Proceedings of the Nutrition Society (1991) **50**, 527–544

PROCEEDINGS OF THE NUTRITION SOCIETY

The annual meeting of the Irish Group of the Nutrition Society was held at The University of Ulster at Coleraine, Northern Ireland on 19–21 June 1991

Symposium on 'Diabetes and hormonal control of glucose homeostasis'

Neuropeptides and intestinal tract in the control of glucose homeostasis

Hypothalamic regulatory peptides and the regulation of food intake and energy balance: signals or noise?

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INTRODUCTION

A confusingly large number of neural, metabolic and endocrine factors are thought to regulate how much an animal eats and how this is varied according to the animal's energy stores. In recent years, peptides with experimental actions which suggest that they may be involved in the control of energy balance have attracted much attention. The list of these peptides is long (Table 1) and continues to lengthen, the main inclusion criterion being the peptide's ability to alter feeding behaviour or energy expenditure, or both, when it is injected into various anatomical sites. Such studies suggest that many peptides may serve to regulate nutritional state throughout a wide range of species, including man and the slug as well as the familiar laboratory rodents (Morley, 1987).

The main difficulty in this area, as implied by the subtitle of the present article, is in picking out those peptides with a genuine 'regulatory' function against the noisy background of experimental artefact (Williams, 1991). A peptide with a true regulatory function in the nutritional context must fulfil certain criteria. It must be found in the parts of the central nervous system which control energy balance and, when released there, must interact with specific receptors which mediate changes in feeding or thermogenesis. For these events to be physiologically appropriate, the peptidergic system in question must interact with a sensor system which is able to identify and respond to changes in nutritional state, perhaps by recognizing a neural or metabolic signal whose intensity is determined by the size of the body's energy stores. It follows that the activity of the peptidergic system must change in response to alterations in nutritional state, although such changes may be difficult to detect if they are relatively small and involve only localized areas such as individual hypothalamic nuclei. These basic criteria are met by only a few of the peptides listed in Table 1.

Table 1. *A few examples of peptides with experimental central actions relevant to energy balance in the rat**

Peptide	Effect on food intake		Effect on thermogenesis	
	Response	Site of action	Response	Site of action
Bombesin	↓	Hypothalamus	↑, ↓	3Ve
Cholecystokinin	↓ ↓	PVN, other sites	↑	3Ve
Corticotrophin-releasing factor	↓ ↓	PVN	↑ ↑	PVN
Dynorphin	↑	PVN	—	—
β-Endorphin	↑	VMH	—	—
Galanin	↑	PVN	—	—
Insulin	↓	3Ve	↑, ↓	VMH
Neuropeptide Y	↑ ↑	PVN, other sites	↓ ↓	PVN
			↑	MPO
Neurotensin	↓	PVN	↑	3Ve
Somatostatin	↓, ↑	3Ve	↑	3Ve

↑, ↑ ↑, Moderate and pronounced increase respectively; ↓, ↓ ↓, moderate and pronounced decrease respectively; MPO, medial preoptic area; PVN, paraventricular nucleus; VMH, ventromedial nucleus; 3Ve, third ventricle.

* For details, see Morley (1987), Rothwell (1989) and Williams (1991).

In preparing the present contribution, we had to choose between presenting a superficial glance at the entire area and a more detailed review of a discrete topic. In the hope that close focusing will not be mistaken for tunnel vision, we have chosen to do the latter and, specifically, have concentrated on the possible metabolic functions of neuropeptide Y (NPY) in the hypothalamus. We have to declare an interest, in that most of our current research is centred on the nutritional and metabolic actions of hypothalamic NPY. Nevertheless, we hope that the evidence to be presented will justify our conviction that this peptide has a plausible part to play in the control of energy balance.

NPY: AN INTRODUCTION

NPY is a thirty-six-amino-acid peptide first isolated from pig brain by Tatemoto and colleagues (Tatemoto, 1982; Tatemoto *et al.* 1982). The 1200 papers written about it since then are a tribute to the great rapidity with which peptides and their actions can be characterized nowadays, and also reflect the wide range of actions attributed to NPY, both within and outside the brain (Table 2). It is named 'Y' for the single-letter code identifying the tyrosine residues found at the C- and N-termini and also at three other positions in the molecule. The C-terminal tyrosine residue is amidated, a feature common to a wide range of biologically active peptides. NPY is structurally related to pancreatic polypeptide and, like all members of this family, has a folded, hairpin-like shape; both ends of the molecule apparently participate in receptor binding (Fuhlendorff *et al.* 1990). So far, two classes of NPY receptors have been identified. Y_1 receptors, found especially in the cerebral cortex, recognize the complete NPY molecule, whereas Y_2 receptors, found in the hippocampus and many other brain regions, are selective for its C-terminal end (Sheikh *et al.* 1989; Dumont *et al.* 1990).

