CMLS, Cell. Mol. Life Sci. 60 (2003) 350–377
1420-682X/03/020350-28

CMLS Cellular and Molecular Life Sciences

Review

Neuropeptide Y: the universal soldier

T. Pedrazzini [a,*], F. Pralong [b] and E. Grouzmann [a]

[a] Division of Hypertension, University of Lausanne Medical School, 1011 Lausanne (Switzerland), Fax: + 41 21 314 5978, e-mail: thierry.pedrazzini@chuv.hospvd.ch
[b] Division of Endocrinology, University of Lausanne Medical School, 1011 Lausanne (Switzerland)

Received 17 July 2002; received after revision 7 November 2002; accepted 29 November 2002

Abstract. The peptidic neurotransmitter neuropeptide Y (NPY) has received great attention because it has been implicated in the regulation of several organ systems. In particular, NPY is involved in the regulatory loops that control food intake in the hypothalamus and appears also to be important for regulating the activity of neuroendocrine axes under poor metabolic conditions. Furthermore, NPY exerts vasoconstrictive action on the vasculature and potentiates the actions of many other vasoconstrictors. In addition, it was demonstrated to have trophic properties and could therefore contribute to cardiovascular remodeling. These various effects plus a number of others make NPY an attractive target for the potential treatment of human diseases, such as obesity, metabolic disorders, hypertension and heart failure.

Key words. Obesity; metabolic disorders; hypertension; heart failure; leptin; receptor.

Introduction

In the years to come, it is anticipated that heart diseases will become the leading cause of death in developed countries. Similarly, the number of obese people worldwide is increasing dramatically. Obesity represents a major public health problem and also contributes to many pathologies. In particular, it is associated with increased risk of hypertension, cardiovascular diseases and diabetes. Along these lines, the clustering of cardiovascular risk factors such as abdominal obesity, dyslipidemia, elevated blood pressure, insulin resistance and glucose intolerance has been recognized for a long time [1]. This syndrome, referred to as metabolic syndrome, is of essential clinical importance. Fortunately, major advances have been made in identifying the key elements that control cardiovascular and energy homeostasis [2]. Among others, the neuropeptide Y (NPY) could play a central role. NPY is the most abundant peptide in the mammalian brain and is highly conserved throughout evolution. Moreover, NPY has been shown to be important in a variety of physiological situations, including cardiovascular regulation and control of food intake [3], and it is implicated in several regulatory loops that control the activity of neuroendocrine axes. The identification of NPY-specific receptors and of their associated pathways is therefore crucial in understanding the molecular regulation of several organ systems and could provide the basis for development of novel therapeutic approaches.

The NPY family of peptides and receptors

NPY was first isolated from porcine brain based on its physicochemical properties [4]. It is a member of a family that also includes peptide YY (PYY) and the pancreatic polypeptide (PP). NPY is a neurotransmitter that is synthesized and released by neurons. In the periphery, the

* Corresponding author.

sympathetic neurons represent the main source of NPY, where it is colocalized with norepinephrine (NE) [5]. PYY is predominantly synthesized in the digestive tract [6], and PP is found in pancreatic endocrine cells distinct from those producing insulin, glucagon or somatostatin. Interestingly, NPY is highly conserved throughout evolution [7]. For instance, the NPY sequence in *Torpedo marmorata* is identical to the mammalian NPY in 33 out of 36 positions. PYY and NPY presumably evolved by duplication from a common ancestral gene in early vertebrates. However, their corresponding genes are located on different chromosomes. Similarly, the PP gene probably arose by duplication of the PYY gene, and both genes are located close to one another in the same chromosomal segment [7]. PYY and PP respectively share 70 and 50% sequence homology with NPY. Each of these peptides is 36 amino acids long, characterized by a large number of tyrosine residues and amidated at its C-terminal end. Based on the crystal structure of avian PP, a three-dimensional model of NPY, PYY and PP has been proposed and is referred to as the PP-fold model. The common features of the PP-fold family consist in a polyproline helix (residues 1–8) followed by a tight hairpin turn and an antiparallel helix (amino acids 13–32) ending in a short terminal tetrapeptide amide spatially close to the N-terminal portion [8, 9].

