MINIREVIEW

Evidence for a Central Mechanism of Obesity in the Zucker Rat: Role of Neuropeptide Y and Leptin[1] (44090)

B. DOUGLAS WHITE AND ROY J. MARTIN[2]
Department of Foods and Nutrition, University of Georgia, Athens, Georgia 30602

Abstract. **Thirty-five years ago, Lois and Theodore Zucker reported the discovery of a genetic mutation in the rat that resulted in juvenile-onset obesity, increased food intake, decreased energy expenditure, and insulin resistance. The mutation was called fatty (*fa*). The fatty gene is passed on to successive generations by an autosomal recessive mode of inheritance. In the intervening years, much work has been done to characterize the many abnormalities of this animal model of obesity. Nearly 10 years ago, we reviewed the evidence for a central nervous system mechanism in the etiology of obesity in the fatty Zucker rat. Since that time, the discovery of novel peptides and genes has revolutionized the study of the etiology of genetically linked obesities. In this review, we update the evidence for a central nervous system mechanism of obesity in Zucker rats by focusing on the possible role of neuropeptide Y (NPY) and leptin in the etiology of obesity. We also discuss the role of glucocorticoids and insulin in the regulation of NPY.** [P.S.E.B.M. 1997, Vol 214]

The discovery of novel biological peptides and genes has revolutionized work concerning the etiology of genetically linked obesities. This review will focus on recent evidence of a link between obesity in the Zucker rat and two important central nervous system (CNS) active peptides, neuropeptide Y and leptin.

Earlier, we reviewed the evidence for a central nervous system mechanism of obesity in the Zucker fatty (*fa/fa*) rat (1). In that report, we summarized the many peripheral changes in the obese Zucker rat that could be linked to a disruption of the central nervous system. For example, the increased rate of adipose cell lipogenesis in pair-fed obese rats was caused by extremely high levels of insulin secretion. This appears to be the result of increased parasympathetic activity. The increase in energetic efficiency of the Zucker rat was explained by a decrease in sympathetic output to brown fat in the obese rat. Hyperphagia was linked to a disrupted feeding mechanism in the brain. The decrease in bone and muscle growth that occurred in the pair-fed obese Zucker rats was linked to the decrease in growth hormone status and the CNS control of the pituitary release of growth hormone. This review will provide further details of the central nervous system mechanisms that are disrupted in the obese Zucker rat. Specifically, we will provide evidence that obese Zucker rats are insensitive to leptin, potentially an important endogenous inhibitor of neuropeptide Y (NPY), and that elevated NPY activity is central to the alterations seen in obesity.

Role of NPY in the Etiology of Obesity in Zucker (*fa/fa*) Rats

Neuropeptide Y is a 36–amino acid peptide, first isolated in 1982 from porcine brain (2). NPY is one of

---

[1] This manuscript is an update of a previously published minireview entitled, "Evidence for a central mechanism of obesity in the Zucker fatty rat (*fa/fa*)" (Proc Soc Exp Biol Med **183**:1–10, 1986).

[2] To whom requests for reprints should be addressed at Department of Foods and Nutrition, Dawson Hall, University of Georgia, Athens, GA 30602.

P.S.E.B.M. 1997, Vol. 214

0037-9727/97/2143-0222$10.50/0

Table I. Effects of Central NPY Administration That Are in Common with Abnormal Characteristics of Obese Zucker Rats

1. Increased food intake
2. Increased body fat accumulation
3. Decreased sympathetic activity to brown adipose tissue
4. Decreased heat production
5. Increased *de novo* fatty acid synthesis
6. Increased serum triglycerides
7. Increased glucose utilization of white adipose tissue
8. Increased lipoprotein lipase activity of white adipose tissue
9. Increased serum insulin concentration
10. Increased serum corticosterone concentration
11. Decreased sexual behavior
12. Altered circadian rhythms

the most abundant neuropeptides in the mammalian brain, being found extensively throughout the central and peripheral nervous systems (3, 4). NPY was originally isolated based on its chemical structure. It has an amidated C terminus. As a result, NPY has been characterized, both biochemically (5) and molecularly (6), independent of any known physiological function.

Studies examining the potential function(s) of NPY have suggested NPY to have cardiovascular, gastrointestinal, reproductive, endocrine, and behavioral effects (7). Many of the effects of central administration of NPY are similar to the abnormal characteristics of obese Zucker rats (Table I).

Central administration of NPY is one the most potent inducers of food intake known. The orexigenic effect of NPY was first described by Clark *et al.* in 1984 (8). Exogenous NPY administered into the cerebral ventricles or directly into various areas of the hypothalamus elicits robust intermittent feeding for several hours (8–10). The effect of NPY on feeding does not appear to develop tolerance (11). Multiple injections of NPY over a period of several days results in sustained overfeeding and increased body fat accumulation (12, 13). Apparently, NPY is able to override endogenous satiety signals. As in obese Zucker rats, body fat accumulation caused by chronic NPY administration is not due entirely to the increase in food intake. NPY also decreases sympathetic activity to brown adipose tissue (14, 15). This decreases the production of heat, making animals more energy efficient. Obese Zucker rats pair-fed to the level of their lean counterparts gain more body fat than lean rats (16). This is believed to be due, at least in part, to a decrease in the sympathetic activity to brown adipose tissue in obese Zuckers (17, 18).

Jeanrenaud and colleagues have recently examined the metabolic and hormonal effects of chronic central NPY administration in Sprague-Dawley rats and have also noted many similarities to obese Zucker rats (19–21). Chronic NPY infusion increases the activity of the lipogenic enzyme, acetyl coenzyme-A carboxylase, as well as *de novo* fatty acid synthesis in both white adipose tissue and the liver. The latter effect most likely contributes to the increased serum triglyceride concentrations seen after NPY treatment. NPY increases both glucose utilization and lipoprotein lipase activity in white adipose tissue. Both factors would tend to promote lipid accumulation in fat cells.

Central NPY administration also increases serum insulin (19, 22–24) and corticosterone concentrations (19, 25, 26). The elevation of serum insulin and corticosterone concentrations has long been known to be a characteristic of obese Zuckers (27, 28). Contrary to central administration, the direct effect of NPY on pancreatic islets is to inhibit insulin release (22, 29). Increased insulin release with central administration may be due to the NPY-induced decrease in sympathetic activity (14, 15). Central NPY appears to stimulate directly the hypothalamic-pituitary-adrenal axis. NPY-containing nerve terminals are in close association with CRH cell bodies in the PVN (26). The injection of NPY into the PVN causes a rapid increase in serum ACTH and corticosterone levels (26). NPY also increases the content of CRH (30) and the level of CRH gene expression (31), suggesting that NPY increases CRH synthesis. NPY may also act in synergy with CRH to enhance the release of ACTH from the anterior pituitary (32).

The prevention of hyperphagia by pair-feeding does not reverse any of the above metabolic or hormonal changes induced by NPY (19), indicating that NPY *per se* is responsible for these effects and they are not secondary effects of hyperphagia. If NPY treatment is ceased, the abnormalities are normalized (21), which shows that the continued presence of high NPY levels is required in order to maintain these abnormalities.

An early observation of NPY was that ICV administration will inhibit sexual behavior in rats (33). The data suggest that NPY selectively depresses sexual motivation. The inhibitory effect of NPY on copulatory behavior has since been confirmed and the effect has been shown not to be secondarily related to increased feeding (34). Obese Zucker rats have a reduced reproductive capacity (35) and display a decrease in sexual behavior (36). In studies using artificial insemination, sperm derived from obese rats are normal in their capacity to fertilize eggs (36). Thus, obese rats, like rats injected with NPY, appear to have dminished sexual motivation. It is this effect that appears to be the primary cause of their decreased reproductive capacity (36).

