Neuropeptide Y in normal eating and in genetic and dietary-induced obesity

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Neuropeptide Y (NPY) is one of the most potent orexigenic peptides found in the brain. It stimulates food intake with a preferential effect on carbohydrate intake. It decreases latency to eat, increases motivation to eat and delays satiety by augmenting meal size. The effects on feeding are mediated through at least two receptors, the Y1 and Y5 receptors. The NPY system for feeding regulation is mostly located in the hypothalamus. It is formed of the arcuate nucleus (ARC), where the peptide is synthesized, and the paraventricular (PVN), dorsomedial (DMN) and ventromedial (VMN) nuclei and perifornical area where it is active. This activity is modulated by the hindbrain and limbic structures. It is dependent on energy availability, e.g. upregulation with food deprivation or restriction, and return to baseline with refeeding. It is also sensitive to diet composition with variable effects of carbohydrates and fats. Leptin signalling and glucose sensing which are directly linked to diet type are the most important factors involved in its regulation. Absence of leptin signalling in obesity models due to gene mutation either at the receptor level, as in the Zucker rat, the Koletsky rat or the db/db mouse, or at the peptide level, as in ob/ob mouse, is associated with increased mRNA abundance, peptide content and/or release in the ARC or PVN. Other genetic obesity models, such as the Otsuka–Long–Evans–Tokushima Fatty rat, the agouti mouse or the tubby mouse, are characterized by a diminution in NPY expression in the ARC nucleus and by a significant increase in the DMN. Further studies are necessary to determine the exact role of NPY in these latter models. Long-term exposure to high-fat or high-energy palatable diets leads to the development of adiposity and is associated with a decrease in hypothalamic NPY content or expression, consistent with the existence of a counter-regulatory mechanism to diminish energy intake and limit obesity development. On the other hand, an overactive NPY system (increased mRNA expression in the ARC associated with an upregulation of the receptors) is characteristic of rats or rodent strains sensitive to dietary-induced obesity. Finally, NPY appears to play an important role in body weight and feeding regulation, and while it does not constitute the only target for drug treatment of obesity, it may nevertheless provide a useful target in conjunction with others.

Keywords: feeding behaviour; dietary composition and preference; leptin resistance; insulin; hypothalamus; Zucker rat and ob/ob mouse

1. INTRODUCTION

Neuropeptide Y (NPY) was discovered over 20 years ago by Tatemoto & Mutt (Tatemoto et al. 1982). It is a 36 amino acid peptide that belongs to the pancreatic polypeptide family. Its name derives from the single-letter code (Y) for the amino acid tyrosine since it contains several tyrosine residues including an amidated C-terminal tyrosine residue. It is one of the most abundant peptides found in the brain, although it is also present in the peripheral nervous system (Everitt et al. 1984; Allen et al. 1987; Zukowska et al. 2003). It is one of the most conserved peptides during evolution (Larhammar 1996). These traits suggest that it is particularly important for the regulation of essential physiological functions.

In the brain, it is present in the cortex, hippocampus, hindbrain and hypothalamus (Chronwall et al. 1985). In the latter area, it is synthesized in abundance by neurons of the arcuate (ARC) nucleus (Chronwall 1985; Beck 2005). These neurons co-synthesize another orexigenic peptide, agouti-related peptide (AgRP; Hahn et al. 1998). They send their axons towards the paraventricular (PVN) nucleus as well as the dorsomedial (DMN) nucleus and the median preoptic area (Bai et al. 1985; Kerkerian & Pelletier 1986). The PVN also receives projections from NPY-synthesizing neurons present in the brainstem (Sahu et al. 1988), particularly in the A1 and C1 areas (Sawchenko et al. 1985).

The widespread distribution of NPY is associated with diverse biological actions, including cardiovascular regulation, seizure and cognition, stress, modulation of neuroendocrine systems and appetite regulation (Walker et al. 1991; Cox 1993; Aubert et al. 1994; Redrobe et al. 1999; Thorsell & Heilig 2002; Zukowska et al. 2003). Biological actions of NPY are mediated by six receptors named Y1–Y6. The Y6 receptor is not present in all mammalian species (Widdowson et al. 1997b; Burkhoff et al. 1998). These receptors probably derive from three Y receptor genes leading to the Y1, Y2 and Y3 subfamilies.
(Larhammar & Salaneck 2004). All three subfamilies appear to play a role in the regulation of feeding behaviour (Henry et al. 2005).

This review will focus on NPY role in normal eating and in animal models with genetic and diet-induced obesity. It will only address the genetic models with a spontaneous gene mutation and not those resulting from human manipulations. Information on knockout and transgenic models can be found in recent reviews (Beck 2001; Herzog 2004).

2. NPY AND NORMAL FEEDING BEHAVIOUR
(a) NPY is a potent orexigenic peptide
Soon after its discovery, several groups demonstrated that NPY injected into the brain either in the ventricles or in different hypothalamic nuclei induced a robust feeding response (Clark et al. 1984; Levine & Morley 1984; Stanley & Leibowitz 1984). It is effective throughout the day with strong effects observed at the beginning of the dark period (Tempel & Leibowitz 1990; Stanley & Thomas 1993). Food intake is increased several fold and the effect lasts 6–8 hours. The orexigenic effect of NPY has been noted in numerous species—both mammalian or non-mammalian (Parrott et al. 1986; Kuenzel et al. 1987; Morley et al. 1987b; Pau et al. 1988; Miner et al. 1989; Morris & Crews 1990). No effects have been described after peripheral infusion.

Feeding behaviour can be separated into two phases: an appetitive one consisting of searching and acquiring food and a consummatory one consisting of ingestion itself (Craig 1918). Consummatory behaviour may be tested in animals receiving a sucrose solution through an implanted intraoral cannula. There is general agreement that NPY injected in the lateral or third ventricle stimulates appetitive behaviour, e.g. eating pellets from a feeder or drinking sucrose from a bottle (Seeley et al. 1995; Ammar et al. 2000; Benoit et al. 2005). But NPY either stimulates (Benoit et al. 2005), fails to change (Seeley et al. 1995) or inhibits (Ammar et al. 2000) intraoral administration of sucrose. These discrepant observations on consummatory behaviour might be related to the training of the rats required to habituate them to the experimental procedure, as naïve untrained rats ingest significantly more intraoral sucrose solution after NPY injection (Benoit et al. 2005). In hamsters, both appetitive and consummatory ingestive behaviours are stimulated (Day et al. 2005).

The stimulatory effect of NPY on food ingestion is confirmed by observations of the effect of blockade of the NPY system by various means. Continuous central infusion of NPY antibodies suppresses intake during the dark period in a dose-dependent fashion (Dube et al. 1994). Local injection of NPY antibodies into the PVN and ventromedial (VMN) nuclei leads to similar inhibitory effects on food ingestion (Shibasaki et al. 1993; Walter et al. 1994). Injection of NPY antisense oligodeoxynucleotides may also diminish food intake, meal size and duration (Hulsey et al. 1995), although in some studies no effects are reported (Dryden et al. 1998); in any case, the results obtained with this technique must be carefully checked because of the difficulties in using it (toxic effects, penetration, exact targeting). However, a recent study where NPY expression in the ARC was inhibited by a gene therapy approach showed that the treated animals released 50% less NPY, gained less weight and ate less than the controls up to 50 days after treatment (Gardiner et al. 2005).

A map of the hypothalamic areas responding to NPY has been established by injecting nanolitre volumes into well-determined nuclei. The perifornical area, the PVN and VMN are the areas most sensitive to administration of NPY injection (Stanley et al. 1985a,b, 1993; Morley et al. 1987a; Jolicoeur et al. 1995). There are, however, differences in the actions of NPY in different regions. In the PVN, NPY increases the respiratory quotient, in addition to its stimulatory effects on food intake (Menendez et al. 1990; Currie & Coscina 1995), consistent with an increased utilization of carbohydrates as energy source. On the other hand, in the perifornical area, NPY strongly stimulates intake without any modification of the respiratory quotient (Currie & Coscina 1995). Opposite effects have also been observed on body temperature following NPY injection in these two areas (Jolicoeur et al. 1995).