NPY synthesis and processing

The biologically active NPY is derived from a 97-amino acid precursor, pre-pro-neuropeptide Y, following at least four posttranslational enzymatic events (fig. 1) [10]. The 69-residue proNPY is produced after removal of the signal sequence. Then, proNPY undergoes cleavage by the proconverting enzymes PC1/3 and/or PC2 at a single paired basic site (Lys^{38}-Arg^{39}), releasing a 30-amino acid carboxyl terminal peptide, the C-flanking peptide of NPY (CPON) and NPY_{1-39} [9]. The processing of proNPY does not involve furin in the specific cleavage of the dibasic site. In addition, kinetic studies demonstrated that PC1/3 was more efficient than PC2 in cleaving proNPY [11]. NPY_{1-39} is further processed by a carboxypeptidase-like enzyme. The resulting NPY_{1-37} is finally amidated at its C-terminal end by the peptidyl-glycine-α-amidating monooxygenase in a process that removes an additional amino acid and produces the active NPY_{1-36}. Active NPY can be degraded by specific enzymes to generate NPY_{2-36} and NPY_{3-36}. These truncated peptides are thought to be selective ligands for particular NPY receptors. The dipeptidyl peptidase IV (DPPIV, EC 3.4.14.5) represents a likely candidate. Indeed, this exopeptidase is a membrane-bound protease which cleaves proline in penultimate position [12]. By removing the Tyr-Pro dipeptide from the NPY N terminal, DPPIV generates NPY_{3-36}, a

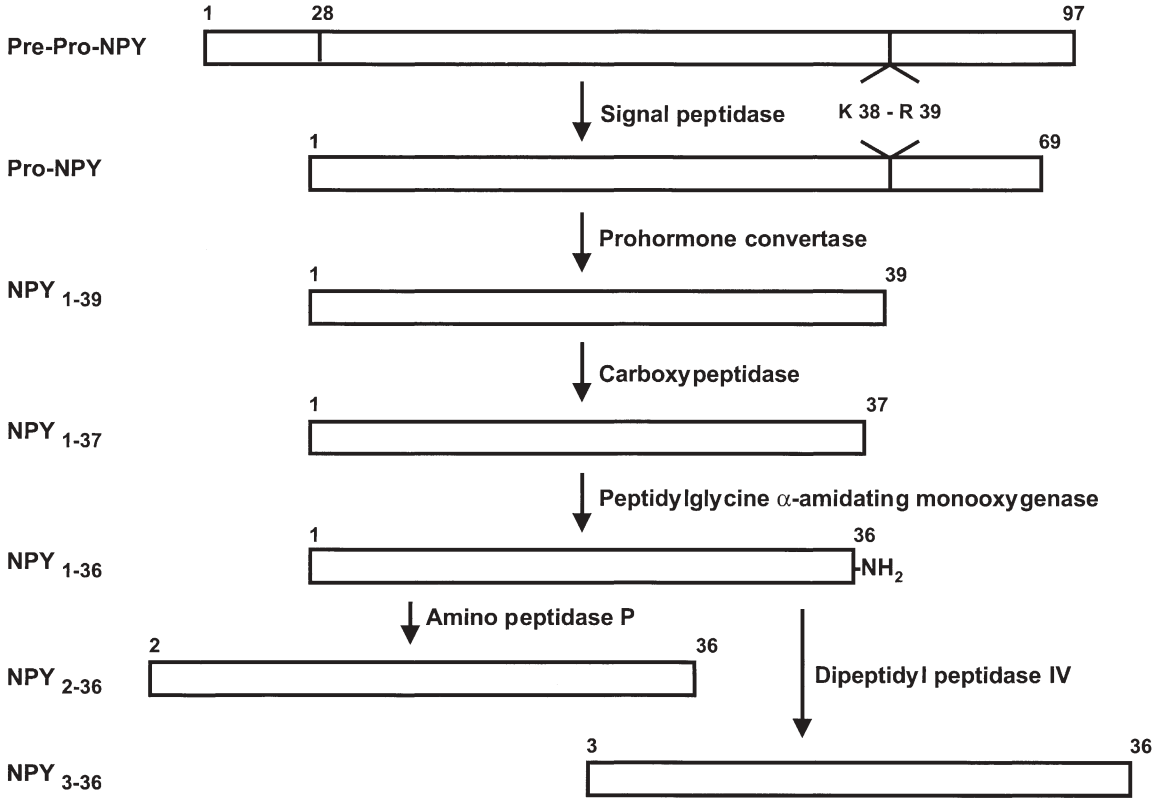

Figure 1. Synthesis and processing of neuropeptide Y. See text for details.

fragment with reduced affinity for the NPY Y1 receptor but that retains its capacity to bind to the NPY Y2 and Y5 receptors [13]. DPPIV is present in the kidneys, liver and ileum, and is abundant on endothelial and epithelial cells, and astrocytes as well as on activated T and B lymphocytes [14]. A soluble form is found in the plasma [15]. Similarly, the aminopeptidase P (AmP, EC 3.4.11.9) can hydrolyze peptide bonds between the first and second amino acid residues at the N-terminal side of a protein provided that the second amino acid is a proline [16]. AmP has thus been shown to remove the N-terminal tyrosine from PYY and NPY in human kidney and jejunum to generate NPY_{2-36} [17], a potentially semiselective Y2 and Y5 agonist. Interestingly, AmP is present in the lungs [18], on astrocytes and neurons, as well as on smooth muscle and endothelial cells [19]. A soluble form of the enzyme exists in the circulation [18]. Nevertheless, the physiological importance of NPY processing to regulate the activation of particular NPY receptors through the generation of selective ligands remains to be established. NPY can be degraded in the central nervous system by proteases present, for instance, in the hippocampal synaptosomes. NPY is efficiently metabolized by a single cleavage between the residues Leu^{30} and Ile^{31}. Thus, catabolic processing of NPY results in formation of a C-terminal truncated fragment NPY_{1-30} and its counterpart, NPY_{31-36}. The enzyme involved in this process appears to possess properties of aspartyl proteases. Moreover, the neutral endopeptidase has a major NPY-hydrolyzing activity in striatal synaptic membrane and in the renal brush border [20]. In contrast, NPY was found to be resistant to the action of two other membrane aminopeptidases, namely the aminopeptidases N and W, and to the action of the angiotensin-converting enzyme.