Not only do obese Zucker rats show alterations in feeding and ambulatory activity, but they also display disruptions in the light : dark cycle of these behaviors. Lean rats show a higher percentage of their activity during the dark period. However, in obese rats, as the obesity progresses, more ingestive and ambulatory activity is shifted toward the light period (37). This disruption of light : dark activity is not due to a disappearance of the circadian rhythm, but rather to a shift and/or a decrease in amplitude of the circadian cycle (38). The suprachiasmatic nucleus (SCN) is believed to play a role in determining light : dark periodicity. NPY-containing cells from the intergeniculate leaflet project to the ventrolateral portion of the SCN. Transitions from light to dark or vice versa have been found to be associated with an increase in the content of NPY in the SCN (39–41). This suggests that NPY may have a role in the process of photic entrainment of circadian rhythms. Other data suggest that NPY may also be involved in nonphotic shifts of the circadian rhythm (42–44). Injections of exogenous NPY into the SCN have been shown to shift activity rhythms of hamsters (42, 45). This effect is not secondary to an increase in motor activity (42).

The above illustrates some of the similarities between the abnormal characteristics of obese Zucker rats and the effects of exogenous NPY administration. These similarities provide indirect evidence consistent with the idea that elevated NPY activity may be causative of the obesity in Zucker rats. More direct evidence comes from the finding that NPY content is elevated in obese Zucker rats. Several studies have found the content of NPY in various hypothalamic nuclei to be elevated in obese Zucker rats. These include the acruate, paraventricular, suprachiasmatic, and medial preoptic areas (46–50). These areas are known to be involved with the regulation of feeding, autonomic nervous system activity, entrainment to circadian rhythms, and reproduction. Phenotypic differences in NPY content have been found in the arcuate nucleus and suprachiasmatic nuclei in animals at 30 days of age (just after weaning), but not at 16 days of age (49, 50). Since obese Zucker rats do not begin to overeat until after weaning (51, 52), these data suggest an association between elevated endogenous levels of NPY and hyperphagia in obese animals.

Alone, changes in tissue content of a neuropeptide can be difficult to interpret, since content is determined by both synthesis and release. In addition to an elevated tissue content of NPY, obese Zucker rats also have increased gene expression of NPY in the arcuate nucleus of the basomedial hypothalamus (53–55). The arcuate nucleus is an important site of NPY synthesis for the hypothalamus (56). This is an important finding because, together with the content data, it suggests that NPYergic activity is elevated in the hypothalamus of obese Zucker rats. One study has suggested that hypothalamic NPY gene expression is not increased until after 11 weeks of age (57). This would argue against NPY's having a role in the development of obesity. Others, however, have found elevated NPY gene expression in younger obese Zucker rats. One study found elevated NPY gene expression in 5-week-old obese Zucker rats (58), while another found elevated NPY gene expression in 6- to 9-day-old animals (59). The latter finding is impressive because these animals are in a pre-obese state. Increases in NPY gene expression precede changes in body composition or circulating insulin (59). This indicates that changes in NPY gene expression occur very early in the life of obese Zucker rats, and supports a role for altered NPY activity in the development of obesity.

Obese Zucker rats have an altered responsiveness to exogenous NPY. In one study, obese Zucker rats failed to show a feeding response to third-ventricular injections of NPY, even with doses up to 30 μg (60). Lean animals, on the other hand, reliably responded to doses as low as 3 μg. This could suggest that in obese rats, NPY receptors are already saturated by high levels of endogenous NPY. Alternatively, it could suggest a decrease in NPY responsiveness with a threshold response of greater than 30 μg. Others have found obese Zucker rats to have a decrease in responsiveness to NPY (61, 62). Though in these two studies obese rats responded as lean rats did to high doses of NPY, they showed a diminished feeding response to low doses. It was suggested that the diminished threshold response in obese rats may be due to either high endogenous levels of NPY or to a decreased number of NPY receptors. Indeed, McCarthy *et al.* reported a decrease in the number of hypothalamic NPY receptors in obese Zucker rats, suggesting that NPY receptors may be downregulated (61). These studies further support the idea of increased NPY activity in obese Zucker rats.

Neuropeptide Y: Regulation by Leptin

How might the mutated fatty (*fa*) gene in obese Zucker rats lead to increased NPY activity? Recent studies concerning the molecular genetics of the *ob* and *db* mutations together with the classic parabiotic studies of obese animals provide a clue. Hervey showed that a normal rat would become anorexic when parabiosed to a rat made hyperphagic by lesioning the ventromedial hypothalamus (VMH) (63). It was proposed that as the VMH-lesioned rat becomes obese, it produces a factor that crosses the parabiotic union and inhibits food intake in the normal rat. The lesion renders the satiety factor ineffective in the lesioned animal. Parabiotic studies have also been used to examine two forms of genetic obesity in mice, obese (*ob*) and diabetic (*db*). Like the Zucker rat, both mouse models are characterized by early-onset obesity, hyperphagia, and hyperinsulinemia (64). Coleman and Hummel showed that, like the VMH-lesioned rat, when a diabetic (*db/db*) mouse is parabiosed to a normal mouse the normal mouse loses weight and dies of apparent starvation (65). This suggests that the diabetic (*db/db*) mouse is producing a satiety/anti-obesity factor, but is unable to respond to it. In contrast, other researchers showed that when an obese (*ob/ob*) mouse is parabiosed to a normal mouse, the rate of weight gain does not decrease in the normal mouse; rather, body weight is decreased in the obese (*ob/ob*) mouse (66–68). Hausberger first suggested that the obese (*ob/ob*) mouse is missing an anti-obesity factor that can be supplied by the normal mouse (66). Further work by Coleman showed that when the obese (*ob/ob*) mouse is parabiosed to a diabetic (*db/db*) mouse the obese (*ob/ob*) mouse loses weight and dies of apparent starvation (69). Together, these data are consistent with the idea that obesity in these two mouse models are caused by a common factor, Obese (*ob/ob*) mice appear unable to produce a satiety/anti-obesity factor, though they are responsive to it. Diabetic (*db/db*) mice, on the other hand, produce the satiety/anti-obesity factor but are unresponsive to it.

The genetic mutation of the obese (*ob/ob*) mouse has been cloned (70). The defective gene codes for a 16-kDa protein that appears to be expressed exclusively in white adipose tissue. The *ob* gene product has been termed leptin (71). Several groups have reported that recombinant leptin decreases food intake and body weight in obese (*ob/ob*) mice (71–75). Because low doses of leptin are more effective when injected into the cerebroventricles of the brain, it appears that lepin has a central site of action (73, 75). Indeed, a saturable unidirectional transport system across the blood brain barrier has been identified for leptin which has an entry rate similar to other blood-borne regulatory compounds (76). Leptin is secreted into the blood, and its concentration reflects the amount of body fat (77, 78). Thus, it appears that leptin provides the brain with information concerning the amount of fat stored in the body. In addition to decreasing food intake, leptin also increases body temperature, suggesting that changes in metabolism also play a role in decreasing body weight (72). As would be expected from the work of Coleman, diabetic (*db/db*) mice fail to response to recombinant leptin (71, 73, 75). Moreover, diabetic (*db/db*) mice have elevated endogenous levels of leptin (71, 77) and increased leptin gene expression (77). A similar increase in circulating leptin levels (77) and gene expression of leptin in white adipose tissue have been found in obese humans (77, 79, 80).

