al. 2001; Lawrence et al. 2002). Recent data also indicate that ghrelin acts in the hypothalamus by altering fatty acid metabolism and AMP-activated protein kinase. It has been demonstrated that ghrelin increases hypothalamic AMP-activated protein kinase phosphorylation levels, activating it. This action may be associated to specific changes in hypothalamic neuropeptides, although the exact molecular mechanisms and anatomical details of this interaction have not been fully identified (Andersson et al. 2004; Kola et al. 2005).

Despite ghrelin having a potent action in regulating food intake, both ghrelin-knock-out mice and mice lacking GHS-R type 1a have normal feeding patterns and body composition on a standard diet (Sun et al. 2003). However, on a high-fat diet the absence of ghrelin ( Wortley et al. 2005) or the ghrelin receptor (Zigman et al. 2005) protects

---

**Fig. 1.** Regulation of growth hormone secretogogue receptor (GHS-R) in the rat hypothalamus by leptin and ghrelin. Effects of intracerebroventricular leptin (■; a, b) and ghrelin (□; c, d) on GHS-R expression in the arcuate nucleus (a, c) and the ventromedial nucleus of the hypothalamus (b, d) in fed and 48 h-fasted rats. (□), Vehicle. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from the corresponding values for fed rats: *P*<0.05. Mean values were significantly different from the corresponding values for vehicle-fed or vehicle-48 h-fasted rats: †P<0.05, ††P<0.01, †††P<0.001.
against early-onset obesity; in both cases this reduced weight gain is associated with decreased adiposity and increased energy expenditure and locomotor activity. These data suggest that ghrelin, like leptin, may play an important role in the development of hypothalamic systems regulating energy balance (Grove & Cowley, 2005). Very interestingly, elimination of ghrelin improves the diabetic phenotype but not the obese phenotype of ob/ob mice (Sun et al. 2006).

Finally, it has recently been reported that obestatin, a new peptide derived from the ghrelin gene, inhibits food intake by acting through the orphan receptor GPR39 (Nogueiras & Tschop, 2005; Zhang et al. 2005). Despite this evidence there are some discrepancies in relation to the anorectic effect of obestatin (Nogueiras et al. 2006) as well as its binding to GPR39 (Holst et al. 2006). If the anorectic effect is confirmed, this finding could provide a new drug target for the treatment of obesity.

Adipose tissue hormones

Originally thought of as an inert tissue involved in the storage of energy, it is now clear that adipose tissue is an active endocrine organ (Casanueva & Diéguez, 1999; Ahima & Flier, 2000a). Adipocyte hormones regulate appetite, glucose homeostasis, lipid metabolism, endocrine function, cardiovascular physiology, reproduction, immune function and development, amongst other functions (Casanueva & Diéguez, 1999; Ahima & Flier, 2000a; Pinto et al. 2004; Horvath & Diano, 2004).

Leptin

Among the adipocyte hormones, the one that has most changed the concept of white adipose tissue as an inert tissue is leptin, the product of the ob (obese) gene (Zhang et al. 1994). Leptin is expressed principally in adipocytes (Zhang et al. 1994), but also at lower levels in the gastrointestinal tract (Bado et al. 1998) and placenta (Señarís et al. 1997; Masuzaki et al. 1997). Plasma leptin levels reflect both energy stores and acute energy balance. Circulating leptin levels are tightly correlated with adipose tissue mass (Maffei et al. 1995), and food restriction results in suppression of circulating leptin (Frederich et al. 1995; Maffei et al. 1995), which can be reversed by refeeding or insulin administration. Peripheral and central leptin administration reduces spontaneous and fasting-induced hyperphagia (Ahima et al. 1996; Ahima, 2000), and chronic peripheral administration reduces feeding, resulting in loss of fat mass and body weight (Halaas et al. 1995).

The complete lack of leptin seen in the ob/ob mouse has profound consequences on body-weight homeostasis, leading to hyperphagia and obesity, as well as neuroendocrine and immune dysregulation, which is normalised by leptin administration (Campfield et al. 1995; Halaas et al. 1995; Pellemounter et al. 1995). In man leptin deficiency causes morbid obesity and hypogonadism (Montague et al. 1997; Strobel et al. 1998), which can be improved by recombinant leptin (Farooqi et al. 1999; Licinio et al. 2004). In the same way, defective leptin receptor signalling also has a profound impact on body weight and endocrine function. A point mutation in the intracellular domain of the long isoform of the leptin receptor (OB-Rb) gene, which prevents signalling, results in obesity in db/db mice (Chen et al. 1996; Lee et al. 1996). Defects in the human leptin receptor have also been reported; as with leptin deficiency, these subjects have hypogonadism and early-onset morbid obesity (Clement et al. 1998, 2002).

Leptin binds and activates a receptor of the cytokine receptor family (Tartaglia et al. 1995). Alternative mRNA splicing and post-translational processing results in several isoforms of the receptor (OB-Ra, OB-Rb, OB- Rc and OB-Rf; Tartaglia, 1997; Chua et al. 1997; Ahima & Flier, 2000b). OB-Rb is the variant implicated in signal transduction (Tartaglia, 1997; Ahima & Flier, 2000b). The other isoforms may act as leptin sequesters and transporters, binding leptin without signal transduction (Friedman & Halaas, 1998; Ahima & Flier, 2000b). Ob-Rb possesses a long intracellular domain that binds to janus kinases (Lee et al. 1996) and to signal transducer and activator of transcription-3 transcription factors (Vaisse et al. 1996; Hakansson et al. 1999), resulting in signal transduction and mediating the action of leptin on feeding (Lee et al. 1996). Activation of the janus kinases/signal transducers and activators of transcription pathway induces expression of suppressor of cytokine signalling-3, a cytokine-inducible inhibitor of signalling; suppressor of cytokine signalling-3 expression is up regulated by leptin in hypothalamic nuclei expressing the Ob-Rb receptor (Ahima & Flier, 2000b; Howard et al. 2004).