Most of the metabolic effects of NPY seem to be mediated by the hypothalamus, a region whose anatomical and functional complexity (all crammed into a volume of about

https://doi.org/10.1079/PNS19910066 Published online by Cambridge University Press

Table 2. *Some neuroendocrine, metabolic and behavioural actions of neuropeptide Y when injected centrally*

Action	Site	Comments
↑ ↑ Feeding	PVN, VMH, DMH, LHA, 3Ve, 4Ve	Most potent central feeding stimulant known Stimulates carbohydrate and fat intake Induces obesity
↑ Drinking	PVN, 3Ve, 4Ve	
↓ Thermogenesis	PVN, 3Ve	Reduces sympathetic activation of BAT (cf. slight increase if injected into MPO)
↑ Insulin secretion	PVN, 3Ve	↑ Glucagon also
↑ ACTH and corticosterone release	PVN, 3Ve	Mediated by CRF release
↓ Growth hormone and prolactin release	3Ve, ARC	
↑ or ↓ LH	3Ve	Depends on sex steroid levels
Shifts circadian rhythms	SCN	

↑, ↑ ↑, Moderate and pronounced increase respectively; ↓, ↓ ↓, moderate and pronounced decrease respectively; PVN, paraventricular nucleus; VMH, ventromedial nucleus; DMH, dorsomedial nucleus; LHA, lateral hypothalamic area; 3Ve, third ventricle; 4Ve, fourth ventricle; BAT, brown adipose tissue; MPO, medial preoptic area; ACTH, adrenocorticotrophic hormone; CRF, corticotrophin-releasing factor; ARC, arcuate nucleus; LH, luteinizing hormone; SCN, suprachiasmatic nucleus.

0·1 ml in the rat) demands a brief introduction. Several discrete areas seem to be involved in regulating nutritional balance, by affecting food intake or energy expenditure, or both (Williams, 1991). The principal nuclei are the paraventricular nucleus (PVN), arising from the top of the third ventricle in the anterior hypothalamus, the plump ventromedial nucleus (VMH) which occupies much of the mediobasal hypothalamus, and the dorsomedial nucleus (DMH) lying above the VMH. In the rat these nuclei lie within a central 2 mm strip conveniently separated from the lateral hypothalamic area (LHA), another appetite-modulating region, by the fornix, a prominent longitudinal fibre bundle. It is now apparent that the notion of 'feeding' and 'satiety' centres (previously identified as the LHA and VMH respectively) is over-simplistic; the distinction is certainly not respected by NPY, which stimulates feeding when injected into all the previously described areas.

The hypothalamus is permeated by a dense NPY-containing innervation arising both within and outside the hypothalamus (Bai *et al.* 1985; Chronwall *et al.* 1985). Within the hypothalamus, NPY is synthesized predominantly in the arcuate nucleus (ARC), a long, thin nucleus wrapped around the base of the third ventricle and lying just above the median eminence in which the hypothalamo–hypophyseal portal vessels originate (Morris, 1989). The ARC produces a dense projection of NPY-containing axons which sweeps up through the LHA to end in the PVN and, to a lesser extent, in the DMH (Bai *et al.* 1985). The NPY mRNA:NPY ratio in the hypothalamus is relatively low, implying that a significant proportion of the peptide is synthesized elsewhere (Higuchi *et al.* 1988· Morris, 1989). A major source appears to be a rostral projection, also terminating in the PVN, arising from groups of cell bodies (designated A1 and C1-3) in the dorsal medulla.

The neurones of this pathway contain catecholamines co-localized with NPY, whereas the intrahypothalamic arcuato-paraventricular projection does not (Sawchenko *et al.* 1985). Other hypothalamic regions rich in NPY include the periventricular area in the walls of the third ventricle, the medial preoptic area (MPO), situated anteriorly, and the suprachiasmatic nucleus (SCN) which indents the top of the optic chiasm (Chronwall *et al.* 1985; Card & Moore, 1988). The SCN apparently functions as a pacemaker for neuroendocrine and metabolic circadian rhythms and, through inputs from the visual pathways, which include a dense NPYergic projection from the lateral geniculate body (Harrington *et al.* 1987), may synchronize these rhythms to light-dark cues.

The NPY-containing systems have intimate anatomical (and probably functional) links with other neurotransmitters and hormones in the hypothalamus. For example, NPY-immunoreactive endings are densely clustered around cell bodies in the PVN which contain corticotrophin-releasing factor (CRF) (Liposits *et al.* 1988) and around oxytocin-containing neurones in the supraoptic nucleus (Willoughby & Blessing, 1987). There are also close connections with nerves containing non-peptide neurotransmitters which influence feeding, notably with noradrenaline and serotonin in the PVN and several other regions (Leibowitz, 1986). In the human hypothalamus, NPY is found in a distribution qualitatively similar to that in rodents (Adrian *et al.* 1983; Corder *et al.* 1990).

APPETITE-STIMULATING AND METABOLIC EFFECTS OF NPY

The discovery of provocatively high NPY concentrations in key regulatory regions of the brain soon inspired a search for its possible biological actions. Several groups have unearthed an impressive array of experimental effects when the peptide is injected into the hypothalamus or cerebral ventricles (Table 2) (Williams & Bloom, 1989). As with all such experiments, the relevance of these experimental effects to the peptide's true biological functions is far from clear.