In the case of intraventricular administration, the precise site of administration of NPY does seem to be important. Thus, NPY stimulates food intake when it is injected into the third or lateral ventricles that are near the hypothalamic region, as well as when it is injected into the fourth ventricle (Corp et al. 1990; Steinman et al. 1994). This suggests that connections between hindbrain and hypothalamus are functionally important for the orexigenic properties of NPY. The importance of these links is indicated by observations of the induction of c-fos expression in the forebrain and in the PVN after NPY injection in the fourth ventricle (Xu et al. 1995), and by observations following brain section. Total transection leads to a decrease in NPY content in the PVN and an increased sensitivity of the operated rats to exogenous NPY injection (Sahu et al. 1988a).

Lesions of the ARC nucleus, either immunologically or chemically (Burlet et al. 1995; Stricker-Krongrad et al. 1996; Bugarith et al. 2005), modify feeding behaviour. A small but durable decrease in food intake is observed in lesioned rats following specific targeting of NPY neurons by neurotoxins, or by less selective destruction of NPY neurons by perinatal injection of toxic doses of monosodium glutamate (MSG; Burlet et al. 1995; Stricker-Krongrad et al. 1996). The decrease in food intake is not directly related to the size of the lesion with, for example, a 10% diminution of intake associated with a 40% or more diminution in NPY content in MSG rats (Stricker-Krongrad et al. 1996). Rats with a toxin-induced lesion in the basomedial hypothalamus remain responsive to NPY but not to leptin (Bugarith et al. 2005).

(b) Mode of action
Careful analyses of the patterns of feeding behaviour reveal that NPY reduces the latency to eat (Clark et al. 1984; Stanley & Leibowitz 1985; Morley et al. 1987b; Corp et al. 1990). Conversely, the absence of NPY in NPY knockout mice is associated with a pronounced delay in the initiation of feeding (Sindelar et al. 2005).
NPY also delays satiety and therefore augments meal size, time spent eating and meal duration (Leibowitz & Alexander 1991; Lynch et al. 1994; Stricker-Krongrad et al. 1994). These effects are independent of the type of food, as they are observed with either solid dry food or sweetened condensed milk solution.

NPY also modifies the motivation to eat (Flood & Morley 1991). Its effect is comparable to that of a 36–48 hour food deprivation (Jewett et al. 1995). This has been shown in three paradigms: lever pressing, appetitive passive avoidance and quinine-adulterated milk. NPY injected into the third ventricle induces a higher consumption of milk when the animals have to work in a lever press apparatus to obtain it. The potency of NPY in inducing intake is evident even when substantial effort (number of lever-pressing events) is required to obtain small amounts of food (Jewett et al. 1992, 1995). NPY-injected mice also tolerate more electric shocks to the tongue while drinking milk and they overcome the unpleasant bitter taste of quinine added to the milk (Flood & Morley 1991). In addition, NPY presumably plays a role in mediating food anticipatory responses, as rats conditioned with daily NPY injections and a restricted feeding schedule eat more after saline injection into the third ventricle than rats conditioned with saline (Drazen et al. 2005).

(c) NPY modulates dietary preferences
Several studies have shown that centrally administered NPY modifies qualitative components of the ingested food in addition to increasing the ingested quantity. When the rats have the choice between three sources of pure macronutrient (casein, lard, mixture of sucrose–cornstarch–dextrose) correctly supplemented with vitamins and minerals, they preferentially increase their intake of carbohydrates after acute NPY injection (Stanley et al. 1985a,b; Stanley et al. 1989a). Similar results are obtained with a choice between two macronutrients, but only if carbohydrates constitute one of the two choices (Stanley et al. 1985a,b). These effects are partly dependent on the previous dietary preference of the rats (Welch et al. 1994). A preferential intake of carbohydrates is also observed when the rats have to choose between two nutritionally complete diets: a high-carbohydrate (HC) diet and a high-fat (HF) diet (Welch et al. 1994). When the rats have the same choice (HF diet versus HC diet), and when NPY is continuously injected into a brain ventricle through osmotic minipumps, both HF intake and HC intake are stimulated (Beck et al. 1992b), but the effect on HF intake is short (2 days), whereas that on HC intake is still observed after 9 days of injection (Beck et al. 1992b).

The nature of the carbohydrate plays a role in the orexigenic effects of NPY. When rats can choose between a HF diet and a HC diet, the animals eat more of the HC diet if the source of carbohydrate is sucrose or polydextrose compared with cornstarch (Glass et al. 1997). The change in palatability due to increased sweetness of the sucrose or polydextrose diets versus cornstarch might be an element of this preference. This is supported by an experiment where different solutions either sweetened with sucrose or saccharin were tested in fully satiated rats. NPY increased the intake of the different sucrose solutions (2–10%) as well as the preferred saccharin solution (Lynch et al. 1993). Orosensory mechanisms might therefore play a role on the stimulatory effects of NPY on carbohydrate intake.

(d) Orexigenic effects are mediated through Y1 and Y5 receptors
At the time of early studies on the NPY system, only two receptor subtypes were known: Y1 and Y2. These were differentiated on the basis of their affinity for NPY fragments (Leibowitz & Alexander 1991; Widdowson 1993). The maximal stimulation is obtained with the complete amino acid sequence (McLaughlin et al. 1991). The loss of several amino acids at either end of the peptide is associated with a diminution of the orexigenic effect. NPY1–27 is, for example, less potent than the entire NPY (Paez et al. 1991). There is, however, an exception since NPY2–36 induces the same or an even greater feeding response than intact NPY (Jolicour et al. 1991; Kalra et al. 1991a; Stanley et al. 1992). Y1 agonists like [D-Arg25]-NPY stimulate food ingestion (Mullins et al. 2001). Potent Y1 antagonists such as 1229U91 and BIBO 3304 are able to inhibit fasting- and NPY-stimulated food intake (Kanatani et al. 1996, 2001; Wieland et al. 1998; Daniels et al. 2001). Comparable effects are observed after injection of Y1 antiserum oligodeoxynucleotides (Lopez-Valpuesta et al. 1996; Schaffhauser et al. 1998).

NPY13–36 does not bind to Y1 receptors, but it is a full agonist at the Y2 receptor. It does not stimulate food intake but inhibits NPY release from hypothalamic slices in vitro (King et al. 1999, 2000). This might contribute to an anorexigenic response. In this context, it is worth noting the inhibitory role of the Y2 ligands, such as PYY3–36 on food intake in humans and rodents that has been recently emphasized (see Tschop et al. 2004; leRoux & Bloom 2005 for more details). Chronic activation of Y2 receptors leads to hypophagia and transient weight loss (Henry et al. 2005) and a normal feeding behaviour requires the presence of the Y2 receptor (Naveilhan et al. 1999).

The variable efficiency of NPY fragments in stimulating food intake, and more particularly that of NPY2–36, suggested that feeding may be elicited through a variant of the Y1 receptor. Subsequently, a further receptor, Y5, was cloned (Gerald et al. 1996) that is present in areas involved in feeding regulation (PVN, lateral hypothalamus (LH)), sometimes in association with the Y1 receptor (Parker & Herzog 1999; Durkin et al. 2000). It is now well established that the Y5 subtype is implicated in feeding behaviour (for review see Balasubramaniam 1997; Blomqvist & Herzog 1997; Inui 1999). Like the Y1 receptor, it is present in the parvo cellular part of the PVN (Wolak et al. 2003). The effects of selective receptor agonists suggest that the Y5 receptor might mediate the consummatory behaviour, whereas the appetitive behaviour might be more related to the Y1 receptor (Day et al. 2005). The Y5 receptor could also play a role in the NPY-induced reduction of energy expenditure (Hwa et al. 1999) and it is downregulated.
by NPY release induced by diet restriction (Widdowson et al. 1997a).