Plasma leptin crosses the BBB via a saturable process (Banks et al. 1996; Banks 2001a,b), thought to be mediated by OB-Ra and OB- Rc (El Haschimi et al. 2000; Ahima & Flier, 2000b). OB-Rb is widely expressed in the hypothalamus (being most abundant in the ARC, the VMH and the DMH) the LHA and the medial preoptic area (Fei et al. 1997; Elmqquist et al. 1998; Hakansson et al. 1998, 1999). OB-Rb is also expressed in feeding-modulating neurons in the brainstem (Elmqquist et al. 1997; Mercer et al. 1998). In the ARC OB-Rb mRNA is expressed by the two major neuronal groups: neurons co-expressing the orexigenic neuropeptides NPY and AgRP (Mercer et al. 1996; Cheung et al. 1997); a distinct second population of neurons co-expressing the anorexigenic CART/POMC and cocaine- and amphetamine-regulated transcript (CART). Leptin inhibits the activity of orexigenic AgRP/NPY neurons and reduces expression of AgRP and NPY (Stephens et al. 1995; Hahn et al. 1998; Elias et al. 1999), while activating anorectic CART/POMC neurons (Schwartz et al. 1997; Kristensen et al. 1998; Swart et al. 2002). In the LHA leptin receptor is expressed in neurons expressing the orexigenic neuropeptides melanin-concentrating hormone (MCH) and the OX, which are inhibited by leptin (Qu et al. 1996; López et al. 2000). When leptin levels are low, such as in food restriction and fasting, the expression of orexigenic neuropeptides is increased and orexigenic neurons are activated; in contrast, anorexigenic neuropeptides are decreased and anorexigenic neurons are inhibited. When plasma leptin levels are high, as in the satiated animal, the anorectic pathways are switched on and the
orexigenic pathways are switched off (Friedman & Halaas, 1998; Kalra et al. 1999; Saper et al. 2002; Flier, 2004).

The role of leptin in human obesity is intriguing. As described earlier, while there are individuals with defects in leptin synthesis or leptin signalling, these cases are extremely rare. The majority of obese individuals are characterised by high levels of leptin (Maffei et al. 1995; Considine et al. 1996), suggesting leptin insensitivity or resistance; in fact, leptin administration to obese subjects has only a moderate effect on body weight (Heymsfield et al. 1999; Fogteloo et al. 2003). In rodents diet-induced obesity has also been correlated with the development of leptin resistance (Van Heek et al. 1997; Levin & Dunnett, 2002). Leptin resistance may develop via different mechanisms. Peripher al leptin resistance may be the result of impairment in the function of the saturable leptin transporters in the BBB (Burguera et al. 2000; Furuhata et al. 2000; Levin et al. 2004). Central leptin resistance may develop as a result of impaired leptin signalling via OB-Rb in the hypothalamus, which could be related to a decrease in OB-Rb expression (Garcia et al. 2000; Seeber et al. 2002; Lopez et al. 2005a), a defect in the intracellular signalling mechanism of the Janus kinases/signal transducers and activators of transcription pathway or the over-expression of suppressor of cytokine signalling-3 (El Haschimi et al. 2000; Howard et al. 2004; Ladyman & Gratten, 2004; Levin et al. 2004; Munzberg et al. 2004; Munzberg & Myers, 2005).

The role of leptin in the hypothalamus is not only associated with food-intake regulation. Leptin also contributes to the adaptation of the neuroendocrine axis to fasting (Ahima et al. 1996; Casanueva & Dieguez, 1999). Additionally, leptin is a neurotrophic factor during the development of the hypothalamus, mediating neuronal plasticity (Bouret et al. 2004a,b; Bouret & Simerly, 2004; Pinto et al. 2004). The importance of this function of leptin in the context of obesity is still not clear, but it has been proposed that perturbations in perinatal nutrition that alter leptin levels may have long-term consequences for the formation and function of hypothalamic circuits regulating feeding and body weight in adulthood (Lopez et al. 2005a).

**Adiponectin**

Adiponectin, also termed adipocyte complement-related protein, apM1 or adipQ, is a 244-amino acid protein secreted from adipose tissue (Hu et al. 1996; Berg et al. 2002; Tsao et al. 2002), the placenta (Caminos et al. 2005) and cardiomyocytes (Pineiro et al. 2005). Adiponectin has four domains: an amino-terminal signal sequence; a region without homology to other known proteins; a collagen-like region; a carboxy-terminal globular domain. The globular domain forms homotrimers, and additional interactions with collag enous segments cause the formation of higher-molecular-weight complexes (Pajvani et al. 2003).

Adiponectin is important in the regulation of energy homeostasis (Scherer et al. 1995). Plasma levels of adiponectin are inversely correlated with adiposity in several species, including man (Hu et al. 1996; Arita et al. 1999; Hotta et al. 2001) Adiponectin is increased after food restriction in rodents (Berg et al. 2001, 2002). Peripheral administration to rodents has been shown to attenuate body-weight gain, by increased O2 consumption, without affecting food intake (Berg et al. 2001; Fruebis et al. 2001; Yamauchi et al. 2003). This effect on energy expenditure appears to be mediated by the hypothalamic melanocortin system, without affecting other neuropeptide systems regulated by leptin (Qi et al. 2004). Circulating adiponectin levels negatively correlate with insulin resistance (Hotta et al. 2001), and treatment with adiponectin can reduce body-weight gain, increase insulin sensitivity and decrease lipid levels in rodents (Berg et al. 2001; Yamauchi et al. 2001; Qi et al. 2004; Winzell et al. 2004). Adiponectin-knock-out mice have severe diet-induced insulin resistance (Maeda et al. 2002). The mechanism by which adiponectin improves insulin resistance and glucose metabolism is not fully understood, but some of these effects may be mediated by activation of AMP-activated protein kinase (Yamauchi et al. 2002).

Adiponectin binds and activates two known membrane receptors, adipoR1 and adipoR2 (Yamauchi et al. 2003). AdipoR1 is highly expressed in skeletal muscle; it has a high affinity for the globular domain of adiponectin and low affinity for the full-length ligand. AdipoR2 is highly expressed in the liver and preferentially binds to the full-length ligand. Adiponectin receptors have also been detected in the hypothalamus (Qi et al. 2004) and the placenta (Caminos et al. 2005). Very interestingly, it has recently been reported that adiponectin does not cross the BBB but modifies cytokine expression in the brain endothelial cells, making unlikely a direct effect of adiponectin in the CNS (Spranger et al. 2006).

