Introduction

Of the many hypothalamic neurotransmitters involved in the control of food intake and metabolism, neuropeptide Y (NPY) has perhaps attracted more interest than any other substance since its discovery in 1983 [1]. The powerful effects of centrally administered NPY on food intake [2], and the fact that repeated injections into the paraventricular nucleus of the hypothalamus (PVN) led to obesity, were soon recognized [3]. It was also observed that the weight gain in animals treated with NPY was disproportionately high, despite the increase in calories consumed, suggesting specific metabolic effects favouring weight gain besides the orexigenic effect. NPY synthesis and content are increased in the hypothalamus of genetically obese rodents [4,5], which are known to have metabolic alterations contributing to weight gain, and cannot be solely attributed to hyperphagia. In keeping with these observations, centrally administered NPY has been shown to have many effects on peripheral metabolism, such as stimulating release of insulin, increasing hepatic glucose production, reducing brown adipose-tissue thermogenesis and increasing lipoprotein lipase activity. Release of several anterior pituitary hormones, such as corticotropin (ACTH), growth hormone and thyroid-stimulating hormone, is also modulated by NPY and it remains to be seen whether the observed metabolic effects are mediated by the autonomic nervous system or via alterations in hormonal status. This article will review the known metabolic actions of NPY with particular relevance to those that may be involved in its tendency to produce obesity.

Anatomy of the NPY system

NPY is an almost ubiquitous neuropeptide in the central nervous system (CNS), but particularly high concentrations are found in the hypothalamus of both rodents and humans [6,7]. Most hypothalamic NPY synthesis occurs in the arcuate nucleus of the hypothalamus, and fibres from this nucleus project to the PVN, which also receives NPYergic fibres from catecholamine-containing cells projecting from the A1, C1, C2 and C3 groups in the brainstem. Because of its key role in the regulation of autonomic function and pituitary hormone secretion, the PVN has received most attention, although the closely related perifornical area may be a more potent site for NPY-induced feeding [8].

Effects of NPY on energy metabolism

Increased food intake in cafeteria feeding generally increases thermogenic activity, whereas genetically obese animals, such as the ob/ob mouse and Zucker obese (fa/fa) rat characteristically have a thermogenic defect, and are unable to increase their metabolic rate in a cold environment or in response to increased food intake [9,10]. NPY injected into the PVN decreases levels of thermogenesis as measured by mitochondrial GDP-binding and uncoupling protein mRNA in brown adipose tissue [11,12], which occurs even when NPY-treated animals are pair-fed to control levels. This decrease in thermogenesis may occur because of reduction in the firing rate of sympathetic nerves supplying brown adipose tissue [13]. It is of interest that intracerebroventricular (ICV) and PVN injection of NPY decreased rates of firing of sympathetic nerves supplying brown adipose tissue, whereas injection into the medial preoptic area increased the sympathetic firing rate. The significance of this observation is unclear, but it does show the importance of anatomical specificity when studying actions of NPY injected into the CNS. Thus increased NPY activity in the hypothalamus, specifically in the paraventricular nucleus, would reduce thermogenesis in brown adipose tissue, which could be a further stimulus to weight gain in addition to its orexigenic effect. These observations are at odds with those in another study, when NPY injected into the PVN did not affect total energy expenditure measured using indirect calorimetry; however, in this study a biphasic
effect on the respiratory quotient was observed. At very low doses of NPY (10 pmol) the respiratory quotient was decreased, suggesting decreased carbohydrate utilization and increased fat catabolism. At higher doses (30–156 pmol) the respiratory quotient increased to greater than 1, suggesting catabolism of carbohydrate and storage of energy as fat [14]. This is consistent with the observation that animals made obese by repeated NPY injection have increased body fat.