One of the most striking actions of NPY is its stimulation of feeding, which has been demonstrated in several species, including rats, other rodents and chicks (Morley, 1987); so far, both man and the slug have managed to evade investigation. The powerful feeding response seen when NPY is injected into the third ventricle has been localized to the PVN (the most sensitive site), VMH, DMH and LHA (Levine & Morley, 1984; Stanley & Leibowitz, 1984, 1985; Stanley *et al.* 1985*a*, 1986; Morley *et al.* 1987); administration into the fourth ventricle also stimulates feeding (Steinman *et al.* 1987). NPY injected into these sites stimulates both active food-seeking behaviour and feeding itself. NPY and its close structural relative, peptide YY (PYY), are the most powerful appetite stimulants known, being over 100 times more powerful than noradrenaline on a molar basis (Stanley & Leibowitz, 1985; Morley, 1987). Food intake can be increased several-fold, the effect lasting for several hours after a single central NPY injection.

NPY induces hyperphagia even when rats are satiated or during the light phase (when food intake is normally low) and can over-ride the anorectic effect of cholecystokinin, although the powerful central appetite-suppressing agent, CRF, can block NPY-induced feeding (Morley, 1987; Rowland, 1988). With repeated NPY injection into the PVN, the feeding response does not attenuate and the animals even become obese, with a significant increase in body fat content (Stanley *et al.* 1986, 1989). NPY is the only peptide known to have this action. NPY-induced feeding resembles that elicited by central noradrenaline injection in that the PVN is highly sensitive to both and that

carbohydrate intake is preferentially increased (Stanley *et al.* 1985*b*; Leibowitz, 1986); during chronic administration, NPY also stimulates fat intake (Stanley *et al.* 1989). Both transmitters are found in the PVN and are co-stored in the medullo–paraventricular projection, but the balance of evidence suggests that they stimulate feeding independently, noradrenaline acting through α_2-adrenoceptors and NPY through Y_1 receptors (Flood & Morley, 1989). Centrally-injected NPY also stimulates drinking, an action not shared by noradrenaline (Stanley & Leibowitz, 1984; Leibowitz, 1986; Morley, 1987).

Energy intake represents one side of the equation which determines nutritional balance, the other being energy expenditure. Circumstantial evidence has suggested that NPY also reduces energy expenditure; for example, the weight gain in rats with NPY-induced obesity was greater than would be anticipated from the increase in energy consumption (Stanley *et al.* 1986). Recent observations have confirmed directly that NPY injected into the third ventricle reduces energy expenditure (Billington *et al.* 1991), probably by reducing the sympathetic outflow which activates thermogenesis in brown adipose tissue (Egawa *et al.* 1991). NPY administration into the MPO stimulates sympathetic nerve activity, but an inhibitory effect mediated by the PVN apparently predominates (Egawa *et al.* 1991). Menendez *et al.* (1990) reported that NPY injected into the PVN altered carbohydrate oxidation without major effects on total energy expenditure. A preliminary observation suggesting that intracerebroventricular NPY injection slightly increased energy expenditure (Rothwell, 1989) may be explained by the known ability of NPY to release CRF within the hypothalamus (Haas & George, 1987), where CRF exerts a powerful thermogenic effect (LeFeuvre *et al.* 1987).

Centrally-injected NPY has several other actions relevant to nutritional state. Administration into the PVN or third ventricle stimulates insulin secretion (Moltz & McDonald, 1985; Abe *et al.* 1989), in contrast to the peptide's direct inhibitory action at the level of the pancreatic islet, which is rich in NPY-containing nerves (Moltz & McDonald, 1987). NPY injected into the PVN also induces glucagon release and mild hyperglycaemia (Abe *et al.* 1989). In addition, its injection into the PVN stimulates adrenocorticotrophic hormone (ACTH) and corticosterone secretion in the rat and dog, possibly by stimulating the release of CRF (Wahlestedt *et al.* 1987; Inoue *et al.* 1989); the CRF-containing cell bodies in the PVN, which are closely surrounded by NPY-containing nerve-endings, project to the median eminence where they release CRF into the hypothalamo–hypophyseal portal vessels. NPY also potentiates the ACTH release induced by CRF (Inoue *et al.* 1989). The interaction of NPY with CRF and the pituitary–adrenocortical system may be highly relevant to the obesity syndromes in rodents, which are critically dependent on the integrity of the axis: adrenalectomy prevents obesity from developing in the *ob/ob* mouse or *fa/fa* Zucker rat, and corticosterone replacement will restore the metabolic abnormalities of the syndromes (York & Bray, 1972; Bray & York, 1979). This topic is discussed in detail later. Other endocrine changes which follow the central injection of NPY include inhibition of growth hormone and prolactin release and either suppression or stimulation of luteinizing hormone (LH) secretion, depending on the animal's gonadal steroid levels (McDonald *et al.* 1985; Hårfstrand *et al.* 1987; Kalra *et al.* 1988). Another important central action of NPY is its ability to phase-shift circadian rhythms in rodents when injected into the SCN (Albers & Ferris, 1984). In view of its other actions, NPY may govern, at least in part, the circadian rhythmicity of metabolic functions such as feeding, insulin release and adrenocortical secretion.