**Resistin**

Resistin is produced by adipose tissue and appears to be involved in the modulation of insulin sensitivity and adipocyte differentiation (Steppan et al. 2001a,b; Vidal-Puig & O’Rahilly, 2001; Steppan & Lazar, 2002). In addition to adipose tissue, resistin is also expressed in the stomach, intestine, adrenal gland, testis and skeletal muscle (Nogueiras et al. 2003a,b, 2004a).

Resistin expression is regulated in a tissue- and gender-specific manner. Food deprivation leads to a decrease in resistin mRNA expression only in adipose tissue (Nogueiras et al. 2003a,b). Circulating resistin is increased in obese rodents (Steppan et al. 2001a) and man (Savage et al. 2001) and falls after weight loss in man (Valsamakis et al. 2004). Resistin-knock-out mice display increased glucose tolerance on a high-fat diet (Banerjee et al. 2004; Sul, 2004) and transgenic mice over-expressing a dominant negative form of resistin show increased adiposity with elevated plasma leptin and adiponectin levels, as well as enhanced glucose tolerance and insulin sensitivity (Sul, 2004). All this evidence suggests that resistin may contribute to the development of insulin resistance and diabetes in obesity (Steppan et al. 2001a,b; Vidal-Puig & O’Rahilly, 2001; Steppan & Lazar, 2002). In support of this role, recent evidence has shown that resistin inhibits feeding through a hypothalamic mechanism (Tovar et al. 2004).
IL-6 and IL-1

IL-6 is a multifunctional immune-modulating cytokine that has been suggested to have important functions in glucose and lipid metabolism. IL-6 is secreted from adipose tissue into the circulation, and its expression is positively correlated with BMI and total fat tissue. IL-6-knock-out mice develop obesity, which can partly be reversed by IL-6 replacement, suggesting a role for IL-6 in the long-term regulation of adipose tissue mass (Wallenius et al. 2002b). Furthermore, central administration of a low dose of IL-6 decreases feeding and increases energy expenditure in rats, suggesting a central site of action for IL-6 (Wallenius et al. 2002a). Supporting this hypothesis it has also been suggested that IL-6 and IL-6 receptors are expressed in the neurons in the VMH and the DMH (Schobitz et al. 1993). IL-1 is also involved in body-weight homeostasis. IL-1 type I receptor-knock-out mice display an obese and insulin-resistant phenotype. This obese phenotype is characterised by a decrease in leptin sensitivity, fat utilisation and locomotor activity (Garcia et al. 2006).

Pancreatic hormones

Insulin

Insulin is also an adiposity signal. Plasma insulin concentrations correlate with peripheral insulin sensitivity, which in turn is linked to total body fat depots and fat distribution, visceral fat being a key determinant (Schwartz et al. 1992a, 2000).

Insulin secretion by the pancreas increases rapidly after a meal, exerting an anorectic effect via the CNS (Schwartz et al. 1992a, 2000). Insulin enters the CNS via saturable receptor-mediated transport across the BBB (Woods et al. 2003). Central administration of insulin or insulin mimetic reduces feeding and body weight in rodents and primates (Woods et al. 1979; Schwartz et al. 1992a, 2000; Air et al. 2002). Administration of antisense RNA against the insulin receptor induces hyperphagia and increased fat mass (Obici et al. 2002a), and neuron-specific deletion of the insulin receptor results in obesity, hyperinsulinaemia and dyslipidaemia in mice (Bruning et al. 2000). Insulin receptors are widespread in the brain and occur in hypothalamic nuclei involved in food intake (ARC, DMH, PVH and periventricular nucleus; Corp et al. 1986; Marks et al. 1990). In the hypothalamus the actions of insulin on food intake and body weight are mediated by NPY (Schwartz et al. 1992b) and the melanocortin system (Sipols et al. 1995; Obici et al. 2001; Benoit et al. 2002).

Pancreatic polypeptide

Pancreatic polypeptide (PP) belongs to the PP-fold family of peptides, which also includes PYY and NPY (Conlon, 2002). PP is mainly produced by peripheral cells of the islets of Langerhans, the exocrine pancreas and the distal gastrointestinal tract (Small & Bloom, 2004; Stanley et al. 2005). Plasma PP concentrations increase proportionally to energy intake (Small & Bloom, 2004; Stanley et al. 2005), and they appear to be inversely proportional to adiposity, with high levels in anorexic subjects and reduced levels in obese subjects (Lassmann et al. 1980; Fujimoto et al. 1997).

Peripheral PP administration reduces feeding and body weight in obese rodents (Malaisse-Lagae et al. 1977) and feeding in man (Batterham et al. 2003). The anorectic effect of PP is exerted via brainstem pathways (in the area postrema), regulation of hypothalamic neuropeptides (NPY and OX) and modulation of ghrelin expression (Asakawa et al. 2003). The anorectic effect of PP is mediated by Y₅ receptor. In contrast to the peripheral actions, central administration of PP increases food intake (Clark et al. 1984); the receptors mediating this action and the mechanisms involved are unclear.

Neural control of food intake

Hypothalamic regulation of food intake

The CNS receives information from the sensory experience of eating and also from the process of ingestion, absorption, metabolism and energy storage. The original theories explaining the central control of food intake were based on a ‘dual-centre hypothesis’ (Hecherington & Ranson, 1942; Anand & Brobeck, 1951). In this model, based on hypothalamic-lesioning experiments, feeding is controlled by two hypothalamic areas: the lateral hypothalamic ‘feeding centres’ and the ventromedial hypothalamic ‘satiety centres’. Lesions of the LHA decrease food intake and eventually lead to starvation and death. Conversely, lesions of several of the mediobasal hypothalamic nuclei result in obesity. Since then, knowledge concerning the hypothalamic regulation of feeding has increased; however, the main concept is the same, i.e. that anatomically-defined hypothalamic areas regulate food intake. These hypothalamic nuclei form interconnected neuronal circuits that respond to changes in energy status by altering the expression of specific neuropeptides, resulting in changes in energy intake and expenditure (Friedman & Halaas, 1998; Kalra et al. 1999; Schwartz et al. 2000; Saper et al. 2002; Flier, 2004; Abizaid et al. 2006; Morton et al. 2006). Table 1 summarises some hypothalamic neuropeptides and neurotransmitters regulating food intake.