Recent studies have cloned the leptin receptor and have shown it to map to the location of the *db* mutation (81–83). At least six alternative splicing forms of the leptin receptor mRNA have been identified (82). Diabetic (*db/db*) mice have a longer fragment length of one of these forms (Ob-Rb). A guanidine to thymidine point mutation causes a 106-bp insertion that results in truncation of the cytoplasmic domain of the leptin receptor (82). This likely results in defective signal transduction.

As with diabetic (*db/db*) mice, obese (*fa/fa*) Zucker rats parabiosed to lean rats cause weight loss in the lean rats (84). The reduction in body weight is due to a reduction in body fat. Obese Zucker rats have been found to have elevated plasma concentrations of leptin (77) and increased leptin gene expression in white adipose tissue (77, 85). Thus, as with the diabetic (*db/db*) mouse, the Zucker (*fa/fa*) rat appears to overproduce but not respond to leptin. A study using linkage analysis has previously suggested that the fatty (*fa*) mutation of the obese Zucker (*fa/fa*) rat is homologous with the *db* mutation of the diabetic (*db/db*) mouse (86). Recent genetic maps have shown that the *fa* mutation and the leptin receptor are within at least 600 kb of each other (83), further suggesting that the *fa* mutation is a mutation of the gene for the leptin receptor. Two recent studies have found a point mutation in the gene for the leptin receptor in obese Zucker rats (87, 88). A single adenine to cytosine transversion results in a glutamine to proline substitution at residue 269 of the leptin receptor. The site of the mutation is highly conserved, being present in all isoforms of the leptin receptor. It was suggested that this mutation does not alter leptin binding, but rather may interfere with dimer formation (87). This step could be essential in signal transduction. The above data suggest that the *fa* mutation of obese Zucker rats is a point mutation in the gene of the leptin receptor which prevents the animal from responding to circulating leptin.

As with obese (*fa/fa*) Zucker rats, obese (*ob/ob*) and diabetic (db/db) mice have elevated levels of hypothalamic NPY gene expression (89, 90). This is consistent with the idea that the increase in NPY gene expression is related to a functional lack of leptin. High-affinity leptin binding has been found in the hypothalamus (75), as well as other areas such as the choroid plexus (81). The OB-Rb form of the leptin receptor mRNA (the isoform abnormally spliced in diabetic (*db/db*) mice) is highly expressed in the hypothalamus (82). Autoradiography has shown intravenously administered [^{125}I]leptin to bind to the arcuate nucleus and median eminence of the hypothalamus (76). These data suggest that leptin may have a hypothalamic site of action in close proximity to a major site of NPY synthesis.

Leptin treatment in obese (*ob/ob*) mice has been found to decrease NPY gene expression in the arcuate nucleus (75, 91). This appears to be a specific effect of leptin and not a secondary effect due to an alteration in food intake or body weight (91). Leptin has been also found to decrease glucocorticoid-stimulated hypothalamic NPY relase *in vitro* (75). Leptin has no effect on NPY gene expression in diabetic (*db/db*) mice (75, 91), again indicating the unresponsiveness of diabetic (*db/db*) mice to leptin. These findings suggest that leptin functions, at least in part, by inhibiting NPY synthesis and release. Because obese (*ob/ob*) mice do not produce functional leptin, NPY activity is not attenuated resulting in elevated NPY activity. Because obese (*fa/fa*) Zucker rats and diabetic (*db/db*) mice do not respond to circulating leptin, these mutations also lead to increased NPY activity. A chronic increase in NPY activity would be expected to increase food intake, decrease energy expenditure, and create an internal milieu that promotes body fat accumulation.

Recently, NPY-deficient mice were developed from embryonic stem cells in which the NPY gene was disrupted (92). In these mice, there was no detectable NPY mRNA in the brain. Interestingly, except for an increased susceptibility to seizures, these mice developed normally. Food intake, body weight, and body fat were normal as were serum glucose, insulin and corticosterone concentrations. This may indicate that when an animal develops in the absence of NPY, other redundant systems are able to compensate for the lack of NPY. Alternatively, the effect of NPY may be unidirectional. A relative lack of NPY activity may not have much of an effect on the regulation of food intake and body weight compared with the control state, whereas a relative increase in NPY activity may have a great effect. It was noted that NPY-deficient mice are initially more sensitive to the food inhibiting effects of acute leptin treatment. This indicates that some of leptin's effect can be independent of NPY. It also suggests that NPY is able to affect leptin responsiveness.

Neuropeptide Y: Regulation by Glucocorticoids

Neuropeptide Y and obesity are also influenced by other hormones, such as glucocorticoids. It has long been known that many of the abnormal characteristics of obese Zucker rats can be reversed or attenuated by adrenalectomy (93–95). The effects of adrenalectomy are reversed by corticosterone treatment, suggesting that glucocorticoids are necessary for the expression of obesity. If, as is our contention, obesity in the obese Zucker rat is mediated, at least in part, by chronically elevated NPY activity, then it would appear that increased NPY activity or the responsiveness to NPY may be dependent on circulating glucocorticoids. This appears to be the case. Adrenalectomy has been shown to suppress NPY-induced feeding (96, 97), while corticosterone replacement normalizes feeding response (97). This suggests that the response to exogenous NPY is dependent on the presence of circulating glucocorticoids. The mechanism by which glucocorticoids affect NPY responsiveness is not known. Consensus sequences for glucocorticoid-responsive elements have been found within the 5′-flanking region of the Y1-receptor gene (98). Moreover, chronic glucocorticoid treatment increases Y1-receptor gene expression (99) and peptide YY binding (100) within the arcuate nucleus, suggesting that circulating glucocorticoids may influence NPY receptor populations. Glucocorticoids may also have an indirect effect on NPY responsiveness through alterations in hypothalamic corticotropin-releasing hormone (CRH) levels. Inhibition of endogenous CRH activity by injections of the CRH receptor antagonist, α-helical $CRH_{9\text{-}41}$, into the paraventricular nucleus (PVN) (101) or by the destruction of CRH-containing cells in the PVN by immunotargeted cytotoxins (102) result in an enhanced feeding response to exogenous NPY. These data suggest that CRH in the PVN exerts an inhibitory effect on the response to NPY. Adrenalectomy, by attenuating feedback inhibition of glucocorticoids, results in increased levels of CRH in the PVN that could attenuate NPY responsiveness.

Other evidence suggests that glucocorticoids may influence NPY synthesis. In the hypothalamus and brainstem there is a close association between NPY-containing neurons and glucocorticoid receptors (103, 104). In the arcuate nucleus, a major site of NPY synthesis, all NPY immunopositive neurons also contain glucocorticoid receptors (104). Early work with cell cultures showed that dexamethasone can increase NPY gene expression in neuronal cell lines (105) and NPY levels in aggregating fetal brain cells (106). Other investigators have shown *in vivo* dexamethasone administration to increase NPY levels in the arcuate and/or paraventricular nucleus (PV) (107–109). Other studies did not find an increase in NPY content with the glucocorticoid, bethamethasone (110), or corticosterone (100), but did observe a decrease in NPY content in the arcuate nucleus and/or PVN with adrenalectomy. Adrenalectomy has been found to decrease NPY gene expression in the arcuate nucleus in some studies (111, 112), suggesting that NPY synthesis is supported by endogenous levels of corticosterone. Other studies have not found a decrease in NPY gene expression with adrenalectomy, but have shown an increase in gene expression with glucocorticoid treatment (99, 100, 113). This difference with adrenalectomy may reflect methodological differences in the time between adrenalectomy and sample collection or differences between the time of day of sample collection. Overall, these data support a stimulatory role of glucocorticoids in NPY activity.