Overall, the experimental metabolic actions of NPY suggest that it may function in an integrated fashion at several levels to produce a positive energy balance, by increasing energy intake and reducing its expenditure; the concomitant secretion of insulin would promote energy storage, especially through the deposition of triacylglycerols in white adipose tissue.

HYPOTHALAMIC NPY AND CHANGES IN NUTRITIONAL STATE

If NPY had a significant involvement in controlling nutritional state, alterations in energy balance would be expected to induce adaptive changes in the peptide's activity in the relevant parts of the brain. The obvious example, and a common and important threat to the survival of animals in the wild, is shortage of food. This is crucial for rodents, whose life in the metabolic fast lane, combined with their limited energy stores, means that 48 h without food can cause a laboratory rat to lose 20% of its body-weight. Several studies have pointed to a striking increase in hypothalamic NPYergic activity in food-deprived or food-restricted rats. The most consistent changes reported are a rise in NPY levels in the ARC, together with an increased NPY mRNA content indicating enhanced synthesis, and in the PVN (Sahu *et al.* 1988; Calza *et al.* 1989; White & Kershaw, 1989; Beck *et al.* 1990*c*; Brady *et al.* 1990). The increases in the PVN are reversed by refeeding (Sahu *et al.* 1988). There are differences in detail regarding the precise regions involved and the time-course of the changes. Beck *et al.* (1990*c*) reported a dramatic tenfold rise in ARC NPY concentrations after 48 h of starvation, whereas Sahu *et al.* (1988) and ourselves (McKibbin *et al.* 1991*b*) did not find any significant regional NPY changes after 48 h deprivation in Wistar or in Zucker rats (either fatty or lean) respectively. Food restriction causing progressive weight loss over many days appears to be a stronger stimulus than acute starvation, at least in terms of the increase produced in NPY mRNA levels (Brady *et al.* 1990).

The elevated NPY tissue levels together with increased NPY synthesis indicate that food deprivation or restriction stimulate the activity of the hypothalamic NPYergic pathways, particularly the arcuato–paraventricular projection. This would release the peptide in the NPY-sensitive PVN and DMH, so stimulating the search for food and then eating when it again becomes available. Increased hypothalamic NPYergic activity may also reduce resting energy expenditure (Billington *et al.* 1991). Specific hypothalamic NPYergic pathways may, therefore, serve a homeostatic function in defending body-weight against losses. Some possible factors which may sense energy-store depletion and stimulate this system are discussed later (pp. 534–536).

Regional hypothalamic NPY concentrations in laboratory rats show circadian variation which may relate to their daily feeding pattern. The rat eats much of its daily intake within the first few hours of darkness, possibly because of a certain urgency injected by its precarious energy balance. The changes reported after darkness include a gradual fall in NPY concentrations in the SCN when food is freely available (Calza *et al.* 1990) and a specific rise in the LHA, which occurs whether food is presented or not (McKibbin *et al.* 1991*c*). These relatively rapid changes, which take place within a few hours, seem more likely to be due to alterations in local NPY release rather than to changes in synthesis, especially as concentrations in the ARC do not appear to be affected. As the SCN receives NPYergic afferents from the visual pathways (Harrington *et al.* 1987), NPY changes in this nucleus may be involved in recognizing the transition to darkness, while

those in the NPY-sensitive PVN and LHA may relate to the increased feeding at this time. Detailed studies of NPY turnover will be needed to determine the significance of these observations.

HYPOTHALAMIC NPY AND DIABETES

Insulin-deficient diabetes, such as that induced in normal rats by the β-cell toxin, streptozotocin (STZ), is a potentially rewarding area for studying the role of NPY. Diabetes causes profound energy losses, marked compensatory hyperphagia and major neuroendocrine dysfunction (Williams & Bloom, 1989). After moderate doses of STZ, plasma insulin levels fall rapidly to 10–20% of non-diabetic values and glycaemia rises to 20–25 mmol/l, but the rats do not become heavily ketotic and can survive for long periods without insulin replacement. Some 20–30% of weight is lost within 3 weeks due to unrestrained catabolism and heavy glucose losses in the urine. Food intake doubles, with a selective increase in carbohydrate-rich food, and water intake rises several-fold. Endocrine disturbances include reduced growth hormone and prolactin secretion and reproductive failure, due at least in part to impaired gonadotrophin secretion (Williams *et al.* 1988*b*; Williams & Bloom, 1989). A similar syndrome develops spontaneously in BB rats (first described from the BioBreeding Laboratories) due to autoimmune β-cell destruction analogous to that in human insulin-dependent diabetes (IDDM). Like people with IDDM, diabetic BB rats have minimal endogenous insulin secretion and so require insulin treatment to survive (Williams *et al.* 1989*b*).