The importance of the Y5 receptor is indicated by several different lines of evidence, including the injection of Y5 antisense oligonucleotides (Schaffhauser et al. 1997; Tang-Christensen et al. 1998; Flynn et al. 1999; Campbell et al. 2001), the injection of specific Y5 agonists (Haynes et al. 1998; Wyss et al. 1998a,b; Cabrele et al. 2000; Parker et al. 2000; Lecklin et al. 2003) and antagonists (Polidori et al. 2000; Kask et al. 2001; Yokosuka et al. 2001; Daniels et al. 2002). In contrast, several studies have suggested a minor role of the Y5 receptor in feeding behaviour. First, the Y5 subtype is expressed at low levels in the hypothalamus (Widdowson et al. 1997b; Dumont et al. 1998; Naveilhan et al. 1998). Second, injection of a highly selective Y5 antagonist fails to inhibit NPY-induced feeding and has no effect on food intake when it is chronically administered (Kanatani et al. 2000b; Turnbull et al. 2002) — this contrasts with the effects of other Y5 antagonists that have affinity for receptors that do not belong to the Y family (DellaZuana et al. 2001). Moreover, NPY-induced feeding is markedly reduced in Y1-knockout mice, whereas it is not altered in Y5-knockout mice (Kanatani et al. 2000a), although others have noted that these differences are limited to low doses of NPY (Marsh et al. 1998). Since administration of Y5 agonists induces food intake in Y5-knockout mice, it would seem that either these compounds exhibit some agonist activity at Y1 receptor, or there exists yet another (novel) receptor subtype (Kanatani et al. 2000a). Studies using different NPY analogues support the latter hypothesis (O’Shea et al. 1997; Dumont et al. 2005). Overlapping of the gene structure of the Y1 and Y5 receptors suggests an exclusion of the co-expression of the two genes in certain cell types; coordinate expression might be important for NPY activity (Herzog et al. 1997). This might explain some differences between studies using animals with different genetic backgrounds.

(e) Feeding factors influencing endogenous NPY

(i) Fasting and associated changes in blood parameters

The most important factor that influences the hypothalamic content of NPY is food deprivation. When rats are deprived of food for 24–96 hours, NPY content and expression are markedly increased in the ARC nuclei as shown by numerous studies (Sahu et al. 1988c; Beck et al. 1990c; Brady et al. 1990; Sanacora et al. 1990; White & Kershaw 1990; Chua et al. 1991a; O’Shea & Gundlach 1991; Marks et al. 1992; Lewis et al. 1993; Schwartz et al. 1993; Davies & Marks 1994). Chronic food restriction induces similar changes (McShane et al. 1993; White et al. 1994b; Kim et al. 1998; McShane et al. 1999), but in addition increases NPY expression in the hindbrain which is not observed after fasting (Chua et al. 1991a; Ishizaki et al. 2003). Parallel increases in NPY content are observed in the PVN after fasting (Sahu et al. 1988c; Calza et al. 1989; Beck et al. 1990c). Refeeding rapidly returns the abundance of NPY in the ARC to initial values (Sahu et al. 1988c; Beck et al. 1990c; Davies & Marks 1994). In the PVN, NPY remains elevated after 6 hour refeeding, which may be related to the fact that body weight of the rats has not returned to normal in this period (Beck et al. 1990c); normalization is, however, achieved after 24 hours.

The changes in NPY content are also reflected in measurements of NPY release both in vitro and in vivo using the push-pull (PP) cannula technique. In vitro, NPY release from microdissected PVN of fasted rats is higher than that measured from ad libitum fed rats (Dube et al. 1992). In vivo, the PP technique allows measurements of peptide in the extracellular space in specific brain regions. Fasting is associated with increased NPY release in the PVN and food ingestion induces a diminution in NPY release (Kalra et al. 1991b; Stricker-Krongrad et al. 1993; Lambert et al. 1994). A similar situation exists in rats used to a daily scheduled feeding for a few hours. Thus, NPY release and hypothalamic expression are enhanced in advance of scheduled feeding time and food ingestion rapidly decreases NPY release in the PVN (Sahu et al. 1992; Yoshihara et al. 1996). The NPY release in response to hyper-osmotic KCl is also associated with feeding episodes (Stricker-Krongrad et al. 1993). All these experiments provide support for the idea of a primary role of the ARC–PVN axis in the regulation of food intake by NPY (cf. figure 1) and a modulatory role of the brainstem in this regulation.

Diurnal variations in hypothalamic NPY in the rat are consistent with this view, as NPY content peaks one hour before dark onset (Jhanwar-Uniyal et al. 1990) in the PVN and decreases one hour after lights off. There is also a pre-dark period rise in NPY mRNA in the mediobasal hypothalamus, including the ARC nucleus (Akabayashi et al. 1994a). Light transition is a triggering signal for food intake and the beginning of the dark period is characterized by a greater intake of carbohydrates when the rats have to choose between the three macronutrients (Tempel et al. 1989). Food ingested during the first few hours of darkness is associated with a peak in NPY release in the PVN (Stricker-Krongrad et al. 1997) and a sharp decrease in NPY mRNA (Akabayashi et al. 1994a). When NPY is absent, as in the NPY knockout mice, intake during the first four hours of the dark period is reduced by one-third (Sindelar et al. 2005).

Fasting leads to changes in numerous plasma parameters. A decrease in blood glucose concentrations is one of these, and diminished glucose availability to the brain could be a factor in NPY regulation. Glucose-sensitive neurons that contain NPY are present in the ARC nucleus (Muroya et al. 1999), and may have an integrative role in the regulation of energy balance (Wang et al. 2004). After induction of a moderate hypoglycaemia by insulin, the feeding response is much larger in normal mice than in mice lacking NPY (Sindelar et al. 2004). Glucoprivation induced by injection of the metabolic blocker, 2-deoxyglucose (2DG) also induces feeding (Smith & Epstein 1969). It is associated with the activation of hypothalamic NPY neurons (Minami et al. 1995) and increased NPY expression and content in the ARC (Akabayashi et al. 1993; Giraudo et al. 1998; Sergeyev et al. 2000) and in the hindbrain (Li & Ritter 2004). Moreover, 2DG-induced feeding is attenuated by injection of NPY antibodies in the PVN (He et al. 2003).
Figure 1. The hypothalamic neuropeptide Y (NPY) system. Names of the areas involved in the system are indicated in italics: ARC, arcuate nucleus; PVN, paraventricular nucleus; LH, lateral hypothalamus; VMN, ventromedial nucleus; DMN, dorsomedial nucleus. The location of specific receptors is shown. The different receptors are identified once in an area by a sign and the receptor name tied to this sign (e.g. black triangle for MC4-R, black explosion for insulin, diamond for glucocorticoids, etc.). In the other nuclei, their presence is indicated by signs only. Ob-Rb, long form of the leptin receptor; MC4-R, type 4 melanocortin receptor; GHS-R, growth hormone secretagogue (ghrelin) receptor; INS-R, insulin receptor; GC-R, glucocorticoid receptor. The names of the neuronal populations connected to NPY neurons in the ARC are given in small capitals: POMC, proopiomelanocortin; CART, cocaine and amphetamine-related peptide; DYN, dynorphin; GAL, galanin; CRH, corticotropin-releasing hormone; MCH, melanin concentrating hormone; OX, orexins; TRH, thyrotropin-releasing hormone. Agouti-related peptide (AgRP) is co-localized with NPY (for more details, see Beck 2005).

1998), and by deletion of the NPY gene (Sindelar et al. 2004).

Further evidence for the importance of glucose availability comes from experiments in which blood glucose concentrations are increased. After an intravenous (IV) injection of glucose, the stimulation of food intake by NPY is less that after an IV injection of saline (Rowland 1988). This is not observed after fructose injection probably because fructose is not metabolized by the brain. Intraperitoneal injection of 10% glucose solution 3 hours before onset of the dark period induces a diminution in NPY expression during the first hour after dark (Chang et al. 2005). A similar result is obtained after injection of microlitre volumes of a 5% glucose solution in the third ventricle.

Changes in food intake also influence plasma insulin concentrations. The latter are diminished by fasting, and can also be suppressed by administration of streptozotocin (STZ) to destroy insulin-secreting pancreatic beta-cells. Streptozotocin-induced diabetic rats are hyperphagic and less sensitive to NPY (Morgan et al. 1996), while the abundance of NPY, and/or NPY expression and release are markedly increased in the hypothalamus and particularly in the ARC–PVN (Williams et al. 1988, 1989; Sahu et al. 1990; White et al. 1990; Abe et al. 1991; McKibbin et al. 1992; Marks et al. 1993; Lambert et al. 1994; Frankish et al. 1995; Morris & Pavia 2004). These increases occur before the onset of hyperphagia (Sahu et al. 1997). Moreover, NPY receptor number is diminished in STZ rats (Frankish et al. 1993). NPY expression is normalized after insulin treatment. In addition, insulin administered peripherally or centrally attenuates the fasting-induced NPY increase in the ARC (Malabu et al. 1992; Schwartz et al. 1992). Furthermore, hyperphagia is not observed in STZ-treated NPY-deficient mice (Sindelar et al. 2002).