The mechanism by which glucocorticoids stimulate NPY synthesis is not known. Glucocorticoids may have a direct effect on NPY synthesis. Three consensus glucocorticoid response elements have been located within the 5′-flanking region of the NPY gene (114). Transfection studies have shown this region of the NPY gene to confer glucocorticoid inducibility of a reporter gene. Other evidence suggests that there may also be an indirect effect of glucocorticoids on CRH levels that could affect NPY synthesis. Exogenous CRH administration has been shown to decrease NPY gene expression in obese Zucker rats (115). This suggests that adrenalectomy could decrease NPY gene expression by increasing hypothalamic CRH levels secondarily to decreased feedback inhibition from lower glucocorticoid levels.

Evidence has suggested that glucocorticoids act through the Type II corticosteroid receptor subtype to increase NPY synthesis and activity. Dexamethasone or RU362, two synthetic glucocorticoids that act through

Type II corticosteroid receptors, increase NPY levels and NPY gene expression (105–109, 113). The Type I receptor agonist, on the other hand, appears to have no effect (113). In addition, the Type II receptor antagonist mimics the effect of adrenalectomy on NPY-induced feeding, whereas the Type I receptor antagonist is without effect (116). It is of interest that antagonism of Type II corticosteroid receptors has also been found to prevent hyperphagia and decrease body fat in obese Zucker rats (117). Potentially these effects in obese Zucker rats could be mediated through attenuation of elevated NPY synthesis and activity. Direct observation of the effects of Type II receptor antagonism on NPY gene expression in obese Zucker rats, however, has produced equivocal results. Though treatment with the Type II receptor antagonist did appear to equalize NPY gene expression in lean and obese Zucker rats, no significant effect was noted because, in this particular experiment, no difference in NPY gene expression was found between the phenotypes in the control group (54).

Obese Zucker rats have been found to have elevated serum concentrations of corticosterone in the morning resulting in relatively high corticosterone concentrations throughout the day (28, 118, 119). High levels of corticosterone, like those found at the peak of the circadian cycle or during stress, interact with Type II corticosteroid receptors. This receptor subtype is found in abundance in the arcuate nucleus (104). Therefore, a sustained elevation in the serum corticosterone serum concentration would result in greater interaction with Type II receptors and may contribute to the increased NPY synthesis and activity found in obese Zucker rats.

Neuropeptide Y: Regulation by Insulin

As opposed to the stimulatory action of glucocorticoids, insulin appears to inhibit NPY activity and NPY synthesis. High levels of hypothalamic NPY are found in rats made diabetic by streptozotocin treatment (120–123). It is of interest to note that diabetic animals are also hyperphagic. Both the elevated NPY levels and hyperphagia are reversed by insulin treatment (122, 123). Streptozotocin-induced diabetes increases the *in vitro* release of NPY from the PVN as well as the *in vivo* release of NPY as determined by push-pull cannula (124). The increase in NPY content with streptozotocin treatment is mirrored by an increase in gene expression in the arcuate nucleus (125, 126), suggesting that low insulin concentrations increase NPY synthesis. The increase in NPY gene expression can be reversed by either peripheral (125) or central (126) insulin treatment. An elevation in NPY content in the PVN and arcuate nucleus has also been found in spontaneously diabetic (BB) rats (123).

Insulin treatment reverses the increase in NPY content in the PVN and NPY gene expression in the arcuate nucleus associated with fasting (127). Since fasting is associated with a decrease in serum insulin levels, this suggests that increased NPY synthesis during fasting is dependent on low insulin levels. Interestingly, other evidence suggests that increased hypothalamic NPY during fasting is also dependent on the presence of glucocorticoids (128).

Though obese Zucker rats do not express frank diabetes, they are insulin resistant. This suggests that rather than serum insulin levels *per se,* it is glucose availability or utilization in specific brain areas that may be the important factor in insulin's regulation of NPY activity. Studies that have examined the response of NPY gene expression in hyperinsulinemic-euglycemic rats support this view (129). Moreover, 2-deoxyglucose treatment, which decreases glucose utilization and increases food intake, is associated with an increased content (130) and gene expression (131) of NPY in the arcuate nucleus.

The number of insulin receptors is decreased in the brains of obese Zucker rats (132). Central insulin administration inhibits NPY gene expression in food-deprived lean rats but not in food-deprived obese rats (126). Additionally, obese rats, unlike lean rats, do not decrease their food intake in response to insulin treatment (133). Those data suggest an impairment of the action of central insulin in obese Zucker rats, which could contribute to the increased NPY activity found in obese Zucker rats.

Interaction between Glucocorticoids and Insulin

Glucocorticoids and insulin appear to interact with each other to affect NPY activity and feeding. The increase in NPY activity observed with dexamethasone treatment is reversed by the concomitant administration of insulin (109). Glucocorticoids cause a greater increase in feeding in STZ-induced diabetic animals than in animals with normal insulin status (134). Elevated corticosterone levels are required to fully increase NPY levels and food intake in diabetic animals (135). In insulin-intact animals, elevated serum corticosterone levels stimulate insulin secretion, which in turn attenuates further elevations in NPY gene expression and food intake (135). Since obese Zucker rats are insulin resistant and have elevated levels of corticosterone, they would be expected to fully express NPY activity and feeding.

Figure 1 shows a schematic of the proposed relationships among leptin, glucocorticoids, and insulin in the regulation of NPY activity. Leptin is secreted from white adipose tissue in proportion to the amount of fat. Normally, leptin inhibits NPY activity, which would tend to decrease food intake and increase energy expenditure. These effects would lead to an appropriate decrease in body fat (Fig. 2). However, because the fatty (*fa*) mutation of obese Zucker rats alters the leptin receptor, the signal from leptin is not transduced, and NPY activ-

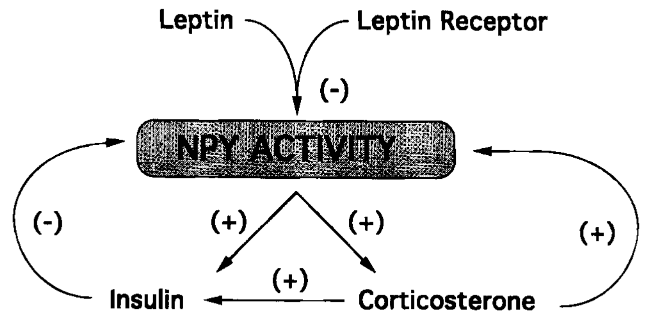

Figure 1. Proposed relationships among leptin, corticosterone, and insulin in the regulation of neuropeptide Y (NPY) activity. Leptin interacting with its receptor leads to an inhibition of NPY activity. Mutations that result in a defect in leptin (*ob/ob*) or the leptin receptor (*db/db, fa/fa*) lead to an increase in NPY activity. Increases in NPY activity lead to an increase in serum corticosterone and insulin concentrations. High levels of corticosterone, interacting with type II corticosteroid receptors, cause a further increase in NPY activity. High levels of corticosterone also increase serum insulin concentrations. Low concentrations of serum insulin or insulin resistance increase NPY activity. Under normal conditions, insulin inhibits NPY activity.