Disturbances of hypothalamic NPY in insulin-deficient diabetes were first revealed by a study of twelve hypothalamic peptides in STZ diabetes of 3–14 weeks' duration (Williams *et al.* 1988*b*). NPY was the only peptide to show consistent changes, with a significant rise in both central and lateral hypothalamic tissue blocks after 3 weeks of diabetes. Central hypothalamic NPY concentrations were also elevated in diabetic BB rats whose insulin dosage was lowered to induce hyperglycaemia and weight loss (Williams *et al.* 1989*b*). More detailed microdissection studies localized NPY increases in STZ-diabetic rats to the ARC, PVN, VMH, DMH and MPO (all in the central hypothalamus) and the LHA (Williams *et al.* 1989*a*). Increased ARC concentrations reflect increased synthesis, as hypothalamic NPY mRNA levels were shown by Northern blotting to have risen fivefold above non-diabetic values (Pierson *et al.* 1988). We proposed that the increased hypothalamic NPYergic activity suggested by these observations could contribute to carbohydrate-specific hyperphagia, polydipsia and impaired pituitary secretion of growth hormone, prolactin and LH in insulin-deficient diabetes (Williams *et al.* 1988*b*, 1989*c*; Williams & Bloom, 1989).

Subsequent studies by other groups and ourselves have confirmed that regional hypothalamic NPY levels are increased, notably in the ARC, PVN, MPO and DMH, in untreated diabetic rats (McKibbin *et al.* 1990; Sahu *et al.* 1990; Abe *et al.* 1991); that hypothalamic NPY mRNA levels are also elevated (White *et al.* 1990); and that these increases are reversed by insulin treatment, which also normalizes food intake and prevents weight loss. Sahu *et al.* (1990) have demonstrated that NPY release from incubated hypothalamic tissue following potassium-induced depolarization (which is a measure of tissue stores of the peptide) is increased in STZ-diabetic rats compared with controls. Duncan Powrie and Paul Shaw (Powrie *et al.* 1991) in our group have found that spontaneous NPY release from single perifused mediobasal hypothalamic fragments

(which occurs in a pulsatile fashion reminiscent of gonadotrophin-releasing hormone secretion) is significantly increased in STZ-diabetes. This is further evidence of increased activity of NPY-containing neurones in hypothalamic regions relevant to both the hyperphagia and the pituitary dysfunction in insulin-deficient diabetes.

Few studies have been performed in other animal models of diabetes. The fatty (*fa/fa*) Wistar rat develops a syndrome resembling non-insulin-dependent diabetes (NIDDM), with obesity, hyperphagia, severe tissue insulin insensitivity and moderate hyperglycaemia, associated with hyperinsulinaemia. These animals show regional hypothalamic NPY increases similar to those in STZ-diabetic and fatty Zucker rats (see p. 537) (Abe *et al.* 1991). As discussed later, hyperinsulinaemia does not exclude the possibility of insulin deficiency at the level of the hypothalamus, and this may resolve the apparent paradox of increased hypothalamic NPY in the presence of raised circulating insulin levels. A preliminary study in mildly diabetic Chinese hamsters, which were slightly obese and had similar insulin levels compared with non-diabetic controls, found reduced whole hypothalamic NPY levels in the diabetics (Williams *et al.* 1988*a*). Regional NPY concentrations clearly have to be measured in this model.

WHAT IS THE METABOLIC SIGNAL REGULATING HYPOTHALAMIC NPY?

The closely similar hypothalamic NPY changes in food deprivation and diabetes suggested that they might be a response to the negative energy balance common to both conditions (Williams & Bloom, 1989; Williams *et al.* 1989*c*). We recently tested this hypothesis by examining the effects on hypothalamic NPY of weight loss induced by intense physical exercise. In this study, performed in collaboration with Professor James Russell (University of Alberta, Edmonton, Canada), rats were trained to run several km/d on a large-diameter exercise wheel. This was enough to expend about 40% of their daily energy intake and, with food intake maintained at non-exercised control values, body-weight fell to 30% below controls after 6 weeks. In running rats, NPY concentrations were significantly higher than controls in the ARC, DMH, LHA and MPO, these changes being virtually identical to those in a separate group of rats which were food-restricted to match the weight loss in the running group. It seems, therefore, that negative energy balance, whether achieved by reduced intake or increased expenditure, stimulates hypothalamic NPYergic activity (Lewis *et al.* 1992).

The nature of the metabolic signal which presumably activates hypothalamic NPY in response to weight loss is not known. One possibility is changes in glucose availability, which has been suggested to determine feeding behaviour. Circulating glucose concentrations are unlikely to regulate hypothalamic NPY, as they are grossly elevated in diabetes but fall in starvation and intense exercise; however, glucose availability to specific regulatory regions of the brain could be important. A feature common to all the previously described conditions is a fall in circulating insulin levels, and we have suggested that this is the change which stimulates hypothalamic NPY (Williams & Bloom, 1989).