**Carbohydrate metabolism**

Animals injected with NPY into the PVN prefer carbohydrate when pure macronutrient diets are available [15], and it has been suggested that NPY may have a specific role to play in the regulation of carbohydrate metabolism. Several studies have shown that NPY injected into several CNS sites, including the third ventricle [16], the PVN [17] and nucleus tractus solitarius [18], increases plasma insulin levels, whether or not food is available [17]. However, the rise in circulating insulin has not been associated with any change in glucose concentrations in these acute studies, although glucose was often only measured at a single time point, so a transient change could have been missed. This lack of change in glucose, despite a 3-fold rise in insulin, suggests either reduced peripheral insulin responsiveness, or an increase in hepatic glucose output. We recently addressed this issue by using [6-3H]glucose as a tracer to measure changes in hepatic glucose output after ICV NPY injection and a hyperinsulinaemic euglycaemic clamp to assess insulin responsiveness. Acute NPY injection into the third ventricle in rats fasted for 6 h and not allowed access to food increased hepatic glucose output which preceded a rise in insulin. Conversely peripheral glucose uptake was increased during the clamp, indicating increased sensitivity to circulating insulin. Both acute experiments were associated with a small rise in glucagon levels [19]. Thus centrally injected NPY has profound effects on secretion of islet hormones and glucose homoeostasis, although the precise mechanisms for these diverse effects remain unknown. These observations would again be consistent with a role for NPY in directing substrate utilization towards carbohydrate, and promoting storage of fat.

**Lipid metabolism**

Few acute studies have focused on effects of NPY on lipid metabolism. Lipoprotein lipase activity and mRNA are increased after PVN injection of NPY [11,12]. This effect seems to be independent of food intake and plasma insulin levels, at least in acute experiments [11], so the effector pathway for this effect is not yet known.

**Hormonal effects**

Centrally injected NPY has profound effects on the secretion of anterior pituitary hormones. ACTH and subsequently corticosterone secretion are increased after ICV injection of NPY, whereas growth hormone, thyroid-stimulating hormone and luteinizing hormone secretion are inhibited [20–23]. Many observed metabolic effects of NPY could clearly be at least partially explained by these changes, but specific experiments to answer this question have not been reported.

**Chronic effects of NPY**

Although repeated PVN NPY injection has long been known to result in obesity and increased body fat [3], it is only recently that the metabolic characteristics of the obese animals produced by such repeated NPY injection have begun to be characterized in detail, and compared with other rodent models of obesity. In a series of elegant studies, Zarjevski and co-workers have studied the effects of chronic NPY infusion into the third ventricle on several aspects of energy, carbohydrate and lipid metabolism. In these studies rats were chronically infused ICV with NPY (10 μg/day) for 7 days using osmotic minipumps, and groups were pair-fed with controls (for either 7 days or 4 days) to try to separate metabolic effects of NPY from those secondary to weight gain [24,25]. Both ad libitum fed and pair-fed NPY-treated animals had increased body fat, and triacylglycerol and insulin levels were approximately three times control values. Blood glucose levels remained normal, suggesting total body insulin resistance. De novo lipogenesis was increased, as was glucose utilization in white adipose tissue, and lipoprotein lipase activity. Corticosterone levels were also increased 4-fold. When the effects on glucose metabolism were studied in more detail [25], it was observed that total body glucose disposal was non-significantly increased during a hyperinsulinaemic euglycaemic clamp, but whereas adipose tissue was four times more insulin sensitive, muscle became insulin resistant. The changes in glucose utilization in fat as measured by the 2-deoxy-D-[1-3H]glucose method were associated with
increased GLUT4 protein and mRNA levels in adipose tissue. No decrease was seen in muscle, however, and the mechanism for decreased insulin sensitivity in muscle remains unknown, although increased substrate cycling or increased corticosterone levels are possibilities. These observations are consistent with the effects observed in acute studies, where the increased peripheral glucose uptake observed during a hyperinsulinaemic clamp may be due to increased glucose uptake in fat. Although fat usually only accounts for 5% of glucose uptake during a clamp, the 4-fold increase in fat glucose uptake seen in the chronic studies would exactly match the 15% increase in total body glucose uptake observed in our acute experiments [19]. If muscle insulin resistance was a result of increased corticosterone, this may not be apparent in an acute experiment.