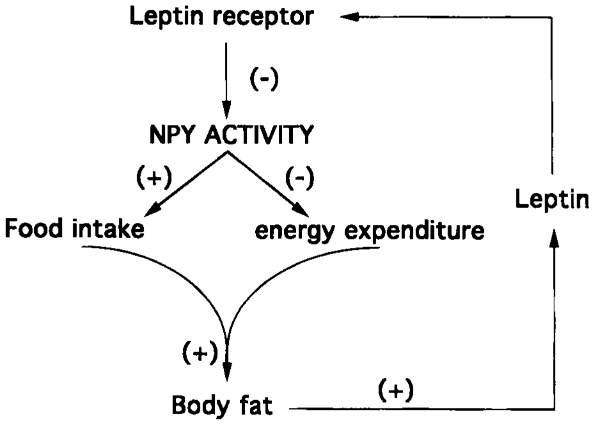

Figure 2. Proposed relationships among leptin, neuropeptide Y (NPY) activity, food intake, energy expenditure, and body fat. Leptin interacting with its receptor leads to an inhibition of NPY activity. Mutations that result in a defect in leptin (*ob/ob*) or the leptin receptor (*db/db, fa/fa*) lead to an increase in NPY activity. Increases in NPY activity increase food intake and decrease energy expenditure. Both processes promote the accumulation of body fat. Leptin is synthesized and secreted from fat in proportion to the amount of body fat.

ity becomes chronically increased. Elevated NPY activity increases serum corticosterone and insulin concentrations (Fig. 1). Chronically high serum corticosterone concentrations would lead to more interaction with Type II corticosteroid receptors in the arcuate nucleus and promote further increases in NPY activity. Elevated serum corticosterone concentrations would also stimulate a further increase in serum insulin levels, possibly by increasing insulin resistance. Normally, elevated insulin levels would decrease NPY activity. However, the developing insulin resistance of obese Zucker rats would prevent the inhibition of NPY, allowing NPY activity to increase further. The result of these interactions is a self-sustaining increase in NPY activity. Chronic elevation of NPY activity would lead to an increase in food intake, decrease in energy expenditure, and provide an internal milieu that promotes the accretion of body fat.

The above is a working hypothesis that is consistent with much of the existing literature. More work is needed to better define the relationships among leptin, glucocorticoids, and insulin in the regulation of neuropeptide Y. This will enhance the understanding of the etiology of obesity in the Zucker rat and possibly lead to a better understanding of some forms of obesity in humans.

---

1. Martin RJ, Harris RBS, Jones DD. Evidence for a central mechanism of obesity in the Zucker fatty rat (fa/fa) Proc Soc Exp Biol Med **183**:1–10, 1986.
2. Tatemoto K, Carlquist M, Mutt V. Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature **296**:659–660, 1982.
3. Chronwall BM, Di Maggio DA, Massari VJ, Pickel VM, Ruggiero DA, O'Donohue TL. The anatomy of neuropeptide Y containing neurons in the rat brain. J Neurosci **15**:1159–1181, 1985.
4. Lungdberg JM, Terenius L, Hokfelt T, Martling CR, Tatemoto K, Mutt V, Polak J, Bloom S, Goldstein M. Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. Acta Physiol Scand **116**:479–480, 1982.
5. Larhammer D, Soderberg C, Blomquist AG. Evolution of the Neuropeptide Y family of peptides. In: Colmers WF, Wahlestedt C, Eds. The Biology of Neuropeptide Y and Related Peptides. Totowa: Humana Press, pp. 1–41, 1993.
6. Allen JM, Balbi D. Structure and expression of the neuropeptide Y gene. In: Colmers WF, Wahlestedt C, Eds. The Biology of Neuropeptide Y and Related Peptides. Totowa: Humana Press, pp 43–64, 1993.
7. Lehmann J. Neuropeptide Y: An overview. Drug Dev Res **19**:329–351, 1990.
8. Clark JT, Kalra PS, Crowley WR, Kalra SP. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology **115**:427–429, 1984.
9. Levine AS, Morley JE. Neuropeptide Y: A potent inducer of consummatory behavior in rats. Peptides **5**:1025–1029, 1984.
10. Stanley BG, Leibowitz SF. Neuropeptide Y injected in the paraventricular hypothalamus: A powerful stimulant of feeding behavior. Proc Natl Acad Sci USA **82**:3940–3943, 1984.
11. Morley JE, Levine AS, Gosnell BA, Kneip J, Grace M. Effect of neuropeptide Y on ingestive behaviors in the rat. Am J Physiol **252**:R599–R609, 1987.
12. Stanley BG, Kyrkouli SE, Lampert S, Leibowitz SF. Neuropeptide Y chronically injected into the hypothalamus: A powerful neurochemical inducer of hyperphagia and obesity. Peptides **7**:1189–1192, 1986.
13. Beck B, Stricker-Krongrad A, Nicolas JP, Burlet C. Chronic and continuous intracerebroventricular infusion of neuropeptide Y in Long-Evans rats mimics the feeding behavior of obese Zucker rats. Int J Obes Relat Metab Disord **16**:295–302, 1992.
14. Billington CJ, Briggs JE, Grace M, Levine AS. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. Am J Physiol **260**:R321–R327, 1991.
15. Egawa M, Yoshimatsu H, Bray GA. Effect of corticotropin releasing hormone and neuropeptide Y on electrophysiological activity of sympathetic nerves to interscapular adipose tissue. Neuroscience **34**:771–775, 1990.
16. Zucker LM. Some effects of caloric restriction and deprivation on the obese hyperlipemic rat. J Nutr **91**:247–254, 1967.