Hypothalamic neuronal pathways regulating appetite

Arcuate nucleus. The ARC is considered as the ‘master hypothalamic centre’ for feeding control. It is situated around the base of the third ventricle and lies immediately above the median eminence. The ARC–median eminence is a circumventricular organ in which the BBB is modified, allowing the entry of peptides and proteins from the circulation, such as PYY, GLP-1, leptin and insulin (Banks et al. 1996; Kastin et al. 2002; Nonaka et al. 2003; Woods et al. 2003).

Two distinct neuronal populations in the ARC integrate peripheral nutritional and/or feeding signals. One set of neurons in the ventromedial part of the ARC express the
orexigenic neuropeptides NPY and AgRP (Broberger et al. 1998b; Hahn et al. 1998). These neurons mostly project to the PVH. In the ventrolateral part of the ARC there is a second population of neurons that express the anorexigenic products of POMC, the precursor of \( \alpha \)-melanocyte-stimulating hormone, and also CART (Elias et al. 1998a; Kristensen et al. 1998). This set of neurons projects more broadly within the CNS to hypothalamic nuclei such as the DMH, the LHA and the perifornical area, as well as the PVH. Thus, AgRP/NPY and CART/POMC neurons act as the primary hypothalamic site of action of peripheral hormones such as insulin and leptin. ARC neurons, in turn, project to secondary hypothalamic nuclei (‘second order neurons’) such as the PVH and the LHA. In these second-order neurons the release of neuropeptides is regulated to modulate energy intake (Schwartz et al. 2000; Flier, 2004).

Paraventricular nucleus. The PVH integrates neuropeptide signals from numerous CNS regions, including the ARC and brainstem (Sawchenko & Swanson, 1983). Administration into the PVH of almost all of the known orexigenic and anorexigenic signalling molecules alters appetite (Kalra et al. 1999). Furthermore, CART/POMC neurons originating in the ARC potentiate inhibitory \( \gamma \)-aminobutyric acidergic signalling in the PVH and reduce feeding, while AgRP/NPY neurons inhibit this \( \gamma \)-aminobutyric acidergic signalling and stimulate food intake (Cowley et al. 1999). Despite the large number of neuropeptides acting on the PVH, recent work suggests that they act to regulate feeding through a common mechanism involving AMP-activated protein kinase (Minokoshi et al. 2004).

The PVH also plays a major role in the integration of food intake and neuroendocrine function. AgRP/NPY and CART/POMC neurons in the ARC project to thyrotrophin-releasing hormone neurons in the PVH (Legradi & Lechan, 1999; Fekete et al. 2000). AgRP/NPY inhibits pro-thyrotrophin-releasing hormone gene expression (Fekete et al. 2002), while \( \alpha \)-melanocyte-stimulating hormone stimulates pro-thyrotrophin-releasing hormone expression and inhibits the fasting-induced suppression of thyrotrophin-releasing hormone (Fekete et al. 2000). The PVH also contains corticotrophin-releasing hormone neurons, which form reciprocal circuits with NPY neurons in the ARC (Kalra et al. 1999).

Ventromedial nucleus of the hypothalamus. The VMH (as distinct from thalamic ventromedial nucleus) has long been considered as the ‘satiety centre’, since the finding that bilateral lesions in this nucleus induce hyperphagia and obesity (Hecherington & Ranson, 1942; Anand & Brobeck, 1951). The VMH mainly receives projections from AgRP/NPY and CART/POMC neurons in the ARC. Additionally, the VMH neurons project their axons to the ARC, DMH and LHA, as well as brainstem regions such as the NTS (Kalra et al. 1999; Pinto et al. 2004; Sternson et al. 2005).

The VMH has been considered as a ‘reception nucleus’ for peripheral signals, as well as central signals. VMH neurons show a high abundance of leptin, ghrelin, oestrogen, thyroid hormone and neuropeptide receptors (Shughrue et al. 1997; Roselli et al. 1997; Kalra et al. 1999; Nogueiras et al. 2004b; King, 2006). However, despite the identification of these receptors, the molecular mechanisms regulating feeding in the VMH have not yet been well
established. Some evidence suggests that brain-derived neurotrophic factor and steroidogenic factor-1 may play crucial roles in mediating body weight in this nucleus. Mice with reduced brain-derived neurotrophic factor receptor expression (Xu et al. 2003), and with reduced brain-derived neurotrophic factor signalling (Xu et al. 2003), and also steroidogenic factor-1 knock-out mice (Majdic et al. 2002), have increased body weight. Furthermore, activation of steroidogenic factor-1 neurons by leptin is required for normal body-weight homeostasis (Dhillon et al. 2006). Finally, it has recently been reported that fatty acid synthase (FAS) and malonyl-CoA levels in this nucleus may play an important physiological role in the regulation of feeding (López et al. 2006).

**Dorsomedial nucleus of the hypothalamus.** Like the VMH, the DMH has long been considered to be an integrative centre, processing information from other hypothalamic areas (Kalra et al. 1999). The DMH is located immediately dorsal to the VMH and has extensive direct connections with other hypothalamic nuclei (e.g. the PVH and the LHA), as well as the brainstem (Kalra et al. 1999; Bellinger & Bernardis, 2002). Destruction of the DMH induces hyperphagia and obesity, although less dramatically than VMH lesions (Bellinger & Bernardis, 2002). Very recent evidence has also demonstrated that the DMH is critical for the expression of food-entrainable circadian rhythms (Gooley et al. 2006).

The DMH contains NPY-expressing cell bodies, which are involved in the hyperphagia observed in pregnant and lactating rats (Li et al. 1998; García et al. 2003). Moreover, CART-expressing neurons are highly abundant in the DMH; the exact function of these cells is unknown, but they are probably involved in fasting-induced responses (Henry et al. 2001).

**Lateral hypothalamic area.** Although the involvement of the LHA, including the perifornical area, in the regulation of feeding has been known for >60 years (Hecherington & Ranson, 1942; Anand & Brobeck, 1951), the molecular mechanisms involved had remained unknown until 15 years ago when MCH was identified as the first orexigenic peptide exclusively expressed in this nucleus (Qu et al. 1996). Other neuropeptides important in the regulation of feeding are also highly expressed in this area, such as galanin (Hakansson et al. 1998), dynorphin (Chou et al. 2001), CART-encoded peptides (Koylu et al. 1998) and OX (de Lecea et al. 1998; Sakurai et al. 1998; López et al. 2005b).