Other relevant factors modified by fasting include glucocorticoids and leptin. Leptin receptors are present in NPY neurons in the ARC nucleus (Mercer et al. 1996a). Expression in adipose tissue and plasma concentrations of leptin are decreased by fasting (Saladin et al. 1995; Hardie et al. 1996), which leads to upregulation of the NPY system with an increased mRNA expression and peptide production in the ARC and an increased release of NPY in the PVN. On the other hand, chronic intracerebroventricular (ICV) administration of NPY augments leptin mRNA in the adipose tissue (Sainsbury et al. 1996). The loop existing between leptin and NPY has been discussed in detail in several recent reviews (Ahima & Flier 2000; Woods & Seeley 2000; Porte et al. 2002; Kalra & Kalra 2003; Sahu 2003; Woods et al. 2006).

Glucocorticoids are involved in body weight regulation (Dallman et al. 2004). There are discrepancies in the published literature on the relation between NPY and glucocorticoids. Glucocorticoid receptors are found on almost all NPY neurons in the ARC (Ceccatelli et al. 1989), and there is evidence of a positive association between NPY and glucocorticoids (Akabayashi et al. 1994a; Tempel & Leibowitz 1994; Wang et al. 1998a, 1999). Thus, adrenalectomy (ADX) has been reported to be associated with a decrease (Pralong et al. 1993; Akabayashi et al. 1994b; Watanabe et al. 1995; Savontaus et al. 2002) or no change (Ponsalle et al. 1992; Larsen et al. 1994;
Influence of diet composition

In addition to energy intake per se, macronutrient composition of the diet also influences NPY abundance in the hypothalamus. Concentration in the paraventricular part of the PVN is lower after two weeks of ingestion of a HC diet than after the ingestion of a HF diet (Beck et al. 1990d). A significant negative correlation exists between NPY concentrations and the carbohydrate-to-fat ratio in the diet (Beck et al. 1992c). When rats have a choice between HC and HF diets, their choice is reflected in PVN NPY concentrations (Beck et al. 1992c). Over longer periods (two months), when the rats are fed from weaning with an obesogenic diet, e.g. a high-starch diet associated with a 25% sucrose solution to drink, the hyperphagia present at the end of the experiment is associated with a significant decrease of NPY content in the ARC nucleus. A further decrease is noted in the ARC as well as in the PVN in normophagic, but obese rats fed a HF diet (Beck et al. 1994a). These changes can be considered as part of a counter-regulatory mechanism necessary for limiting over-consumption of food as well as fat accretion.

These findings are consistent with data obtained in rats exhibiting marked dietary preferences (Beck et al. 2001a). When Long–Evans rats can choose between a HC and a HF diet, food choice among individuals follows a Gaussian distribution with the majority of the animals having no or minor dietary preferences. At each end of the distribution, there are rats showing a clear preference for either carbohydrates or fats. The former are characterized by lower NPY concentrations in the paravascular part of the PVN (Beck et al. 2001a) in the basal state. In feeding periods, such as the beginning of the dark period, carbohydrate-prefering rats have higher hypothalamic NPY concentrations (Jhanwar-Uniyal et al. 1993). During this period, there is also a spontaneous preferential consumption of carbohydrates (Tempel et al. 1989). In relation to these data, it seems likely that the amplitude of NPY variations rather than the absolute content better characterizes preference for macronutrients. Each dietary preference presumably reflects the result of a balance (Beck et al. 2001a) between the actions of NPY and those of other hormones and neuropeptides.

Macronutrient composition has also an impact on the NPY system during development. In the hypothalamus, NPY neurons in the ARC are already present during the last week of gestation and the maturation of the system goes on postnatally during the suckling period (Kagotani et al. 1989; Bouret et al. 2004). The offspring of dams fed on unbalanced diets (HF or HC) during these periods have persistent alterations in the functioning of the NPY system in adulthood (Kozak et al. 1998, 2000, 2005). The changes in sensitivity to NPY injection and an increased peptide release after a glucoprivic challenge are associated with delays in the establishment of the dietary preferences (Kozak et al. 2000, 2005). Under- and over-nutrition during early life or gestational diabetes also strongly influence the NPY system by boosting it whatever the type of the adverse condition (Plagemann et al. 1999a,b, 2000; Lopez et al. 2005). All these data support an important effect of nutritional factors on NPY.

Interaction with other modulators of feeding

Macronutrient composition not only influences the energy content of diets, but is also an essential element in determining palatability. By manipulating it, it is possible to study the hedonic/reward aspects of food. Two systems, the opioid system and the cannabinoid system have been linked to these aspects of feeding behaviour (Glass et al. 1999; Harrold & Williams 2003; Di Marzo & Matias 2005). Both systems operate in areas such as nucleus accumbens and hypothalamus (Harrold et al. 2002; Bodnar 2004) and in the hippocampus, which is also linked to food reward (Tracy et al. 2001). Owing to the widespread distribution of NPY in the brain (Everitt et al. 1984; Chronwall et al. 1985) and particularly in these areas, and as NPY is sensitive to diet composition and modulates motivation for food ingestion, relationships among the three systems appear plausible. In support of this, it has recently been demonstrated that NPY release from rat hypothalamic explants in vitro is stimulated by the cannabinoid agonist, anandamide, and inhibited by the antagonist AM 251 (Gamber et al. 2005). Furthermore, NPY-induced feeding can be strongly decreased with pre-treatment of rats with different (mu, kappa and delta) opioid receptor antagonists (Israel et al. 2005).

Finally, the NPY system interacts with a number of other orexigenic systems (reviewed in Beck 2005). These include the melanocortin system which is also represented in the ARC nucleus. Both systems interact through axons and axonal terminals that form synaptic contacts on each type of neurons (Csiffary et al. 1990). Y1 receptors have also been detected on neurons positive for melanocortin type 4 receptor (MC4-R) in the PVN (Kishi et al. 2005). The precise relations between the two systems are detailed in recent interesting reviews (Seeley et al. 2004; Cone 2005). NPY neurons in the ARC are connected with another orexigenic system: the orexin neurons in the lateral hypothalamus (Elías et al. 1998; Horváth et al. 1999). Orexins are sensitive to fat ingestion (Park et al. 2004) and there are orexin receptors on NPY neurons.
(Funahashi et al. 2003). These connections may link feeding with the wake/sleep cycle (Willie et al. 2001). A recently discovered peptide, ghrelin, also interacts with the NPY system. Ghrelin is the only peripheral peptide that stimulates food intake (Nakazato et al. 2001). It is also linked to diet composition, as it preferentially stimulates fat intake and conversely fat ingestion also linked to diet composition, as it preferentially preferential intake of carbohydrates. Its orexigenic effects are mediated by at least two receptors, the Y1 and Y5 receptors. A negative energy balance with its associated hormonal and metabolic changes is the main factor activating the NPY system. On the other hand, diet composition can influence the hypothalamic abundance of NPY mainly in the PVN. Carbohydrate-rich diets influence NPY in the PVN over both short and long terms, whereas fat-rich diets have predominantly long-term influences. Dietary preference for either carbohydrate or fat is associated with NPY status in the PVN. The NPY system is in close interaction with other brain modulators (including orexins, ghrelin, glucocorticoids) to adapt feeding behaviour to the individual needs and situations.

3. NPY IN GENETIC MODELS OF OBESITY

The identification of leptin and its receptor in the mid-1990s is a pivotal point in studies of genetic models of obesity (Zhang et al. 1994; Tartaglia et al. 1995). It is now clear that leptin is a primary factor regulating body weight (Ahima & Flier 2000) and that genetic models of obesity may include faults in leptin signalling as well as other mediators.