17. Bazin R, Eteve D, Lavau M. Evidence for decreased GDP binding to brown-adipose-tissue mitochondria of obese Zucker (fa/fa) rats in the very first days of life. Biochem J **221:**241–245, 1984.
18. York DA, Marchington D, Holt SJ, Allars J. Regulation of sympathetic activity in lean and obese Zucker (fa/fa) rats. Am J Physiol **249:**E299–E305, 1985.
19. Zarjevski N, Cusin I, Vettor R, Rohner-Jeanrenaud F, Jeanrenaud B. Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. Endocrinology **133:**1753–1758, 1993.
20. Zarjevski N, Cusin I, Vettor R, Rohner-Jeanrenaud F, Jeanrenaud B. Intracerebroventricular administration of neuropeptide Y to normal rats has divergent effects on glucose utilization by adipose tissue and skeletal muscle. Diabetes **43:**764–769, 1994.
21. Vettor R, Zarjevski N, Cusin I, Rohner-Jeanrenaud F, Jeanrenaud B. Induction and reversibility of an obesity syndrome by intracerebroventricular neuropeptide Y administration to normal rats. Diabetologia **37:**1202–1208, 1994.
22. Moltz JH, McDonald JK. Neuropeptide Y: Direct and indirect action on insulin secretion in the rats. Peptides **6:**1155–1159, 1985.
23. Kuenzel WJ, McMurtry J. Neuropeptide Y: Brain localization and central effects on plasma insulin levels in chicks. Physiol Behav **44:**669–678, 1988.
24. Abe M, Saito M, Shimazu T. Neuropeptide Y and norepinephrine injected into the paraventricular nucleus of the hypothalamus activate endocrine pancreas. Biomed Res **10:**431–436, 1989.
25. Harfstrand A, Eneroth P, Agnati L, Fuxe K. Further studies on the effects of central administration of neuropeptide Y on neuroendocrine function in the male rat: relationship to hypothalamic catecholamines. Regul Pept **17:**167–169, 1987.
26. Wahlestedt C, Skagerberg G, Ekman R, Heilig M, Sundler F, Hakaanson R. Neuropeptide Y (NPY) in the area of the hypothalamic paraventricular nucleus activates the pituitary-adrenocortical axis in the rat. Brain Res **417:**33–38, 1987.
27. Zucker LM, Antoniades HN. Insulin and obesity in the Zucker genetically obese rat "fatty." Endocrinology **90:**1320–1330, 1972.
28. Martin RJ, Wangsness, PJ, Gahagan JH. Diurnal changes in serum metabolites and hormones in lean and obese Zucker rats. Horm Metab Res **10:**187–192, 1978.
29. Opara EC, Burch WM, Taylor IL, Akwari OE. Pancreatic hormone response to neuropeptide Y (NPY) perifusion in vitro. Regul Pept **34:**225–233, 1991.
30. Haas DA, George SR. Neuropeptide Y administration acutely increases hypothalamic corticotropin-releasing factor immunoreactivity: Lack of effect in other rat brain regions. Life Sci **41:**2725–2731, 1987.
31. Suda T, Tozawa F, Iwai I, Sato Y, Sumitomo T, Nakano Y, Yamada M, Demura H. Neuropeptide Y increases the corticotropin-releasing factor messenger ribonucleic acid level in the rat hypothalamus. Mol Brain Res **18:**311–315, 1993.
32. Inoue T, Inui A, Okita M, Sakatani N, Oya M, Marioka H, Mizuno N, Oimosi M, Baba S. Effect of neuropeptide Y on the hypothalamic-pituitary-adrenal axis in the dog. Life Sci **44:**1043–1051, 1989.
33. Clark JT, Kalra PS, Kalra SP. Neuropeptide Y stimulates feeding but inhibits sexual behavior in rats. Endocrinology **117:**2435–2442, 1985.
34. Poggioli R, Vergoni A-V, Marrama D, Giuliani D, Bertolini A. NPY-induced inhibition of male copulatory activity is a direct behavioural effect. Neuropeptides **16:**169–172, 1990.
35. Zucker LM, Zucker TF. Fatty, a new mutation in the rat. J Hered **52:**275–278, 1961.
36. Edmonds ES, Dallie SK, Withyachumnarnkul B. Reproductive system of the obese male Zucker rat. Reproductive capacity, artifical insemination and plasma testosterone levels. Biol Reprod **27:**891–897, 1982.
37. Fukagawa K, Sakata T, Yoshimatsu H, Fujimoto K, Shiraishi T. Disruption of light-dark cycle of feeding and drinking behavior, and ambulatory activity induced by development of obesity in the Zucker rat. Int J Obes **12:**481–490, 1988.
38. Fukagawa K, Sakata T, Yoshimatsu H, Fujimoto K, Uchimura K, Asano C. Advance shift of feeding circadian rhythm induced by obesity progression in Zucker rats. Am J Physiol **263:**R1169–R1175, 1992.
39. Jhanwar-Uniyal M, Beck B, Burlet C, Leibowitz SF. Diurnal rhythm of neuropeptide Y-like immunoreactivity in the suprachiasmatic, arcuate and paraventricular nuclei and other hypothalamic sites. Brain Res **536:**331–334, 1990.
40. Calza L, Giardino L, Zanni M, Velardo A, Parchi P, Marrama P. Daily changes of neuropeptide Y-like immunoreactivity in the suprachiasmatic nucleus of the rat. Regul Pept **27:**127–137, 1990.
41. Shinohara K, Tominaga K, Isobe Y, Inouye S-IT. Photic regulation of peptides located in the ventrolateral subdivision of the suprachiasmatic nucleus of the rat: Daily variations of vasoactive intestinal polypeptide, gastrin-releasing peptide and neuropeptide Y. J Neurosci **13:**793–800, 1993.
42. Biello SM, Janik D, Mrosovsky N. Neuropeptide Y and behaviorally induced phase shifts. Neuroscience **62:**273–279, 1994.
43. Mrosovsky N. A non-photic gateway to the circadian clock of hamsters. Ciba Found Symp **183:**154–174, 1995.
44. Huhman KL, Albers HE. Neuropeptide Y microinjected into the suprachiasmatic region phase shifts circadian rhythms in constant darkness. Peptides **15:**1475–1478, 1994.
45. Albers HE, Ferris CF. Neuropeptide Y: Role in light-dark cycle entrainment of hamster circadian rhythms. Neurosci Lett **50:**163–168, 1984.
46. Beck B, Burlet A, Nicolas JP, Burlet C. Hyperphagia in obesity is associated with a central peptidergic dysregulation in rats. J Nutr **120:**806–811, 1990.
47. Beck B, Burlet A, Nicolas JP, Burlett C. Hypothalamic neuropeptide Y (NPY) in obese Zucker rats: Implications in feeding and sexual behaviors. Physiol Behav **47:**449–453, 1990.
48. McKibbin PE, Cotton SJ, McMillan S, Holloway B, Mayers R, McCarthy HD, Williams G. Altered neuropeptide Y concentrations in specific hypothalamic regions of obese (fa/fa) Zucker rats. Possible relationship to obesity and neuroendocrine disturbances. Diabetes **40:**1423–1429, 1991.
49. Beck B, Burlet A, Bazin R, Nicolas JP, Burlet C. Early modification of neuropeptide Y but not of neurotensin in the suprachiasmatic nucleus of the obese Zucker rat. Neurosci Lett **136:**185–188, 1992.
50. Beck B, Burlet A, Bazin R, Nicolas JP, Burlet C. Elevated neuropeptide Y in the arcuate nucleus of young obese Zucker rats may contribute to the development of their overeating. J Nutr **123:**1168–1172, 1993.
51. Boulange A, Planche E, de Gasquet P. Onset of genetic obesity in the absence of hyperphagia during the first week of life in the Zucker rat (fa/fa). J Lipid Res **20:**857–864, 1979.
52. Godbole VY, Grundleger ML, Pasquine TA, Thenen SW. Composition of rat milk from day 5 to 20 of lactation and milk intake of lean and preobese Zucker pups. J Nutr **111:**480–487, 1981.
53. Sanacora G, Kershaw M, Finkelstein JA, White JD. Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology **127:**730–737, 1990.
54. Pesonen U, Rouru J, Huupponen R, Koulu M. Effects of repeated administration of mifepristone and 8-OH-DPAT on expression of preproneuropeptide Y mRNA in the arcuate nucleus of obese Zucker rats. Mol Brain Res **10:**267–272, 1991.
55. Pesonen U, Huupponen R, Rouru J, Koulu M. Hypothalamic neuropeptide expression after food restriction in Zucker rats: Evidence of persistent neuropeptide Y gene activation. Mol Brain Res **16:**255–260, 1992.