MCH and prepro-OX are each expressed by a different cell population, both of which receive projections from AgRP/NPY and CART/POMC neurons in the ARC (Broberger et al. 1998a; Elias et al. 1998b; Horvath et al. 1999). Additionally, both sets of neurons express leptin receptors, indicating that their actions may be integrated (Hakansson et al. 1998, 1999). The LHA also contains a large number of glucose-sensing neurons (Bernardis & Bellinger, 1996). OX neurons in the LHA respond to a fall in glucose levels with an increase in activity (Cai et al. 1999; Moriguchi et al. 1999). LHA neurons project widely to a large number of extrahypothalamic areas. Major targets of the MCH and OX neurons include the brainstem motor systems, sympathetic and parasympathetic preganglionic nuclei in the medulla and spinal cord, the locus coeruleus, the medial raphe nucleus, the tuberomammillary nucleus and the cerebral cortex. All these areas are fundamental in different aspects of food intake regulation, from feeding-related behaviours to arousal and motor activity (Willie et al. 2001; López et al. 2001a, 2005b; Saper et al. 2002; Steininger et al. 2004). Thus, these second-order neurons in the LHA play a fundamental role in integrating information from ARC neurons before sending it to other CNS areas involved in feeding control.

**Hypothalamic neuropeptides regulating food intake**

Neuropeptide Y. NPY is a thirty-six-amino acid peptide belonging to the PP-fold family of peptides, which also includes PYY and PP (Conlon, 2002). NPY is widely distributed in the CNS and is one of the most potent stimulators of food intake; repeated third ventricle or PVH administration of NPY induces striking hyperphagia and obesity (Stanley et al. 1986; Zarjevski et al. 1993). Central administration of NPY also reduces brown fat thermogenesis (Billington et al. 1991), suppresses sympathetic nerve activity (Egawa et al. 1991) and inhibits the thyroid axis (Fekete et al. 2002) in order to reduce energy expenditure. Additionally, NPY induces hyperinsulinaemia (Moltz & McDonald, 1985; Zarjevski et al. 1993), hypocortico-steronaemia (Zarjevski et al. 1993) and reduced plasma testosterone levels (Kalra et al. 1999); effects that are independent of increased food intake. NPY mRNA levels and NPY release in the ARC respond to changes in energy status, being increased after fasting and food restriction and decreased after refeeding (Sanacora et al. 1990; Kalra et al. 1991; Swart et al. 2002).

Regardless of its potent orexigenic effect, NPY-knock-out mice show normal body weight and adiposity (Erickson et al. 1996), probably related to a compensatory and redundant mechanism in the orexigenic pathways, particularly in relation to AgRP. However, it has been reported recently that selective ablation of AgRP/NPY neurons in adult mice results in hypophagia and leanness, demonstrating direct evidence for a critical role of these neurons in the regulation of energy homeostasis (Gropp et al. 2005; Luquet et al. 2005).

NPY, as part of the PP-fold family of peptides, binds and activates G-protein-coupled receptors termed Y<sub>1</sub>–Y<sub>5</sub> (Larhammar, 1996; Kalra et al. 1999). Y<sub>1</sub>–Y<sub>5</sub> receptors are present in rat brain, but Y<sub>4</sub> is only active in mice, being absent in rats and inactive in primates (Inui, 1999). The orexigenic action of NPY is thought to be mediated by hypothalamic Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>4</sub> and Y<sub>5</sub> receptors (Kalra et al. 1999; Williams G et al. 2000, 2001; Stanley et al. 2005).

**Melanocortin system (α-melanocyte-stimulating hormone/pro-opiomelanocortin and agouti-related peptide).** Among the hypothalamic neuropeptide systems that regulate feeding, melanocortins play a prominent role (Kalra et al. 1999; Cone, 1999, 2005; Coll et al. 2004b). The central melanocortin system modulates energy homeostasis through the anorectic actions of the agonist α-melanocyte-stimulating hormone (a POMC cleavage product) and the endogenous orexigenic antagonist AgRP (Kalra et al. 1991).
Five melanocortin receptors (MCnR, n 1–5) have been identified. The feeding-related effects of both α-melanocyte-stimulating hormone and AgRP are mediated via MC3R and MC4R. Both receptors are widely expressed in the hypothalamus and are found in the ARC, VMH and PVH (Kalra et al. 1999; Cone, 1999, 2005; Coll et al. 2004b). MCH-knock-out mice are hypophagic and lean (Shimada et al. 1998). Finally, the double knock-outs leptin/MCH have lower weight gain and adiposity compared with leptin-deficient ob/ob mice (Segal-Lieberman et al. 2003), suggesting that MCH is a downstream mediator of leptin effects on feeding. Finally, MCH receptor1-knock-out mice display a lean phenotype as a result of increased energy expenditure (Marsh et al. 2002).

**Orexins.** The OX (OX-A and OX-B), or hypocretins (hypocretins 1 and 2), are neuropeptides derived from the common precursor prepro-OX (also called prepro-hypocretin) expressed in the LHA/perifornical area (de Lecea et al. 1998; Sakurai et al. 1998). Two different OX receptors have been cloned, termed OX 1 receptor (or hypocretin receptor 1) OX 2 receptor (or hypocretin receptor 2). Although OX expression in the brain is only located in the LHA–perifornical area (Broberger et al. 1998b; Elias et al. 1998b; Horvath et al. 1999), OX receptors show a widespread distribution in the CNS, with high levels of abundance in some hypothalamic nuclei (ARC, DMH, LHA, PVH and VMH; Marcus et al. 2001; Backberg et al. 2002). OX receptors are also present in the adrenal gland (López et al. 1999), pituitary (Blanco et al. 2001) gut (Kirchgessner & Liu, 1999), testis, kidney, ovary and placenta (Johren et al. 2001).