(a) Models with a leptin-signalling failure

The Zucker fa/fa rat, the db/db mouse and the ob/ob mouse are among the most widely used rodent models of obesity (Bray 1977; Argiles 1989; Robinson et al. 2000). Zucker (fa/fa) rats become obese due to a missense mutation in the leptin receptor gene, which diminishes but does not completely eliminate responsiveness to leptin (Chua et al. 1996a, b; Iida et al. 1996; Phillips et al. 1996). The obese Zucker does not respond to ICV injections of leptin (Seeley et al. 1996), except at very high doses (Dryden et al. 1999), and leptin inhibits a much lower proportion of the ARC neurons (Nagamori et al. 2003).

The mouse homologue of the Zucker rat is the db/db mouse (Truett et al. 1991; Chua et al. 1996a). The obese cp/cp JCR : LA corpulent rat and the Koletsky rat are other models with mutations in the leptin receptor genes (Takaya et al. 1996; Kahle et al. 1997). However, the nonsense mutation in the Koletsky rat results in a premature stop codon in the extracellular domain of the leptin receptor gene. All isoforms of the receptor are affected (Takaya et al. 1996; Wu-Peng et al. 1997), leading to a total insensitivity of the Koletsy rat to exogenous leptin (Wildman et al. 2000).

In the obese ob/ob mouse, a nonsense mutation in the coding region of the leptin gene prevents the production of a functional peptide (Zhang et al. 1994). However, when leptin is injected ICV in ob/ob mice, it regulates feeding behaviour (Schwartz et al. 1996; Smith et al. 1998), proving that receptor mechanisms are functional. It is worth noting that all these models share with human obesity the characteristics of hyperphagia, hypertriglyceridemia, hyperleptinemia and hyperinsulinemia (Bray 1977; Argiles 1989; Williams et al. 1992; Hiraoka et al. 1997; Robinson et al. 2000; Keen-Rhinehart et al. 2004a).

(i) The obese Zucker rat

In the obese Zucker rat, the central NPY system is profoundly altered. This dysregulation was described several years before the discovery of leptin, and this was thought at the time to be the primary cause for the development of obesity. The NPY changes in the brain are site-specific. Thus, while studies of blocks of hypothalamic tissue failed to detect differences in NPY content between lean and obese phenotypes (Williams et al. 1991a), microdissection of individual hypothalamic nuclei revealed significantly higher NPY concentrations in the PVN and ARC nuclei of obese rats (Beck et al. 1990b,c, 1993; McGibbon et al. 1991). Parallel studies have demonstrated that the mRNA encoding NPY is also increased in abundance in the ARC nucleus of the fa/fa rat (Sanacora et al. 1990; Mercer et al. 1996b), suggesting that with respect to NPY expression obese rats appear to be constantly in a deprivation state. Leptin-receptor gene transfer into the ARC nucleus of female fatty Zucker rats induced a diminution of NPY mRNA abundance in this nucleus (Keen-Rhinehart et al. 2004b), supporting the specificity of changes in the NPY system. The hyperphagia of Zucker rats might also relate to the GABAergic system since NPY inhibits the firing of GABA (gamma amino butyric acid) neurons in the ARC and PVN (Prochuk & Colmers 2004; Acuna-Goycolea et al. 2005). Moreover, muscimol, a GABA agonist stimulates food intake when it is injected into hypothalamic nuclei (Kelly et al. 1979). Interestingly,
the obese rat has a similar feeding response to lean rats, but NPY is less effective at PVN GABA synapses in obese compared with lean Zucker rats (Pronchuk & Colmers 2004). NPY could therefore modulate feeding through this pathway.

The main metabolic changes are exhibited very early (three to five weeks of age) in the life of these animals (Krief & Bazin 1991). The NPY increases are also apparent early in life. In terms of content and expression, the increase is significant 30 days after birth when weaned rats begin to overeat (Bchini-Hooft van Huijsduijnen et al. 1993; Beck et al. 1993). These increases are still evident later in life up to 40 weeks of age (Sanacora et al. 1992; Bchini-Hooft van Huijsduijnen et al. 1993; Jhanwar-Uniwal & Chua 1993). New molecular genetic methods (Smoller et al. 1993; Chua et al. 1996b) have allowed studies of the fa genotype at earlier stages than more classic approaches, such as oxygen consumption or inguinal fat pad weights (Kaplan 1979; Lavau & Bazin 1982). They reveal that NPY mRNA abundance in the ARC nucleus of fa/ fa pups is increased as early as post-natal day 9 (Kowalski et al. 1999), whereas hypothalamic concentration is decreased (Ster et al. 2003), consistent with an augmented NPY release. However, it is difficult to link food intake and NPY changes before weaning, as discordant results have been reported concerning the onset of hyperphagia during this period (Buchberger & Schmidt 1996; Kowalski et al. 1998). This discrepancy may simply be related to the method of determination of food intake in the pups: one study in which suckling and pup weight were measured found no increase, whereas another examining ingestion independent of the dam found that hyperphagia emerges between post-natal days 9 and 12. Nonetheless, during this early period, NPY could contribute to fat deposition by its metabolic and antithermogenic effects (Godbole et al. 1978).

Early increases in NPY content and expression in the Zucker fatty rat have physiological consequences for NPY receptor function. First, receptor-binding studies have shown that the hypothalamic receptor density is reduced in obese rats (McCarthy et al. 1991). Studies employing antagonists and masking techniques were able to show that binding to Y5 receptors was likely to be reduced in obese rats (Widdowson 1997), and this was subsequently confirmed by measurement of NPY receptor expression by RT-PCR (Beck et al. 2001b). It appears that both Y1 and Y5 receptors are downregulated. The downregulation of NPY receptors leads to a decreased sensitivity of obese rats to exogenous NPY, so that higher NPY doses are required to induce the same food intake as in lean rats (McCarthy et al. 1991; Stricker-Krongrad et al. 1994), and in some cases, NPY is even unable to stimulate feeding (Brief et al. 1992). The decrease in NPY binding and receptors is, however, not sufficient to normalize food intake.

The diminution of the receptor-binding capacity in obese rats also accounts for the fact that weak antagonists or agonists are unable to modify the feeding behaviour in obese rats (Beck et al. 1994b), even if they are Y5-specific (Wyss et al. 1998a; B. Beck, unpublished observations). However, in circumstances where there is extreme overeating, a potent NPY antagonist was able to markedly diminish food intake up to 24 hours after central injection (Ishihara et al. 1998). Intracerebroventricular NPY antiserum also curtailed daily food intake and promoted weight loss in female obese Zucker rats (MarinBivens et al. 1998).

The functionality of the NPY system is also dependent on nutritional and hormonal state. Thus, in lean rats, food deprivation induces an increase in hypothalamic NPY mRNA abundance (Sanacora et al. 1990; Berelowitz et al. 1992). Peptide content is not changed even after restriction, indicating clear differences with the Sprague–Dawley rat (McKibbin et al. 1991; Beck et al. 1992a). The same situation occurs in the obese Zucker rat, although at higher levels for the peptide content (McKibbin et al. 1991; Beck et al. 1992a). The high NPY mRNA abundance in obese rats is either further increased by deprivation (Korner et al. 2001) or shows a tendency to increase (Sanacora et al. 1990; Berelowitz et al. 1992), after long (two weeks) and severe (50%) food restriction (Pesonen et al. 1992). These data suggest increased NPY synthesis, transport and release in the Zucker rat. Measurements of the in vivo NPY release in the PVN by the PP cannula technique support this hypothesis. Whereas the basal NPY efflux measured in vitro is not different between lean and obese rats (Kalra et al. 1994), the in vivo release at different times in the light/dark cycle is significantly enhanced in obese rats (Dryden et al. 1995; Stricker-Krongrad et al. 1997). Moreover, at the light-to-dark transition, which is characterized by peak concentrations in the PVN and ARC nucleus (Jhanwar-Uniwal et al. 1990) and by feeding episodes (Tempel et al. 1989), the obese rat does not show the typical peak of NPY release observed in its lean counterpart (Stricker-Krongrad et al. 1997). It seems then that NPY is released in an unsynchronized manner in the obese rat which may contribute to the disappearance of the dark/light rhythm of feeding behaviour in Zucker fa/fa rats (Becker & Grinker 1977; Alingh Prins et al. 1986).