56. Bai FL, Yamano M, Shiotani Y, Emson PC, Smith AD, Powell JF, Tohyama M. An arcuato-paraventricular and -dorsomedial hypothalamic neuropeptide Y-containing system which lacks noradrenaline in the rat. Brain Res **331**:172–175, 1985.
57. Jhanwar-Uniyal M, Chua SC Jr. Critical effects of aging and nutritional state on hypothalamic neuropeptide Y and galanin gene expression in lean and genetically obese Zucker rats. Mol Brain Res **19**:195–202, 1993.
58. Sanacora G, Finkelstein JA, White JD. Developmental aspect of differences in hypothalamic preproneuropeptide Y messenger ribonucleic acid content in lean and genetically obese Zucker rats. J Neuroendocrinol **4**:353–357, 1992.
59. Chung WK, Truett GE, Smoller JW, Hirsch J, Leibel R. Increased hypothalamic preproneuropeptide Y mRNA in six to nine day old fatty (fa/fa) rats. (abstract) FASB J **6**:A1785, 1992.
60. Brief DJ, Sipols AJ, Woods SC. Intraventricular neuropeptide Y injections stimulate food intake in lean, but not obese Zucker rats. Physiol Behav **51**:1105–1110, 1992.
61. McCarthy HD, McKibbin PE, Holloway B, Mayers R, Williams G. Hypothalamic neuropeptide Y receptor characteristics and NPY-induced feeding responses in lean and obese Zucker rats. Life Sci **49**:1491–1497, 1991.
62. Stricker-Krongrad A, Max JP, Musse N, Nicolas JP, Burlet C, Beck B. Increased threshold concentrations of neuropeptide Y for a stimulatory effect on food intake in obese Zucker rats—changes in the microstructure of the feeding behavior. Brain Res **660**:162–166, 1994.
63. Hervey GR. The effects of lesions in the hypothalamus of parabiotic rats. J Physiol **145**:336–352, 1959.
64. Coleman DL. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia **14**:141–148, 1978.
65. Coleman DL, Hummel KP. Effects of parabiosis of normal with genetically diabetic mice. Am J Physiol **217**:1298–1304, 1969.
66. Hausberger FX. Parabiosis and transplantation experiments in hereditarily obese mice. (abstract) Anat Rec **130**:313, 1958.
67. Haessler HA, Crawford JD. Alterations in the fatty acid composition of depot fat associated with obesity. Ann N Y Acad Sci **131**:476–484, 1965.
68. Chlouverakis C. Insulin resistance of parabiotic obese-hyperglycemic mice (ob/ob). Horm Metab Res **4**:143–148, 1972.
69. Coleman DL. Effects of parabiosis of obese with diabetic and normal mice. Diabetologia **9**:294–298, 1973.
70. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature **372**:425–432, 1994.
71. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. Science **269**:543–546, 1995.
72. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science **269**:540–543, 1995.
73. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science **269**:546–549, 1995.
74. Weigle DS, Bukowski TR, Foster DC, Holderman S, Kramer JM, Lasser G, Lofton-Day CE, Prunkard DE, Raymond C, Kuijper JL. Recombinant *ob* protein reduces feeding and body weight in the *ob/ob* mouse. J Clin Invest **96**:2065–2070, 1995.
75. Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, MacKellar W, Rosteck PR Jr., Schoner B, Smith D, Tinsley FC, Zhang X-Y, Heiman M. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature **377**:530–532, 1995.
76. Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent of insulin. Peptides **17**:305–311, 1996.
77. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM. Leptin levels in human and rodent: Measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. Nature Med **1**:1155–1161, 1995.
78. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS. Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. Nature Med **1**:1311–1314, 1995.
79. Lonnqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nature Med **1**:950–953, 1995.
80. Hamilton BS, Paglia D, Kwan AYM, Deitel M. Increased *obese* mRNA expression in omental fat cells from massively obese humans. Nature Med **1**:953–956, 1995.
81. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. Cell **83**:1263–1271, 1995.
82. Lee G-H, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM. Abnormal splicing of the leptin receptor in diabetic mice. Nature **379**:632–635, 1996.
83. Streamson CC Jr., Chung WK, Wu-Peng XS, Zhang Y, Liu S-M, Tartaglia L, Leibel RL. Phenotypes of mouse *diabetes* and rat *fatty* due to mutations in the OB (Leptin) receptor. Science **271**:994–996, 1996.
84. Harris RBS, Hervey E, Hervey GR, Tobin G. Body composition of lean and obese Zucker rats in parabiosis. Int J Obes **11**:275–283, 1987.
85. Moinat M, Deng C, Muzzin P, Assimacopoulos-Jeannet F, Seydoux J, Dulloo AG, Giacobino J-P. Modulation of *obese* gene expression in rat brown and white adipose tissues. FEBS Lett **373**:131–134, 1995.
86. Truett GE, Bahary N, Friedman JM, and Leibel RL. Rat obesity gene fatty (*fa*) maps to chromosome 5: Evidence for homology with the mouse gene diabetes (*db*). Proc Natl Acad Sci U S A **88**:7806–7809, 1991.
87. Phillips MS, Liu Q, Hammond HA, Dugan V, Hey PJ, Caskey CT, Hess JF. Leptin receptor missense mutation in the fatty Zucker rat. Nat Genet **13**:18–19, 1996.
88. Iida M, Murakami T, Ishida K, Mizuno A, Kuwajima M, Shima K. Phenotype-linked amino acid alteration in leptin receptor cDNA from Zucker fatty (fa/fa) rat. Biochem Biophys Res Commun **222**:19–26, 1996.
89. Wilding JP, Gilbey SG, Bailey CJ, Batt RA, Williams G, Ghatei MA, Bloom SR. Increased neuropeptide-Y messenger ribonucleic acid (mRNA) and decreased neurotensin mRNA in the hypothalamus of the obese (ob/ob) mouse. Endocrinology **132**:1939–1944, 1993.
90. Chua SC, Brown AW, Kim J, Hennessey KL, Leibel RL, Hirsch J. Food deprivation and hypothalamic neuropeptide gene expression: Effects of strain background and the *diabetes* mutation. Mol Brain Res **11**:291–299, 1991.
91. Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Prunkard DE, Porte D, Woods SC, Seeley RJ, Weigle DS. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in *ob/ob* mice. Diabetes **45**:531–535, 1996.
92. Erickson JC, Clegg KE, Palmiter RD. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. nature **381**:415–418, 1996.
93. Yukimura Y, Bray GA, Wolfsen AR. Some effects of adrenalectomy in the fatty rat. Endocrinology **103**:1924–1928, 1978.