We have recently performed two studies to test this hypothesis. The first examined the effects on hypothalamic NPY levels of insulin-induced hypoglycaemia, which causes marked hyperphagia and, with repeated insulin administration for several days, leads to significant weight gain. In contrast to the other hyperphagic conditions described previously, which are all characterized by insulin deficiency, hyperphagia in

hyperinsulinaemic, hypoglycaemic rats was not accompanied by any increases in regional hypothalamic NPY levels. This implies that hypothalamic NPY is not simply activated under any circumstances which cause hyperphagia, and lends support to our hypothesis that insulin deficiency may be a specific stimulus (Corrin *et al.* 1991). In our second study, we aimed to normalize glycaemia in STZ-diabetic rats without correcting insulin deficiency. We found that this could be achieved by restricting the diabetic rats' food intake to non-diabetic values or below. Normoglycaemic, food-restricted rats showed regional hypothalamic NPY increases which were similar in distribution and at least as great as those in untreated diabetics. By contrast, regional NPY levels were lowered to normal when normoglycaemia was restored with insulin treatment. This again suggests that insulin deficiency, rather than changes in glycaemia, stimulates hypothalamic NPY (McKibbin *et al.* 1991*c*).

Other metabolic and hormonal factors undoubtedly modulate hypothalamic NPY, for example, glucocorticoids increase NPY and NPY mRNA levels (Corder *et al.* 1988; Dean & White, 1990), and their possible roles in signalling changes in nutritional state will require investigation. However, the suggestion that insulin deficiency is a specific signal which activates hypothalamic NPY is consistent with the proposal, first made some years ago, that insulin acts on the brain as a 'satiety' factor to regulate feeding.

INSULIN AND THE REGULATION OF ENERGY BALANCE

Insulin is well qualified to convey information about nutritional state (Grossman, 1986; Baskin *et al.* 1987; Woods *et al.* 1990). Its circulating levels generally parallel body fat content and, as noted previously, they fall under conditions of weight loss. Short-term information about energy intake is also provided by the rapid, dose-related rise in circulating insulin concentrations after eating. However, the possible role of insulin in modulating feeding behaviour remains uncertain, for two main reasons.

The first source of confusion is that different doses of insulin produce opposite effects on feeding. Systemic dosages high enough to cause hypoglycaemia stimulate feeding, presumably in response to neuroglycopenia which may be detected by neurones in 'classical' hypothalamic regions such as the VMH, in other parts of the brain, or even in extracerebral sites such as the liver (Friedmann & Granneman, 1983; Cane *et al.* 1986). By contrast, subhypoglycaemic insulin dosages suppress feeding in rats, and intracerebroventricular administration of insulin to rats or baboons suppresses food intake in a dose-related fashion (Woods *et al.* 1979; Vanderweele *et al.* 1980; Brief & Davis, 1984); conversely, injection of insulin antibodies into the VMH in rats stimulates feeding (Strubbe & Mein, 1977). The second obstacle to this hypothesis is doubt that circulating insulin could gain access to the brain in order to influence feeding behaviour. It has been generally assumed that the blood–brain barrier is impervious to molecules as large as insulin. However, the barrier seems to be selectively leaky, particularly in certain specialized circumventricular regions (including the median eminence), so providing the potential for sensing circulating hormones and metabolites. Porte and Woods and their colleagues (Baskin *et al.* 1983, 1987; Corp *et al.* 1986; Schwartz *et al.* 1990; Woods *et al.* 1990) have amassed evidence that insulin can enter the cerebrospinal fluid (CSF) and have shown that the hypothalamus and other brain regions contain both insulin and insulin receptors. They have postulated that circulating insulin is an indicator of nutritional state and acts on the brain as a satiety signal, modulating food intake

according to need. Low circulating insulin levels might, therefore, be a stimulus to eating in starvation and diabetes. There is some evidence that the brain insulin system is responsive to changes in nutritional state, as insulin binding in the hypothalamus alters in starvation (Melnyk & Martin, 1984), but the hypothesis remains controversial.

The central mechanisms through which insulin might inhibit feeding are not known, although some brain regions involved in metabolic regulation are apparently insulin-sensitive. For example, the firing rate of certain neurones in the LHA is influenced by ambient insulin concentrations, and specific glucose-sensing hypothalamic regions may depend on insulin to facilitate glucose entry (Oomura, 1987). We suggest that this nutritional feedback loop is completed by NPYergic neurones which are activated by insulin deficiency.

Insulin and NPY have reciprocal effects on energy expenditure, which may also be interlinked. Insulin has been postulated by Rothwell & Stock (1988) to be a centrally-acting thermogenic agent which activates the sympathetic outflow to brown adipose tissue; these findings have, however, been disputed by Sakaguchi & Bray (1987). According to Rothwell & Stock's (1988) hypothesis, insulin deficiency could, therefore, reduce thermogenesis, and this effect could also be mediated by increased NPYergic activity in the hypothalamus.