**OX** are important regulators of the sleep–wake cycle and the absence of OX signalling causes narcolepsy (Willie et al. 2001; Taheri et al. 2002; Sutcliffe & de Lecea, 2002). However, evidence also links OX to endocrine function (López et al. 1999, 2001b, 2004, 2005b; Barreiro et al. 2004; Seoane et al. 2004) and food intake regulation (Sakurai et al. 1998; López et al. 2001a, 2005b). Central administration of OX to rats stimulates feeding via a NPY-dependent mechanism (Sakurai et al. 1998; Dube et al. 2000; Ida et al. 2000; Jain et al. 2000; Yamanaka et al. 2000; López et al. 2002). Other evidence has linked the feeding actions of OX-A to opioids, corticotrophin-releasing hormone (Ida et al. 2000), urocortin and melanocortins (Wang & Kotz, 2002). Finally, prepro-OX-knock-out mice (Willie et al. 2001) and the OX/ataxin-3 transgenic mice in which OX-containing neurons are ablated (Hara et al. 2001) are hypophagic.

**OX** neurons are also responsive to peripheral signals regulating food intake. The expression of prepro-OX is increased in fasting and restored to normal by leptin (Sakurai et al. 1998; López et al. 2000; Zhu et al. 2002; Yamanaka et al. 2003). OX neurons in the lateral hypothalamus are also sensitive to glucose, being activated during hypoglycaemia (Cai et al. 1999; Griffond et al. 1999; Moriguchi et al. 1999). It has been also proposed that visceral feeding-related signals regulate OX actions, which are thought to act via the vagus nerve and the NTS. Thus, stimuli acting as ‘terminate-eating’ signals, such as gastric distension and glucose concentrations in the portal vein, appear to be important in the regulation of OX (Cai et al. 2001, 2002).

Despite evidence supporting the orexigenic effect of OX, it has been proposed (Hagan et al. 1999) that these effects are secondary, and are related to the state of arousal and vigilance necessary for normal feeding. However,
Fig. 2. Fatty acid synthesis pathway. Fatty acid synthesis is catalysed by acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) in the cytoplasm. ACC catalyses the carboxylation of acetyl-CoA to malonyl-CoA. Acetyl-CoA and malonyl-CoA can be used as the substrates for the production of palmitate by the seven enzymic reactions catalysed by FAS. The synthesis step of malonyl-CoA is a reversible regulated mechanism, and malonyl-CoA decarboxylase (MCD) converts malonyl-CoA back to acetyl-CoA. The inhibition of FAS (by using cerulenin, C75 or tamoxifen) increases the levels of malonyl-CoA in the hypothalamus, altering the concentration of long-chain fatty acid (LCFA)-CoA, which reduces feeding. The link between this effect and the neuropeptide changes is unknown. POMC, pro-opiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; AgRP, agouti-related peptide; NPY, neuropeptide Y.

OX-A-induced feeding that is independent of arousal activation has been reported (Kotz et al. 2002).

Cocaine- and amphetamine-regulated transcript. CART is the third-most-abundant transcript in the hypothalamus and is expressed in the ARC, DMH, LHA and PVH (Kristensen et al. 1998; Elias et al. 2001; Hunter et al. 2004). Food deprivation decreases ARC expression of CART, while peripheral leptin treatment in ob/ob mice increases CART expression (Kristensen et al. 1998). Central administration of CART-(1–102) and CART-(82–103) inhibits feeding (Kristensen et al. 1998) and CART-knockout mice display a predisposition to become obese on a high-fat diet (Asnicar et al. 2001), an age-related increase in body weight and impaired glucose metabolism (Wierup et al. 2005), supporting the role of CART in the hypothalamic mechanism regulating food intake.

Lipid sensing in the hypothalamus

Although circulating lipids have for some time been hypothesised as signalling molecules that inform the hypothalamus of metabolic status, this function has only recently been definitively demonstrated. Following an elegant experimental approach Rossetti and colleagues (Obici et al. 2002b; Morgan et al. 2004) have shown that central administration of long-chain fatty acids such as oleic acid inhibits food intake via AgRP/NPY neurons in the ARC; this effect is not produced by medium-chain fatty acids. The physiological relevance of these data is intriguing. Since circulating NEFA can access the brain it is likely that the anorectic action of long-chain fatty acids may play an important role in the regulation of energy balance by acting as a ‘nutrient abundance’ signal (Lam et al. 2005a,b). Impairment of hypothalamic lipid-sensing in rats induces obesity (He et al. 2006), as well alterations in plasma glucose (Pocai et al. 2006), indicating that this mechanism may be important in the physiological regulation of metabolism and body-weight homeostasis.

Fatty acid synthesis pathway in the hypothalamus

Recent reports demonstrate that the enzymes of the fatty acid synthesis pathway (Fig. 2) are expressed in the hypothalamus. Acetyl-CoA carboxylase, FAS and malonyl-CoA decarboxylase mRNA and proteins have been detected in the ARC, DMH, PVH and VMH (Kim et al. 2002; López et al. 2006). The anatomical location of these enzymes suggests that they may play a role in the hypothalamic mechanism regulating feeding. This notion is further supported by evidence demonstrating that peripheral and central administration of the FAS inhibitors cerulenin, C75 and tamoxifen reduces food intake and body weight through a malonyl-CoA-dependent mechanism (Loftus et al. 2000; Hu et al. 2003; Lelliott et al. 2005; López et al. 2006). The anorectic action of FAS inhibitors is linked to decreased expression of AgRP/NPY and elevated expression of CART/POMC in the neurons of the ARC, although the molecular mechanisms of this interaction have not yet been completely defined (Loftus et al. 2000; Shimokawa et al. 2002; López et al. 2006). Additionally, it has been demonstrated that nutritional status regulates hypothalamic malonyl-CoA levels and FAS expression in a nucleus-specific manner, with FAS mRNA levels down regulated by fasting and up regulated by refeeding, an effect specific to the VMH (Fig. 3; López et al. 2006). This evidence suggests that the regulation of FAS could be a physiological mechanism of food-intake control and that the increase in malonyl-CoA induced by FAS inhibition may act as central lipid-sensing signal.