Effector pathways
Centrally injected NPY clearly has a wide range of effects on many systems concerned with metabolic regulation. However, the complexity of these systems, and their interdependence, makes it difficult to determine which of these effects are direct actions of NPY and which are the result of secondary changes. There seems little doubt that the effects on thermogenesis are mediated via a reduction in sympathetic nervous system activity, but this is the only action where a direct measurement has been made [13]. The effect on insulin secretion is also likely to be neurally mediated, possibly by stimulation of the parasympathetic nervous system, but this has not been tested, for example in vagotomized animals. The observation of increased hepatic glucose production raises the possibility that increased insulin secretion could be a result of this. Many of the other observed effects could be secondary to either increased insulin levels (e.g. increased lipoprotein lipase activity in adipose tissue) or increased levels of corticosterone. Studies in corticosterone-replaced adrenalectomized animals or in insulin-treated diabetic animals would be necessary to resolve these issues.

NPY receptors
Ligand binding and studies of biological activity in a variety of tissues suggest the existence of several subtypes of NPY receptor. The best characterized are the NPY Y1 and Y2 receptors [26]. The Y1 receptor has been cloned and belongs to the family of G-protein-linked receptors with seven transmembrane domains [27,28]. Activation of this receptor causes inhibition of adenylate cyclase and mobilization of intracellular Ca [29,30]. The NPY analogue [Leu"Pro"]NPY is a selective ligand for the Y1 receptor [31]. Loss of one or more amino acids (e.g. NPY-(2-36) or -(3-36)) results in almost complete loss of biological activity at this receptor [28].

The NPY Y2 receptor is typically a pre-junctional receptor. It is able to bind C-terminal fragments of NPY such as NPY-(13-36), which only bind the Y1 receptor weakly. Y2 receptors are widely expressed in the CNS, notably in the hippocampus, but also in the hypothalamus, where they constitute over 80% of NPY-binding sites [32]. Both Y1 and Y2 receptors are peptide YY (PYY) preferring in receptor-binding studies, and PYY is at least as effective as stimulating food intake as NPY, but PYY is not present in the hypothalamus. A third (Y3) receptor has been characterized pharmacologically which binds NPY more avidly than PYY [33]. Claims that this receptor has also been cloned have since been refuted [34,35].

Hypothalamic NPY binding is predominantly Y2, but receptor autoradiography shows low levels of NPY binding in the PVN [36], one of the main sites of NPY action on food intake, and the only hypothalamic site of c-fos activation after ICV NPY [37]. The Y2 agonist NPY-(13-36) only stimulates food intake weakly, even at high doses [38]. ICV antisense injections which effectively reduce NPY Y1 receptor expression in the cortex and attenuate NPY-induced anxiety do not influence food intake in the rat [39]. Others have observed that NPY-(2-36), whilst ineffective in most Y1 systems (e.g. SK NMC cells), is equipotent with NPY at stimulating food intake in the rat [40]. Thus NPY-induced feeding is not mediated by typical Y1 or Y2 receptors. The receptor subtypes involved in the metabolic responses are unknown, and it remains a possibility that further receptor subtypes that mediate these effects will be identified.

Pathophysiological role in obesity
Hypothalamic neuropeptide Y concentrations and mRNA are increased in several genetic models of obesity, including the fatty Zucker rat and the ob/ob and db/db mice [5,41,42]. Given the striking similarities between the observed actions of cen-
trally injected NPY on food intake and metabolism and the abnormalities seen in such rodents, it seems possible that NPY may play a critical role in the development of obesity in such animals. This suggestion has recently been given a significant boost by the identification of the genetic defect leading to obesity in one of these models, the ob/ob mouse [43] (Figure 1). The obese gene codes for a 167-amino acid protein, leptin, which is expressed only in adipose tissue. Ob/ob mice are unable to make leptin, and circulating leptin is absent in these animals. Replacement of leptin using recombinant protein injected subcutaneously in ob/ob mice causes weight loss, and the increased levels of NPY mRNA are decreased to normal [44]. Leptin-binding sites are present in hypothalamic tissue [44]. Whether leptin directly regulates NPY synthesis in the hypothalamus or whether some intermediate process is involved remains to be seen. Insulin and corticosteroids are also involved in the regulation of hypothalamic NPY synthesis, with insulin decreasing and corticosteroids increasing hypothalamic NPY mRNA [45]. It has previously been demonstrated that genetically obese animals fail to suppress NPY synthesis in response to insulin infused into the cerebrospinal fluid [46], and failure to respond to the anorectic effect of insulin has been proposed as one mechanism for increased NPY synthesis in the Zucker rat [47]. Further experiments are essential to clarify the relationships between insulin and leptin in terms of regulation of hypothalamic NPY. Parabiosis experiments suggest that the db/db mouse has a defect in the leptin receptor (as may the Zucker rat), and when this receptor is cloned further definition of this pathway may be possible.