HYPOTHALAMIC NPY DISTURBANCES IN OBESITY

Diseases of nutrition raise two of the most challenging questions about putative regulators of energy balance. First, could a disturbance of a given regulatory system be the cause of obesity or anorexia? Second, could these disorders be treated by manipulating the activity of the system? This is particularly relevant to the common and currently insoluble problem of human obesity.

Hypothalamic NPY has been investigated in several rodent models of obesity. Spontaneous obesity in these animals is conferred by homozygosity for recessive genes such as *fa* (fatty) or *cp* (corpulent) in the rat, and *ob* (obese) or *db* (diabetes) in the mouse. Weight gain is due predominantly to reduced energy expenditure and is exacerbated by hyperphagia. Peripheral tissues (brown adipose tissue, skeletal muscle and liver) are variably insensitive to insulin and circulating insulin levels are greatly elevated; glucose tolerance may be essentially normal (e.g. in *fa/fa* Zucker and *cp/cp* rats) or diabetes may result (in *fa/fa* Wistar rats and *ob/ob* or *db/db* mice). Many of these metabolic abnormalities are attributed to an imbalance in the autonomic nervous system, with a relative decrease in sympathetic tone which causes reduced thermogenesis in brown adipose tissue, and increased parasympathetic activity, which stimulates insulin secretion. The hypothalamo–pituitary–adrenocortical axis seems to be closely involved in the pathogenesis of these syndromes. Corticosterone secretion is increased compared with lean littermates, and adrenalectomy prevents, whereas glucocorticoid replacement restores, obesity and hyperinsulinaemia. Reproductive function is poor in obese rodents. The neuroendocrine and metabolic features of these syndromes have been thoroughly reviewed (Bray & York, 1979; Bray *et al.* 1989).

An initial screening study found no differences in central or lateral hypothalamic NPY levels between fatty and lean Zucker rats, although fatty rats showed a significant rise in the central hypothalamus when they were food-restricted to reduce their weight to lean levels (Williams *et al.* 1991*a*). However, more detailed studies have provided evidence of

increased NPYergic activity in specific, discrete hypothalamic regions of fatty Zucker rats, similar to those involved in food-restricted or diabetic rats. The most consistent increases are in the ARC, PVN, DMH and MPO (Beck *et al.* 1990*a*,*b*; McKibbin *et al.* 1991*a*), and NPY mRNA levels in the ARC are also elevated (Sanacora *et al.* 1990). Fatty and lean Zucker rats also differ with respect to the changes in hypothalamic NPY and NPY mRNA induced by food restriction with or without refeeding, further indicating abnormal NPY regulation in the fatty rats (Sanacora *et al.* 1990; McKibbin *et al.* 1991*a*). We have recently shown that hypothalamic NPY receptor numbers are reduced (with no change in affinity for NPY), and that the feeding response to relatively low NPY doses injected intracerebroventricularly is attenuated, in fatty Zucker rats compared with lean controls (McCarthy *et al.* 1991). These findings are consistent with increased release of endogenous NPY within the hypothalamus, causing down-regulation of NPY receptor numbers and blunted responsiveness to exogenous NPY. Overactivity of hypothalamic NPY could contribute to hyperphagia, reduced energy expenditure and obesity in the fatty Zucker rat, and could also play a part in the increased insulin secretion, enhanced adrenocortical activity and poor reproductive function. Increased regional hypothalamic NPY levels have also been reported in fatty Wistar rats, which display an obesity/NIDDM-like syndrome (Abe *et al.* 1991).

Could overactivity of the NPYergic system be the primary genetic defect conferred by the homozygosity for the *fa* gene? We have addressed this question in a recent study (in collaboration with Professor James Russell) of obese JCR:LA-corpulent rats, phenotypically similar to the fatty Zucker but homozygous for the *cp* gene rather than the *fa* gene. Like fatty Zucker rats, obese *cp/cp* rats showed significantly higher NPY levels in the ARC than in lean controls, and also showed abnormal NPY responses to food restriction (Shellard *et al.* 1992). This suggests that increased hypothalamic NPYergic activity is a common feature of certain obesity syndromes and so is unlikely to be their primary cause. Possible causes of increased hypothalamic NPY in these models include increased corticosterone levels and, in agreement with our working hypothesis, insulin deficiency at the level of the brain. This may seem inconsistent with the gross hyperinsulinaemia of the obese rats, but there is evidence that brain insulin levels and brain insulin-binding capacity are both reduced in fatty Zucker rats (Baskin *et al.* 1985; Figlewicz *et al.* 1985). Furthermore, intracerebroventricular insulin administration does not suppress feeding in fatty Zucker rats as it does in lean rats, suggesting that the obese rat's brain (like its peripheral tissues) may be insensitive to insulin (Ikeda *et al.* 1986). A state of apparent insulin deficiency might, therefore, be registered, despite hyperinsulinaemia, so activating the hypothalamic NPYergic pathways. Direct evidence that central insulin levels affect NPY has recently been provided by Schwartz *et al.* (1991), who found that NPY infused intracerebroventricularly reduced NPY mRNA levels in the ARC in lean Zucker rats, but not in fatty Zucker rats. This suggests that insulin normally exerts a regulatory inhibitory action on hypothalamic NPY and that fatty rats are resistant to this action.