Brainstem regulation of food intake

The brainstem plays an essential role in the regulation of body-weight homeostasis. The NTS is anatomically close to the area postrema, a circumventricular organ, like the ARC, with a partial BBB (Stanley et al. 2005). Consequently, the NTS is in a perfect location to receive peripheral circulating signals, as well as vagal afferents from the gastrointestinal tract and the glossopharyngeal nerves (Kalia & Sullivan, 1982; Sawchenko, 1983).

The NTS contains GLP-1, NPY and melanocortin neuronal circuits. GLP-1-expressing neurons comprise the main brainstem circuit modulating feeding. In the CNS GLP-1 is only synthesised in the caudal NTS. GLP-1 fibres project to the PVH and DMH, with fewer projections to the ARC (Stanley et al. 2005). Central administration of GLP-1 into either the third ventricle or the fourth ventricle reduces food intake, and treatment with the GLP-1 receptor...
antagonist exendin (9-39) increases appetite (Turton et al. 1996). This finding suggests a role for endogenous GLP-1 in energy homeostasis. NPY neurons from the brainstem project to the PVH (Sawchenko et al. 1985), and extracellular NPY levels within the NTS are nutritionally-regulated (Yoshihara et al. 1996). Y_1 and Y_5 receptors are also located in the NTS (Harfstrand et al. 1986; Dumont et al. 1998; Glass et al. 2002). POMC-derived peptides are synthesised in the NTS of the rat (Kawai et al. 1984; Bronstein et al. 1992; Fodor et al. 1996). Brainstem POMC neurons are activated by feeding and also by CCK treatment (Fan et al. 1997). MC4R are also expressed in the NTS (Mountjoy et al. 1994) and act to modulate energy intake. Fourth-ventricle administration of a MC3R/MC4R agonist decreases food intake, and MC3R/MC4R-antagonist administration to these areas increases food intake (Williams DL et al. 2000).

Prolactin-releasing peptide is expressed in the NTS, in addition to the hypothalamic DMH (Lee et al. 2000). Prolactin-releasing peptide expression is reduced in fasting, and central administration of prolactin-releasing peptide decreases appetite by a corticotrophin-releasing hormone- and CCK-mediated mechanism (Seal et al. 2001; Ellacott et al. 2002; Lawrence et al. 2004). Chronic administration of prolactin-releasing peptide does not affect food intake (Ellacott et al. 2003), suggesting a role in short-term appetite regulation instead of long-term control of body weight.

**Reward and regulation of food intake**

Even in the absence of an energy deficit, the rewarding nature of food may act as a stimulus to feeding. Several signals are able to modulate reward pathways.
The reward circuitry is complex, involving interactions between several signalling systems, including the opioid, dopaminergic and endocannabinoid (EC) systems (Cota et al. 2003a,b; Flier, 2004; Di Marzo & Matias, 2005; Lichtman & Cravatt, 2005; Fulton et al. 2006; Hommel et al. 2006).

**Opioids.** Opioids play an important role in the regulation of feeding. The anatomical site for opioid action is the nucleus accumbens (Zhang & Kelley 2000; Zhang et al. 2003). Mice lacking enkephalin or β-endorphin lose the reinforcing property of food, despite the palatability. This effect is overridden after fasting, indicating that homeostatic mechanisms can override the hedonistic pathway (Hayward et al. 2002). In man opiate antagonists decrease food palatability without altering subjective hunger (Yeomans et al. 1990; Drewnowski et al. 1992).

**Endocannabinoids.** The effect of marijuana (Cannabis sativa) to increase appetite has been known for many years (Di Marzo & Matias, 2005; Lichtman & Cravatt, 2005). The primary constituent of cannabis is Δ9-tetrahydrocannabinol; this molecule, along with other naturally-occurring and synthetic cannabinoids (CB), binds to two separate G-protein-coupled receptors: the CB1 receptor, which is located in the CNS and periphery; the CB2 receptor, which is primarily found in cells of the immune system (Matsuda et al. 1990; Devane et al. 1992; Munro et al. 1993). These receptors also bind endogenous ligands, the EC, which include the fatty acid amide N-arachidonoyl ethanolamine (anandamide) and the monocacylglycerol 2-arachidonoylglycerol (Di Marzo et al. 2001; Cota et al. 2003a; Di Marzo & Matias, 2005; Lichtman & Cravatt, 2005).

Central and peripheral administration of EC stimulates food intake (Williams et al. 1998; Koch, 2001; Cota et al. 2003a). This orexigenic effect is mediated via CB1 receptors in the hypothalamus, which co-localise with CART, corticotrophin-releasing hormone, MCH and OX (Cota et al. 2003b). Additionally, CB1-knock-out mice display hypophagia and leanness (Cota et al. 2003b), and leptin-deficient signalling is associated with high hypothalamic EC levels (Di Marzo et al. 2001). Recent evidence (Verte et al. 2004) has also shown that the EC receptors are located downstream from the melanocortin system.

Together this evidence supports the important role of the EC system in the regulation of feeding. In fact, there is currently a CB1 selective antagonist, Rimobabant (SR141716), in use in phase III clinical trials that may be a potentially promising anti-obesity drug (Di Marzo & Matias, 2005).

**Dopamine.** The dopaminergic system is also important in the rewarding circuitry of feeding regulation (Fulton et al. 2006; Hommel et al. 2006). In fact, mice lacking tyrosine hydroxylase, the enzyme synthesising dopamine, are hypophagic (Szczypka et al. 2001). These actions are mediated via D1 and D2 receptors (Szczypka et al. 2001).

**Serotonin.** Serotonin plays an important role in regulating both rewarding and homeostatic mechanisms (Halford & Blundell, 2000b). Serotonin actions of food intake are mediated via the melanocortin system (Heisler et al. 2002, 2003). In fact, the currently-discontinued anorectic agent fenfluramine mediates its actions via serotonin and melanocortins (Heisler et al. 2002).

**Conclusions**

Multiple, redundant and complex peripheral neural circuits participate in the regulation of food intake and bodyweight homeostasis. All this evidence indicates that obesity, and associated metabolic alterations, is complex, multifactorial and chronic pathology. Thus, the search for, and development of, new weight-loss drugs is made extremely complicated. In fact, the efficacy of drugs acting on a single molecular target might be limited by compensatory feedback mechanism. In the near future combined therapies that act on both peripheral and central targets should be sought. Understanding these molecular networks regulating food intake could lead to the design of better therapeutic targets for weight loss.