94. Fletcher JM. Effects of adrenalectomy before weaning in the genetically obese Zucker rat (fa/fa). Br J Nutr **56:**141–151, 1986.
95. Freedman MR, Horwitz BA, Stern JS. Effects of adrenalectomy and glucocorticoid replacement on development of obesity. Am J Physiol **250:**R595–R607, 1986.
96. Kalra SP, Dube MG, Kalra PS. Continuous intraventricular infusion of neuropeptide Y evokes episodic food intake in satiated female rats: Effects of adrenalectomy and cholecystokinin. Peptides **9:**723–728, 1988.
97. Stanley BG, Lanthier D, Chin AS, Leibowitz SF. Suppression of neuropeptide Y-elicited eating by adrenalectomy or hypophysectomy: Reversal with corticosterone. Brain Res **501:**32–36, 1989.
98. Eva C, Oberto A, Sprengel R, Genazzani E. The murine NPY-1 receptor gene. Structure and delineation of tissue-specific expression. FEBS Lett **314:**285–288, 1992.
99. Larson PJ, Jessop DS, Chowdrey HS, Lightman SL, Mikkelsen JD. Chronic administration of glucocorticoids directly upregulates prepro-neuropeptide Y and Y1-receptor mRNA levels in the arcuate nucleus of the rat. J Neuroendocrinol **6:**153–159, 1994.
100. Akabayashi A, Watanabe Y, Wahlestedt C, McEwen BS, Paez X, Leibowitz SF. Hypothalamic neuropeptide Y, its gene expression and receptor activity: Relation to circulating corticosterone in adrenalectomized rats. Brain Res **665:**201–212, 1994.
101. Heinrichs SC, Menzaghi F, Pich EM, Hauger RL, Koob GF. Corticotropin-releasing factor in the paraventricular nucleus modulates feeding induced by neuropeptide Y. Brain Res **611:**18–24, 1993.
102. Menzaghi F, Heinrichs SC, Pich EM, Tilders FJH, Koobs GF. Functional impairment of hypothalamic corticotropin-releasing factor neurons with immunotargeted toxins enhances food intake induced by neuropeptide Y. Brain Res **618:**76–82, 1993.
103. Harfstrand A, Cintra A, Fuxe K, Aronsson M, Wikstrom AC, Okret S, Gustafsson JA, Agnati LF. Regional differences in glucorticoid receptor immunoreactivity among neuropeptide Y immunoreactive neurons of the rat brain. Acta Physiol Scand **135:**3–9, 1989.
104. Ceccatelli S, Cintra A, Hokfelt T, Fuxe K, Wikstrom AC, Gustafsson JA. Coexistence of glucocorticoid receptor-like immunoreactivity with neuropeptides in the hypothalamic paraventricular nucleus. Exp Brain Res **78:**33–42, 1989.
105. Higuchi H, Yang HY, Sabol SL. Rat neuropeptide Y precursor gene expression, mRNA structure, tissue distribution, and regulation by glucocorticoids, cyclic AMP, and phorbol ester. J Biol Chem **263:**6288–6295, 1988.
106. Barnea A, Cho G, Hajibeigi A, Aguila MC, Magni P. Dexamethasone-induced accumulation of neuropeptide-Y by aggregating fetal brain cells in culture: A process dependent on the developmental age of the aggregates. Endocrinology **129:**931–938, 1991.
107. Corder R, Pralong F, Turnill D, Saudan P, Muller AF, Gaillard RC. Dexamethasone treatment increases neuropeptide Y levels in rat hypothalamic neurones. Life Sci **43:**1879–1886, 1988.
108. McKibbin PE, Cotton SJ, McCarthy HD, Williams G. The effect of dexamethasone on neuropeptide Y concentrations in specific hypothalamic regions. Life Sci **51:**1301–1307, 1992.
109. Wilding JPH, Gilbey SG, Lambert PD, Ghatei MA, Bloom SR. Increases in neuropeptide Y content and gene expression in the hypothalamus of rats treated with dexamethasone are prevented by insulin. Neuroendocrinology **57:**581–587, 1993.
110. Pralong FP, Corder R, Gaillard RC. The effects of chronic glucocorticoid excess, adrenalectomy and stress on neuropeptide Y in individual rat hypothalamic nuclei. Neuropeptides **25:**223–231, 1993.
111. White BD, Dean RG, Martin RJ. Adrenalectomy decreases neuropeptide Y mRNA levels in the arcuate nucleus. Brain Res Bull **25:**711–715, 1990.
112. Watanabe Y, Akabayashi A, McEwen BS. Adrenal steroid regulation on neuropeptide Y (NPY) mRNA: differences between dentate hilus and locus coeruleus and arcuate nucleus. Mol Brain Res **28:**135–140, 1995.
113. White BD, Dean RG, Edwards GL, Martin RJ. Type II corticosticoid receptor stimulation increases NPY gene expression in basomedial hypothalamus of rats. Am J Physiol **266:**R1523–R1529, 1994.
114. Misaki N, Higuchi H, Yamagata K, Miki N. Identification of glucocorticoid responsive elements (GREs) at far upstream of rat NPY gene. Neurochem Int **21:**185–189, 1992.
115. van Huijsduijnen OB-H, Rohner-Jeanrenaud F, Jeanrenaud B. Hypothalamic neuropeptide Y messenger ribonucleic acid levels in pre-obese and genetically obese (fa/fa) rats: Potential regulation thereof by corticotropin-releasing factor. J Neuroendocrinol **5:**381–386, 1993.
116. Tempel DL, Leibowitz SF. Glucocorticoid receptors in PVN: Interactions with NE, NPY, and Gal in relation to feeding. Am J Physiol **265:**E794–E800, 1993.
117. Langley SC, York DA. Effects of antiglucocorticoid RU 486 on development of obesity in obese fa/fa Zucker rats. Am J Physiol **259:**R539–R544, 1990.
118. Fletcher JM, Haggary P, Wahle KWJ, Reeds RJ. Hormonal studies of young lean and obese Zucker rats. Horm Metab Res **18:**290–295, 1986.
119. Guillaume-Gentil C, Rohner-Jeanrenaud F, Abramo F, Bestetti GE, Rossi GL, Jeanrenaud B. Abnormal regulation of the hypothalamo-pituitary-adrenal axis in the genetically obese fa/fa rat. Endocrinology **126:**1873–1879, 1990.
120. Williams G, Steel JH, Cardoso H, Ghatei MA, Lee YC, Gill JS, Burrin JM, Polak JM, Bloom SR. Increased hypothalamic neuropeptide Y concentration in diabetic rats. Diabetes **37:**763–772, 1988.
121. Williams G, Gill JS, Lee YC, Cardoso HM, Okpere BE, Bloom SR. Increased neuropeptide Y concentrations in specific hypothalamic regions of streptozotocin-induced diabetic rats. Diabetes **38:**321–327, 1989.
122. Sahu A, Sninsky CA, Kalra PS, Kalra SP. Neuropeptide-Y concentration in microdissected hypothalamic regions and in vitro release from the medial basal hypothalamus-preoptic area of streptozotocin-diabetic rats with and without insulin substitution therapy. Endocrinology **126:**192–198, 1990.
123. Abe M, Saito M, Ikeda H, Shimazu T. Increased neuropeptide Y content in the arcuato-paraventricular hypothalamic neuronal system in both insulin-dependent and non-insulin-dependent diabetic rats. Brain Res **539:**223–227, 1991.
124. Sahu A, Sninsky CA, Phelps CP, Dube MG, Kalra PS, Kalra SP. Neuropeptide Y release from the paraventricular nucleus increases in association with hyperphagia in streptozotocin-induced diabetic rats. Endocrinology **131:**2979–2985, 1992.
125. White JD, Olchovsky D, Kershaw M, Berelowitz M. Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in streptozotocin-diabetic rats. Endocrinology **126:**765–772, 1990b.
126. Schwartz MW, Marks JL, Sipols AJ, Baskin DG, Woods SC, Kahn SE, Porte D Jr. Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) but not obese (fa/fa) Zucker rats. Endocrinology **128:**2645–2647, 1991.
127. Schwartz MW, Sipols AJ, Marks JL, Sanacora G, White JD, Scheurink A, Kahn SE, Baskin DG, Woods SC, Figlewicz DP, Porte D Jr. Inhibition of hypothalamic neuropeptide Y gene expression by insulin. Endocrinology **130:**3608–3616, 1992.
128. Ponsalle P, Srivastava LS, Uht RM, White JD. Glucocorticoids are required for food deprivation-induced increases in hypothalamic neuropeptide Y expression. J Neuroendocrinol **4:**585–591, 1992.

129. Cusin I, Dryden S, Wang Q, Rohner-Jeanrenaud F, Jeanrenaud B, Williams G. Effect of sustained physiological hyperinsulinaemia on hypothalamic neuropeptide Y and NPY mRNA levels in the rat. J Neuroendocrinol **7**:193–197, 1995.
130. Akabayashi A, Zaia CTBV, Silva I, Chae HJ, Leibowitz SF. Neuropeptide Y in the arcuate nucleus is modulated by alterations in glucose utilization. Brain Res **621**:343–348, 1993.
131. He B, White BD, Edwards GL, Martin RJ. 2-Deoxy-D-glucose and NPY gene expression in the arcuate nucleus and specific brainstem nuclei in the rat. (abstract) FASEB J **9**:A582, 1995.
132. Figlewicz DP, Dorsa DM, Stein LJ, Baskin DG, Paquette T, Greenwood MRC, Woods SC, Porte D Jr. Brain and insulin liver binding is decreased in Zucker rats carrying the "fa" gene. Endocrinology **117**:1537–1543, 1985.
133. Ikeda H, Nishikawa K, Matsuo T. Feeding responses of Zucker rats to 2-deoxy-D-glucose, norepinephrine, and insulin. Am J Physiol **239**:E379–E384, 1980.
134. Dallman MF, Strack AM, Akana SF, Bradbury MJ, Hanson ES, Scribner KA, Smith M. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Front Neuroendocrinol **14**:303–347, 1993.
135. Strack AM, Sebastian RJ, Schwartz MW, Dallman MF. Glucocorticoids and insulin: Reciprocal signals for energy balance. Am J Physiol **268**:R142–R149, 1995.