A similar NPY profile is found in the other animal models with a leptin receptor mutation. NPY mRNA abundance is increased in db/db mice (Chua et al. 1991b; Schwartz et al. 1996; Yamamoto et al. 1999; Bates et al. 2003), and this is further augmented by food deprivation or restriction (Chua et al. 1991a; Yamamoto et al. 2000). In cp/cp rats, NPY concentrations are elevated in the ARC nucleus as in Zucker rats, although food restriction does not further enhance NPY in the ARC (Williams et al. 1992). In the Koletsky rat, there is also a large augmentation of both NPY mRNA and peptide content (Keen-Rhinehart et al. 2004a). Gene therapy which restores leptin receptor expression in this rat leads to a significant reduction in NPY mRNA expression (Morton et al. 2003), adding weight to view that the leptin receptor is important in NPY regulation. Moreover, in keeping with this idea, deletion of the leptin receptor in mice induces an increase in NPY mRNA expression and obesity (Cohen et al. 2001).

Chronic NPY infusion in brain ventricles of normal rats through osmotic minipumps mimics the constant elevated central NPY levels measured in the obese...
Zucker rat and Koletsky rat, and induces hyperphagia, disrupts nycthemeral feeding rhythms and leads to increased body weight and adiposity (Beck et al. 1990a, 1992b) as well as numerous other hormonal and metabolic changes, including elevated plasma insulin, leptin and corticosterone concentrations (McMinn et al. 1998; Raposinho et al. 2001; Baran et al. 2002). Adrenalectomy prevents the deleterious effects induced by chronic NPY administration (Sainsbury et al. 1997). In contrast to Zucker rats, however, NPY mRNA abundance is reduced by overfeeding in NPY-injected Long–Evans rats, which might be related to the high concentrations of insulin and active leptin in these rats (McMinn et al. 1998; Raposinho et al. 2001) unlike obese fa/fa rats (Schwartz et al. 1991). It should also be noted that NPY effects diminish after 9–10 days of infusion, reflecting a possible downregulation of receptors. This extinction of the effects is not observed in overweight rats after repeated (three times per day) injection of NPY (Stanley et al. 1989a) that allows a return to basal state between injections. Similar effects on body weight, adiposity and respiratory quotient are observed after chronic infusion for 6 days of Y1 and Y5 agonists (Mashiko et al. 2003; Henry et al. 2005), thereby confirming the role of both receptors in this system.

(ii) The obese ob/ob mouse
The hypothalamic NPY status of obese mice is somewhat comparable to that of the Zucker fa/fa rat. Hypothalamic NPY gene expression is upregulated (Wilding et al. 1993; Schwartz et al. 1996; Mercer et al. 1997; Stricker-Kronrad et al. 2002) and peptide content in the ARC nucleus is significantly increased (Jang & Romans 1998), although no change in the peptide concentrations are noted in either the whole hypothalamus or the PVN in the fed state (Williams et al. 1991b; Jang & Romans 1998; Stricker-Kronrad et al. 2002). Peptide content is neither modified by food deprivation nor by refeeding in obese mice unlike their lean counterparts (Jang & Romans 1998). There is a downregulation of the Y5 receptor level (Xin & Huang 1998), but no change in the feeding response to ICV injection of NPY (Morley et al. 1987b; Walker & Romans 1993). These data are consistent with an increased transport and release of NPY. The importance of NPY in ob/ob mice is furthermore demonstrated by the attenuation of the obese phenotype in the ob/ob mice crossed with NPY null mice (Erickson et al. 1996; Marsh et al. 1998). However, the ob/ob phenotype is unlikely to be mediated by NPY signalling through Y5 receptors because Y5 receptor-deficient ob/ob mice are equally obese (Marsh et al. 1998). Delivery of biologically active leptin through gene therapy in ob/ob mice induces a decrease of NPY expression in the ARC associated with body weight loss and diminished food intake (Dhillon et al. 2000).

(b) Other genetic models
(i) The Otsuka–Long–Evans–Tokushima Fatty rat
The Otsuka–Long–Evans–Tokushima Fatty (OLETF) rat is characterized by a lack of functional cholecystokinin (CCK-A) receptors (Miyašaka et al. 1994; Takiguchi et al. 1998; Moran & Bi 2006). The OLETF rats have therefore a peripheral satiety deficit that results in increased meal size and overeating (Moran et al. 1998), together with decreased responsiveness to ingested fat (Schwartz et al. 1999) and increased preference for sucrose (De Jonghe et al. 2005). It is moderately obese and diabetic and at least in part decreased physical activity favours weight gain (Sei et al. 1999). Defects in CCK-1 receptor signalling appear to be the major cause of hyperphagia in this rat. But it should be noted that a similar phenotype is not found in CCK-1 receptor null mice which are normoglycaemic and have a normal body weight (Kopin et al. 1999). This suggests the existence of either species differences or additional mutations independent of CCK in the OLETF rat. A very recent paper has described a mutated GPCR10 receptor in OLETF rats as responsible for its hyperphagia (Watanabe et al. 2005) even if the ligand of this receptor, prolactin-releasing peptide, is a poor mediator of food intake (Beck et al. 2004).

The NPY status in the OLETF rat is interesting. NPY mRNA expression is decreased in the ARC nucleus, whereas it is increased in the dorsomedial hypothalamus (DMH). The latter over-expression is already evident at five-week-old preobese rats (Bi et al. 2001). Food deprivation does not modify NPY mRNA in either area (Bi & Moran 2003), but exercise induces an increase in the ARC nucleus only (Bi et al. 2005). The OLETF rat is more sensitive to NPY injection than its lean control (Moran et al. 2002). This is in accordance with the observations made in rats with a NPY decrease in the ARC–PVN axis (Sahu et al. 1988a; Stricker-Kronrad et al. 1996). The role of NPY in the DMH remains to be clarified.

(ii) The yellow agouti mouse
The yellow agouti A yellow mouse is another well-studied model of obesity. The product of the agouti gene interacts with α-melanocyte stimulating hormone (α-MSH) at the level of melanocortin type 1 receptors to determine the coat colour of the animals. The agouti gene is expressed throughout the body of the animals. In the hypothalamus, its product, AgRP, interacts with α-MSH at MC4-R. This leads to the development of obesity associated with moderate hyperphagia and decreased thermogenesis (Moustaid Moussa & Claycombe 1999). Feeding behaviour of the obese agouti mouse is characterized by a durable preference for fat (Koegler et al. 1999). Over-expression of AgRP in transgenic mice results in the same syndrome (Graham et al. 1997) as well as the genetic deletion of the MC4-R (Huszar et al. 1997) and the long-term blockage of the MC4-R through HS014, a specific antagonist (Kask et al. 1999). The abundance of NPY mRNA in the ARC nucleus is similar to that in the lean mouse, but NPY is expressed at high levels in the DMN nucleus (Kesterson et al. 1997). The role of NPY in this model remains to be fully explored since ΔNPY-NPY knockout mice exhibit greater food intake and body weight then A yellow mice (Hollopeter et al. 1998).

(iii) The fat and tubby mice
In contrast to the yellow agouti mouse, the other obesity mutations in mouse models are recessive. The

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Table 1. Characteristics of the hypothalamic NPY system in some genetic animal models. (ARC, arcuate nucleus; PVN, paraventricular rat; OLETF rat, Otsuka–Long–Evans–Tokushima Fatty rat; CPE, carboxypeptidase E; n.d., not described to our knowledge.)