**Acknowledgements**

This work has been supported by grants from Instituto Salud Carlos III, Spanish Ministry of Health, Xunta de Galicia, DGICYT (BFU 2005-06287) and the European Union (LSHM-CT-2003-503041 and QLK6-2001-02288). CD and ML are members of the CIBER of Obesity and Nutrition (ISCIII). LMW is funded by the Scottish Executive Environment & Rural Affairs Department (SEERAD).

**References**


Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T et al. (2001) Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like...


Cota D, Marsicano G, Tschop M, Gruber Y, Flachskamm C, Schubert M et al. (2003b) The endogenous cannabinoid...


Fei H, Okano HJ, Li C, Lee GH, Zhao C, Darnell R & Friedman JM (1997) Anatomical localization of alternatively


Griffond B, Risold PY, Jacquemard C, Colard C & Fellmann D (1999) Insulin-induced hypoglycemia increases preprohypo-
neurons are mandatory for feeding. Nature Neuroscience 8, 1289–1291.
Grove KL & Cowley MA (2005) Is ghrelin a signal for the develop-
ment of metabolic systems? Journal of Clinical Investi-
gation 115, 3393–3397.
Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, Van Der Ploeg LH & Howard
AD (1997) Distribution of mRNA encoding the growth
hormone secretagogue receptor in brain and peripheral tissues. Brain Research Molecular Brain Research 48, 23–29.
Gutzwiller JP, Goke B, Drew E, Hildebrand P, Ketterer S,
humans. Gut 44, 81–86.
et al. (1999) Orexin A activates locus coeruleus cell firing and
increases arousal in the rat. Proceedings of the National Academy of Sciences USA 96, 10911–10916.
Hagan MM, Castaneda E, Sumaya IC, Fleming SM, Galloway
hippocampal acetylcholine release in vivo: implications for
Hahn TM, Breininger JF, Baskin DG & Schwartz MW (1998)
Coexpression of Agrp and NPY in fasting-activated hypo-
Hakansson M, de Lecea L, Sutcliffe JG, Yanagisawa M &
Meister B (1999) Leptin receptor- and STAT3-immuno-
reactivities in hypocretin/orexin neurons of the lateral hypo-
Hakansson ML, Brown H, Ghilandi N, Skoda RC & Meister B
(1998) Leptin receptor immunoreactivity in chemically defined
target neurons of the hypothalamus. Journal of Neuroscience 18, 559–572.
Halaas JL, Gaiwala KS, Maffe i M, Cohen SL, Chait BT,
Weight-reducing effects of the plasma protein encoded by the
Haldor HC & Blundell JE (2000a) Separate systems for serotonin
circulating levels of ghrelin in human obesity. Gastroenterology 122, 345–354.
Härlström A, Fuxe K, Agnati LF, Benfenati F & Goldstein M
(1986) Receptor autoradiographical evidence for high densities
of 125I-neuropeptide Y binding sites in the nucleus tractus
solitarius of the normal male rat. Acta Physiologica Scandi-
Hayward MD, Pintar JE & Low MJ (2002) Selective reward
He W, Lam TK, Obici S & Rossetti L (2006) Molecular disrup-
tion of hypothalamic nutrient sensing induces obesity. Nature
Neuroscience 9, 227–233.
Hechinger A & Ranson S (1942) The spontaneous activity
Heisler LK, Cowley MA, Kishi T, Tectoh LH, Fan W, Low MJ
et al. (2003) Central serotonin and melanocortin pathways
Heisler LK, Cowley MA, Tectoh LH, Fan W, Low MJ, Smart JI
et al. (2002) Activation of central melanocortin pathways by
Henry BA, Rao A, Ikenasio BA, Mountjoy KG, Tlbrook AJ &
Clarke IJ (2001) Differential expression of cocaine- and
amphetamine-regulated transcript and agouti related-protein in
Herrmann C, Goke R, Richter G, Fehmann HC, Arnold R &
insulin-releasing polypeptide plasma levels in response to
nutrients. Digestion 56, 117–126.
Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R,
obese and lean adults: a randomized, controlled, dose-
Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T,
Free fatty acids regulate gut incretin glucagon-like peptide-1
secretion through GPR120. Nature Medicine 11, 90–94.
Holst B, Egerod KL, Schild E, Vickers SP, Cheetham S,
Gerlach LO, Storjohann L, Stidsen CE, Jones R, Beck-
Sickinger AG & Schwartz TW (2006) GPR39 signaling is
Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW,
Gao XB, Thurmon JJ, Marinelli M & DiLeo RJ (2006)
Leptin receptor signaling in midbrain dopamine neurons
Minireview: ghrelin and the regulation of energy balance –
a hypothalamic perspective. Endocrinology 142, 4163–4169.
regulating energy homeostasis. Neuroscience 120, 235–246.
Horvath TL, Diano S & van den Pol AN (1999) Somatic
interaction between hypocretin (orexin) and neuropeptide Y
cells in the rodent and primate hypothalamus: a novel circuit
implicated in metabolic and endocrine regulations. Journal of
Neuroscience 19, 1072–1087.
Hotta K, Funahashi T, Bodkin NL, Ortmeeyer HK, Arita Y,
of the adipocyte protein adiponectin are decreased in parallel
with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes 50, 1126–1133.
Howard JK, Cabe B, Oksanen LJ, Tzameli I, Bjorbaek C &
Flier JS (2004) Enhanced leptin sensitivity and attenuation of
diet-induced obesity in mice with haploinsufficiency of Socs3.
Molecular mechanisms and physiology of food intake


Lawrence CB, Liu YL, Stock MJ & Luckman SM (2004) Anorectic actions of prolactin-releasing peptide are mediated...
M. López et al.

López M, Seoane L, Señaris R & Diéguez C (2001b) Prepro-orexin mRNA levels in the rat hypothalamus, and orexin receptors mRNA levels in the rat hypothalamus and adrenal gland are not influenced by the thyroid status. Neuroscience Letters 300, 171–175.
Luquet S, Perez FA, Hnasko TS & Palmerd RD (2005) NPY/AgRP neuropeptides are essential for feeding in adult mice but can be ablated in neonates. Science 310, 683–685.
M. López et al.