Obesity due to voluntary over-feeding with a high-fat diet is reportedly associated with moderately increased NPY concentrations in the PVN and reduced levels in the LHA, compared with rats fed freely or with a high-carbohydrate diet (Beck *et al.* 1990*d*). The relationship of these changes to those occurring in spontaneous obesity is not yet clear.

Hypothalamic NPY disturbances may be related to changes in other appetite-modulating neurotransmitters in obese rodents. The close anatomical links with the

CRF-containing pathways are particularly interesting, as the two peptides have opposing effects on energy balance: CRF reduces food intake and increases energy expenditure by activating the sympathetic outflow to brown adipose tissue (Brown *et al.* 1982; Arase *et al.* 1988). Fatty Zucker rats display reduced whole hypothalamic CRF concentrations (Nakaishi *et al.* 1991) and continuous intracerebroventricular infusion of CRF into fatty Zucker rats apparently increases sympathetic nervous activity and energy expenditure, reduces food intake and decreases body-weight towards lean values (Rohner-Jeanrenaud *et al.* 1989). This hints at a specific defect of CRFergic activity, but whether this is primary, secondary or unrelated to the increased NPYergic activity is not known. The same uncertainty applies to other peptide abnormalities in fatty Zucker rats, which include reduced regional neurotensin levels (Beck *et al.* 1990*a*), increased central hypothalamic neuromedin B concentrations (Williams *et al.* 1991*a*) and altered cholecystokinin receptor characteristics (Finkelstein *et al.* 1984). Regional hypothalamic NPY concentrations have not yet been studied in obese mice, although a screening study found similar central and lateral NPY hypothalamic concentrations in *ob/ob* and lean mice (Williams *et al.* 1991*b*).

FAT RATS, FAT PEOPLE AND BROAD HORIZONS

It seems reasonable to conclude that hypothalamic NPY may help to regulate nutritional state in rodents and that overactivity of this system may contribute to obesity in certain models. Can these conclusions be extrapolated to man, and could they be exploited to treat obesity?

Fat rats and fat people obviously differ in several important respects. Reduced thermogenesis is the main cause of weight gain in obese rodents but its role in human obesity remains controversial (and is probably less important). Furthermore, with the obvious but rare exception of Cushing's syndrome, glucocorticoids do not seem to be of central importance in human obesity. So far, no systematic studies of NPY in human obesity or diabetes have been reported, although increased CSF levels of NPY (and of CRF) have been described in anorexic patients; NPY concentrations fell to normal after refeeding (Fava *et al.* 1989), suggesting a dynamic response to starvation similar to that in rodents. This potentially exciting territory awaits further exploration.

Should NPY have similar actions in man, NPY antagonists could prove to be useful anti-obesity drugs as they might both reduce food intake and perhaps increase energy expenditure. Interestingly, injection into rats of fenfluramine, a serotonergic anorectic drug in wide clinical use, causes rapid and dramatic falls in NPY levels in several hypothalamic regions (Rogers *et al.* 1991), suggesting that existing appetite-modulating agents may interact with the NPYergic system. The possible therapeutic use of NPY inhibitors has been brought a step nearer to reality by Tatemoto (1990), who developed two relatively small peptide antagonists, designated PYX_1 and PYX_2, and by the discovery of a non-peptide compound, He90481, with the same property (Michel & Motulsky, 1990). However, considerable practical problems remain. To be suitable for oral use, any peptide drugs will have to resist enzymic degradation in the gut and be reliably absorbed, although the intranasal route (which is effective for larger peptides such as insulin and glucagon) may be a viable alternative. An NPY-blocking appetite-suppressant would have to target the hypothalamus selectively, without interfering with the actions of NPY in the periphery (where it is a potent vasoconstrictor) or in other

brain regions. Despite these difficulties, several pharmaceutical companies are actively investigating this area and, given the remarkable speed of advances in peptide chemistry, it is possible that appetite-modulating drugs based on NPY may find clinical application within the next few years. If this is the case, the remarkable activity of the first decade of the history of NPY will have been amply rewarded.

The authors are very grateful for the help and encouragement provided by their colleagues, particularly Helen Leitch, Lyndsey Shellard, Diane Lewis, Paula Rogers, Susan Corrin, Paul Shaw and Duncan Powrie in the Department of Medicine at Liverpool University; Drs Brian Holloway and Rachel Mayers of ICI Pharmaceuticals, Macclesfield; and Professor James Russell and Dorothy Koeslag of the Department of Surgery, University of Alberta, Edmonton. Financial support for the present studies comes from the British Diabetic Association, Cancer Research Campaign, Mersey Regional Health Authority, the Wellcome Trust, ICI plc and Servier Laboratories UK Ltd. The authors are indebted to Caroline Williams for typing the manuscript with skill and good humour.

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Printed in Great Britain