<table>
<thead>
<tr>
<th>mutation at:</th>
<th>food intake</th>
<th>ARC content</th>
<th>ARC mRNA expression</th>
<th>Y1/Y5 receptors</th>
<th>PVN release</th>
</tr>
</thead>
<tbody>
<tr>
<td>leptin receptor gene</td>
<td>Zucker fa/fa rat</td>
<td>↑ + +</td>
<td>↑ + +</td>
<td>↑ + +</td>
<td>Y1: ↓/Y5: ↓ +</td>
</tr>
<tr>
<td>db/db mouse</td>
<td>↑ + +</td>
<td>↑ + +</td>
<td>↑ + +</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Koletsky rat</td>
<td>↑ +</td>
<td>↑ +</td>
<td>↑ +</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>leptin gene</td>
<td>ob/ob mouse</td>
<td>↑ + +</td>
<td>↑ or →</td>
<td>↑ + +</td>
<td>Y5: ↓ +</td>
</tr>
<tr>
<td>CCK-A receptor</td>
<td>OLETF rat</td>
<td>↑ +</td>
<td>n.d.</td>
<td>↓</td>
<td>n.d.</td>
</tr>
<tr>
<td>tub gene</td>
<td>tubby mouse</td>
<td>↓</td>
<td>n.d.</td>
<td>↓</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Tubby mouse is characterized by an autosomal recessive mutation, which results in maturity-onset obesity (Coleman & Eicher 1990). The tub gene is well expressed in the brain (Kleyn et al. 1996; Nobentrauth et al. 1996). Its deletion is sufficient to give rise to the full spectrum of the tubby phenotype (Stubdal et al. 2000). In old mice, when obesity is present, NPY mRNA abundance is increased in the DMN and VMN nuclei and diminished in the ARC nucleus (Guan et al. 1998). The number of NPY-immunoreactive fibres is also decreased in the ARC (Backberg et al. 2004). It is unlikely that the decreased abundance of NPY mRNA in the ARC has an impact on the development of the obese phenotype because NPY mRNA is also decreased in the ARC in young non-obese animals. Thus the downregulation of the proopiomelanocortin (POMC) mRNA in the ARC (Guan et al. 1998) is thought to be more important in the development of the obese tub phenotype.

The fat mutation maps to the gene encoding carboxypeptidase E (CPE). CPE is widely distributed in the brain and is involved in the processing of peptides by selective cleavage at sites containing basic amino acids (Fricker & Leiter 1999). The CPE (fat) mouse has extremely low levels of CPE which together with other carboxypeptidases apparently are evidently sufficient for viability. The fat mouse only develops late-onset overeating and its obesity might therefore be related to defects in nutrient partitioning (Leiter et al. 1999). To our knowledge, the NPY status of this mouse is not known even though changes in some other regulatory peptides, such as thyrotropin-releasing hormone, neuropeitin, CCK8, POMC, opioid or melanin-concentrating hormone, have been described (Rovere et al. 1996; Wang et al. 1998b; Shen et al. 1999; Boudarine et al. 2002; Nillni et al. 2002). AgRP which is co-localized with NPY in the ARC is not modified (Wilson et al. 1999).

In summary (cf. table 1), the animal models of obesity with a deficiency in leptin signalling are characterized by an upregulation of the NPY system which contributes to their hyperphagia and excessive weight gain. The other genetic models of obesity present different and distinctive pictures with respect to NPY status. They are indeed characterized by a diminution in NPY expression in the ARC nucleus and by a significant increase in the DMN. A similar profile is also described in the obese hyperphagic UCP-1/Δ mice (Tritos et al. 1998). ARC, PVN and DMN belong to complex pathways involved in the control of food intake. NPY neurons in the ARC nucleus project their axons towards the DMH and PVN (Bai et al. 1985) and DMN neurons directly innervate the PVN (Thompson et al. 1996). NPY neurons in the DMH are also differentially regulated as they do not co-express leptin receptors (Bi et al. 2003) unlike NPY neurons in the ARC (Mercer et al. 1996a). In the other genetic models, an NPY increase in the DMH might therefore be a compensatory mechanism for the ARC deficiency in order to maintain adequate NPY levels in the PVN for the feeding regulation. Measurement of NPY release in the PVN could indicate if the peptide has a real importance in the hyperphagia of OLETF rats, agouti and tubby mice.

4. NPY IN DIET-INDUCED OBESITY (DIO)

Single gene mutations at the level of either the leptin gene or the NPY gene are very rare in human obesity (Hung et al. 2004; Farooqi & O’Rahilly 2005). Obesity is for the most part a polygenic disease (Perusse et al. 2005) and the environment, especially the nutritional conditions, in which genes function is particularly important. A plethora of studies have shown that obesity can be induced by ingestion of unbalanced diets either HF, HC or high-energy (HE) palatable (‘cafe-teria’) diets (Rothwell & Stock 1979; Louis-Sylvestre et al. 1984; Sclafani 1987; Warwick & Schiffman 1992). The fat content is a primary factor for the development of metabolic syndrome (Ghibaudo et al. 2002), and a 40–45% fat diet is already obesogenic as shown in...
multiple studies described below. The nature of the fat source is important and lower effects on weight gain are measured after a diet containing 32% fish oil than after the same diet containing either maize oil or beef tallow (Jang et al. 2003).

Several approaches have been used to study the relation between NPY and dietary-induced obesity. One approach consists in feeding animals with unbalanced diets (mostly HF diets) and then following peptide variations in parallel with body weight, adiposity and metabolic factors. A second depends on the selection of individuals with a susceptibility to obesity and their comparison with individuals resistant to obesity. A third is similar but uses rodent strains either resistant or sensitive to HF diets instead of individuals of the same strain.

As already indicated, hypothalamic NPY is sensitive to diet composition. In rats fed either HC or HF diets, it varies differentially according to the duration of the dietary exposure. There is a decline in NPY levels after short (two weeks) and long (two months) periods of ingestion of carbohydrate-rich diets by Long–Evans rats (Beck et al. 1990d, 1992c, 1994a). A transient decrease in NPY mRNA is noted after 2 days of ingestion of a HF diet (Ziotopoulou et al. 2000). During these 2 days, the rats are hyperphagic. After one or two weeks, NPY expression or content are unchanged or return to baseline in HF rats (Beck et al. 1992c; Lin et al. 2000b; Ziotopoulou et al. 2000; Hansen et al. 2004; Tabb et al. 2004), except when the HF diet consists of highly saturated fat (Wang et al. 2002) or when the fat content is increased to 77% (Giraudo et al. 1994). An absence of change in NPY mRNA expression is also noted after one week of a high-palatable (fat and sweet) diet (Kim et al. 1998). A significant decrease is once again observed after 8–10 weeks in HF diet-fed rats when the fat content is 45% or greater than 60% (Beck et al. 1994a; Lin et al. 2000b). The reduction in mRNA abundance is probably related to the substantial (more than 200%) increase in circulating leptin concentrations at eight weeks and the progressive development of leptin insensitivity with age (Qian et al. 1998). After 12 weeks, a decrease in NPY overflow from hypothalamic slices is observed (Hansen et al. 2004). An upregulation of Y5 receptors in adult rats fed for six weeks on HE diet is consistent with a diminished NPY release (Widdowson et al. 1997a). At 16–17 weeks, NPY concentration is diminished in HF fed rats in the PVN (Hansen et al. 2004; Velkoska et al. 2005) and in the ARC with a 30% fat diet (Hansen et al. 2004). At 19 weeks, however, when central leptin resistance is well established, NPY mRNA expression is not further decreased (Stricker-Krongrad et al. 1998; Lin et al. 2000a). Leptin resistance is specifically located in the ARC (Munzberg et al. 2004) and the sensitivity of the leptin system appears to be an essential element in the development of obesity (Ren et al. 2005). This link with leptin resistance is further supported by the absence of effect of HF diets in the obese Zucker rat (Mercer et al. 1996b).

A comparable diminution is present in rats fed a HE palatable diet (Widdowson et al. 1999; Archer et al. 2004) but in this case somewhat earlier, e.g. after five weeks. This is probably related to the greater sensitivity of the NPY system to the sucrose content of the diet. The interaction of NPY with palatable diets is supported by the decreased intake of such a diet in NPY knockout mice (Sindelar et al. 2005).

All these dietary treatments induce increased adiposity although they do not systematically lead to increased body weight or overeating, when given early in life e.g. from weaning (Oscai et al. 1984; Beck et al. 1994a; Archer et al. 2004). All these data are consistent with the existence of a counter-regulatory mechanism mediated at least partly by depressed NPY diminishing energy intake and limiting the development of obesity in rats consuming HF or HE diets. This is not, however, sufficient to totally counteract the obesogenic drive. Reducing energy intake from HF diets can attenuate but not suppress obesity (Petro et al. 2004). Data obtained in STZ diabetic rats support the downregulation of the NPY system by HF diets. When the STZ rats are fed a HF diet, they reduce their caloric intake in association with a decrease in NPY mRNA expression in the hypothalamus (Chavez et al. 1998). An intact hepatic vagus nerve is necessary for these changes (laFleur et al. 2003).