What is the normal physiological role of hypothalamic NPY in metabolic regulation?

NPY concentration, mRNA and release from the arcuate/PVN system are all increased in food-deprived animals [48–50]. The actions of NPY on energy metabolism would serve to conserve energy in the face of reduced dietary intake, but the switch to carbohydrate metabolism, storage of fat and increased insulin release appear paradoxical. It must be remembered, however, that the physiological milieu in a starved animal is rather different from the partially satiated models in which most of these studies have been carried out, and the effects of relatively large injections of NPY, given at a time when endogenous levels would be expected to be low, may be totally different in a food-deprived animal, when endogenous NPY is high. Thus it could be hypothesized that, when food is available, increased NPY levels may serve to increase insulin secretion, and direct metabolism towards storage of energy as fat. If food is not available, the high NPY levels will conserve energy by reducing thermogenesis, but effects on insulin secretion and lipogenesis will become less significant as substrate availability is low.

Conclusions

Hypothalamic NPY plays a fundamental role in the regulation of feeding behaviour and has profound metabolic effects which lead to increased body fat, weight gain and many of the biochemical features of obesity. Hypothalamic NPY is increased in obese rodents, and may be a major transducer in the pathways signalling body fat to the hypothalamus, and in regulating body fat content. Further delineation of the physiological and pathophysiological role of NPY will come with further physiological studies to investigate the hormonal and neural pathways through which NPY mediates its multiple metabolic effects, the identification of the NPY receptor subtypes through which these responses are conducted,
and with the development of effective antagonists.

43 Zhang, Y., Proenca, R., Maffei, M., Barone, M.,
Central glucagon-like peptide-1 in the control of feeding
I. Gunn, D. O'Shea, M. D. Turton, S. A. Beak and S. R. Bloom
Division of Endocrinology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0NN, U.K.

Introduction
Appetite, energy balance and body weight gain are modulated by diverse neurochemical and neuroendocrine signals from different organs in the body and diverse regions of the brain. The hypothalamus at the base of the forebrain plays an important integrative role receiving input from and acting through a variety of systems that involve a close interaction between nutrients, amines, neuropeptides and hormones [11]. When the homoeostasis of these neuroendocrine-neurochemical systems is perturbed, abnormal eating patterns such as those seen in anorexia nervosa, bulimia, diabetes and obesity can develop [1–3].

Several neuropeptides are known to be involved in the regulation of appetite and body weight. Neuropeptide Y (NPY), galanin and opioids increase food intake [4–7] whereas corticotrophin-releasing factor, neotensin, and cholecystokinin have been shown to reduce feeding [5,8–10]. The hypothalamus co-ordinates the interactions between these peptide systems, and raised levels of NPY and neotensin are found in the hypothalamus of diabetic and genetically obese animal models [11–13].

Glucagon-like peptide-1 (7–36) amide (GLP-1)
GLP-1 is the biologically active product of differential post-translational processing of preproglucagon in the central nervous system and gut. Although it is structurally similar to glucagon, GLP-1 shares few of its actions [14,15]. That GLP-1 must have a critical function is inferred by the complete conservation of its sequence through all mammalian species studied. GLP-1 is released into the blood stream in response to a meal [15]. Systemically it is known to be a powerful stimulant of insulin release from pancreatic β-cells and inhibitor of gastric acid secretion and gastric emptying in vitro [16,17]; however, a physiological role for GLP-1 in the central nervous system has not yet been established. We have previously shown that GLP-1 and its specific binding sites are present in the rat hypothalamus [18,19]. A similar distribution of specific hypothalamic receptors has been shown in rat and man.

Exendin (9–39)
Exendin is a peptide which has recently been isolated from Gila monster venom [20]. Its fragment exendin (9–39) has high sequence homology to GLP-1 and acts as a highly specific GLP-1 receptor antagonist in vitro [21]. We have shown that exendin (9–39) specifically blocks GLP-1-induced insulin secretion in the rat at a ratio of 15:1, and in vitro in a clonal β-cell line at