Further support to this hypothesis is provided by the study of individuals or strains either susceptible (DIO-S) or resistant (DIO-R) to dietary obesity. The Sprague–Dawley rat strain has been widely used for obesity studies. It has been characterized by the existence of obesity-prone and obesity-resistant rats (Levin et al. 1983, 1985; Levin 2006). These rats were detected after several months of exposure to HF diets. This bimodal distribution has, however, not always been confirmed (Archer et al. 2003) perhaps because of the different origin of the rats. Therefore, in some studies (Chang et al. 1990; Gao et al. 2002; Farley et al. 2003), rats characterized as DIO-S or DIO-R are taken at each end of the body weight distribution of a large population.

The DIO-S rats are hyperphagic and their hyperphagia is due to an increased meal size (Farley et al. 2003). They have also a different energy metabolism with a lower energy expenditure and an increased respiratory quotient indicating a lower use of fat as fuel substrate favouring therefore fat storage (Chang et al. 1990; Gao et al. 2002).

Cross-breeding of high weight gaining rats over several generations reinforces their characteristics and leads to a strain of DIO-S rats that are heavier than DIO-R rats even on a low-fat (LF) diet (Levin et al. 1997, 2003; Levin & Dunn-Meynell 2002c). Obesity induced during gestation in DIO-S dams fed a HE diet further enhances obesity in offspring (Levin & Govek 1998). The DIO-S rats are characterized by higher (greater than 40%) NPY mRNA expression in the ARC in the preobese state (Levin & Dunn-Meynell 1997; Levin 1999; Gao et al. 2002) and by the absence of regulation of this expression by 48 hour fast or 50% food restriction (Levin & Dunn-Meynell 2002b; Levin et al. 2004), or insulin-induced hypoglycaemia (Tkacs & Levin 2004). These changes might be due to the early loss of sensitivity to leptin (Levin & Dunn-Meynell 2002b; Levin et al. 2004) and to a decreased responsiveness of hypothalamic neurons to
glucose (Levin et al. 1998). However, when obesity is well established in 22-week-old rats, NPY mRNA expression is about 20% lower than in DIO-R rats (Levin 1999), although the NPY system becomes responsive to diet restriction and palatability (Levin 1999; Levin & Dunn-Meynell 2002a) in order to defend body weight. Leptin delivery directly in the PVN through gene therapy reverses dietary obesity and normalizes the metabolic parameters (Bagnasco et al. 2003). Exercise, which induces a lower weight gain, is not associated with any NPY change in the ARC and DMN in DIO-S rats (Levin & Dunn-Meynell 2004). However, in the case of the selected DIO-S mice, there is leptin-resistance after 22 weeks on a 40% fat diet, NPY mRNA expression is increased and the whole NPY system is disturbed as Y5 mRNA expression is 36% higher (Huang et al. 2003).

Increased activity of the NPY system has been described in other rodent strains. It is well known that some rat (Schemmel et al. 1970; Bray et al. 1987) and mouse (West et al. 1992; Kobayashi et al. 2004) strains are more susceptible to develop obesity on a HF diet. This susceptibility is often associated with an increased consumption of fat in a food choice paradigm (Smith et al. 2000). The Osborne–Mendel rat is one of these DIO-S rats. Its NPY mRNA expression is about 50% higher than in S5B/P1 rats (Schaffhauser et al. 2002). Despite this increase, Y1 and Y5 mRNA expression is raised by 16–71% with the highest augmentation measured on a LF diet (Schaffhauser et al. 2002). This is the opposite to the receptor downregulation existing in the Zucker rat (Beck et al. 2001b). This rat remains, however, responsive to central leptin injection even after five weeks on HF diet (Lin et al. 1998).

The C57BL/6J and DBA mouse strains belong to the group of DIO-S strains whereas the A/J and SWR/J mice are DIO-resistant (West et al. 1992; Surwit et al. 1995; Tortoriello et al. 2004). On a HF diet for 14 weeks, NPY mRNA expression decreases in A/J mice but not in C57BL/6J mice (Bergen et al. 1999). This is not observed after a shorter exposure to HF diet (Bullen et al. 2004). The NPY system is also upregulated in DBA/2J mice (Tortoriello et al. 2004). These central NPY changes might be related to the high circulating concentrations of leptin (Surwit et al. 1997) and the maintenance of leptin responsiveness in A/J mice (Watson et al. 2000) while C57BL/6J mice are less responsive to leptin in the fed state (Takahashi et al. 2002). Leptin responsiveness is also normal in the DIO-R SWR/J mice (Takahashi et al. 2002), leading to a suppression of NPY mRNA expression when leptin levels are elevated.

In summary, it is evident that there is an adaptation of the NPY system when normal rats are fed an energy-dense food. The diminution in hypothalamic NPY is, however, not sufficient to totally stop excessive weight gain in most cases. In DIO-S strains or individuals however, there is a clear link between the obesity of these animals and increased activity of the NPY system and disturbed receptor regulation. Decreased sensitivity to leptin appears to be a major factor explaining these changes. Other factors are, however, probably involved in the short term resistance to DIO in some strains (Bullen et al. 2004). Some of these factors can be linked to energy expenditure through the uncoupling proteins whereas some others remain unknown (Surwit et al. 1998; Bullen et al. 2004).

5. CONCLUSION
This review is focused on the role of NPY in feeding regulation in normal and obese animals (figure 2). Notwithstanding evidence for the importance of NPY, it is obvious that feeding results from interaction between numerous neuropeptides and neurotransmitters in the central nervous system as well as peripheral hormones (Kalra et al. 1999; Schwartz et al. 2000). NPY is implicated in the changes that occur in many

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obesity models (Beck 2000). The melanocortins have a particular and direct interaction with NPY (Seeley et al. 2004; Cone 2005) and there are also strong interactions with the stress hormone corticotropin-releasing hormone (Heilig 2004). All these systems exhibit plasticity in the face of changes in nutritional and environmental factors, the specific response depending on the situation and on individual sensitivity. Given the complexity of this system it is not particularly surprising to find discrepancies between studies as described in this review. Or, for that matter, between different models—for example, animals with hypothalamic lesions induced by gold thioglucose (GTG) or MSG develop obesity in the presence of lower hypothalamic NPY content and/or mRNA expression (Kerkerian & Pelletier 1986; Marks et al. 1996; Stricker-Krongrad et al. 1996; Bergen et al. 1998), and are associated with different feeding behaviours (hyperphagia in the GTG model and hypophagia in MSG-treated animals). The compensatory mechanisms that are likely to be involved in these cases are also well exemplified in knockout models (Beck 2001; Herzog 2004) and double knockout mice (Qian et al. 2002). For this reason, efficacious treatment of obesity through a specific agonist or antagonist may be difficult to achieve; instead, it may be particularly surprising to find discrepancies between studies as described in this review. Or, for that matter, depending on the situation and on individual sensitivity.

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REFERENCES


DellaZuana, O. et al. 2001 Reduced food intake in response to CCP 71635A may be due to mechanisms other than NPYY5 receptor blockade. Int. J. Obes. 25, 84–94. (doi:10.1038/sj.ijo.0801472)


Flood, J. F. & Morley, J. E. 1991 Increased food intake by neuropeptide Y is due to an increased motivation to eat. Peptides 12, 1329–1332. (doi:10.1016/0196-9781(91)90215-B)


Phil. Trans. R. Soc. B (2006)

NPY, feeding behaviour and obesity B. Beck 1177


NPY, feeding behaviour and obesity

B. Beck 1179


Phil. Trans. R. Soc. B (2006)


Stanley, B. G., Magdalin, W., Seirafi, A., Thomas, W. J. & Leibowitz, S. F. 1993 The perifornical area—the major focus of (a) patchily distributed hypothalamic neuropeptide Y-sensitive feeding system(s). Brain Res. 604, 304–317. (doi:10.1016/0006-8993(93)90382-W)


Surwit, R. S., Petro, A. E., Patel, H. R., Qi, Y., Dushay, J. & Ahima, R. S. 2002 Divergent effects of leptin in mice susceptible or


Turnbull, A. V. et al. 2002 Selective antagonism of the NPYY5 receptor does not have a major effect on feeding in rats. *Diabetes* 51, 2441–2449.