Normal NPY concentrations in the human plasma have been reported to range from 0.25 to 129 pM [21–25]. This variability in the measurement of circulating NPY concentrations likely reflects cross-reactivities of antisera directed against NPY toward the pro-peptide and NPY fragments as well as to PYY and PP. Measurements of plasma NPY concentrations in rats vary in the literature from 25 pM to 3000 pM. In this species particularly, blood sampling might pose a problem. For instance, rat platelets contain high NPY levels and hemorrhage causes the release of NPY from the sympathetic nerve endings [26].

NPY receptors

The study of the different NPY receptors has largely been facilitated by the synthesis of selective agonists and antagonists. In fact, NPY receptors were originally defined according to their capacity to bind particular NPY analogs or truncated peptides [27]. The NPY_{13-36} peptide was the first NPY receptor agonist that allowed discrimination between the Y1 and Y2 subtypes based on its capacity to inhibit the twitch response in rat vas deferens and inability to promote vasoconstriction [28]. Thus, it was proposed that receptors that are activated solely by intact NPY would be named Y1, whereas those that are activated by NPY as well as its C-terminal fragments would be designated Y2. Then, the [Leu^{31}, Pro^{34}] NPY-substituted peptide was synthesized and shown to be a selective agonist for the NPY Y1 receptors [29]. However, molecular cloning of the different receptors known to date revealed the existence of at least 5 seven-transmembrane domain receptors [30–33]. Each of these receptors could be responsible for particular NPY functions, and therefore it appeared that more specific compounds needed to be generated.

The NPY Y1 receptor

The first NPY receptor identified was cloned from a rat complementary DNA (cDNA) library and shown to encode the NPY Y1 receptor [34]. In humans, the gene is located on chromosome 4q(31.3–32) [35]. Exons 2 and 3 are two coding exons separated by a small intron of about a hundred base pairs. Three variants of the human NPY Y1 receptor have been identified that depend on alternative transcription of noncoding exons 1A, 1B and 1C. Distinct promoters, some of them positioned between exons, are used alternatively and allow tissue-specific expression [36]. NPY Y1 expression has been detected in a variety of tissues including brain, heart, kidneys and gastrointestinal tract. Some established cell lines such as SK-NMC cells [37], a neuroblastoma cell line, and HEL cells [38], an erythroleukemia cell line, constitutively express Y1. Analysis of stably transfected cells enables rank ordering of binding potency, which appeared to be NPY = PYY = [Leu^{31}, Pro^{34}]NPY > C-terminal NPY fragments > PP [39]. However, with the discovery and cloning of the Y4 and Y5 receptors, the classical Y1 agonist [Leu^{31}, Pro^{34}]NPY was found to bind significantly to these two receptor subtypes and thus to be less selective than previously thought. New compounds became available and among them, [D-Arg^{25}]NPY, [D-His^{26}]NPY and Des-AA^{11-18}[$Cys^{7,21}$, D-Lys^{9}(Ac),D-His^{26},Pro^{34}]NPY demonstrated high affinities for the Y1 receptor and inhibited forskolin-stimulated cyclic AMP (cAMP) production at subnanomolar concentrations [40]. Therefore, these peptides seem to be highly selective for the NPY Y1 receptor. Interestingly, these agonists stimulate feeding dose dependently after intracerebroventricular administration, suggesting a role for the Y1 receptor in mediating food intake in rodents (see also below). Recently, [Phe^{7}, Pro^{34}]NPY was also reported to specifically activate Y1 [41].

The prototype of NPY Y1-mediated responses is vasoconstriction [42]. However, this receptor has been involved in several other NPY-induced responses, such as

stimulation of food intake and activation of neuroendocrine axes. Because of the NPY Y1 receptor's potential as a therapeutic target, large random screening was initiated to discover nonpeptidic antagonists. BIBP3226, the first Y1 antagonist, demonstrated a K_i in the nanomolar range [43]. Its affinity for other NPY receptors is not significant. Unfortunately, the bioavailability of this compound is quite low, and its poor solubility precludes its wide use in vivo. Nevertheless, peripheral administration of BIBP3226 blunts the effect of exogenous NPY on blood pressure [44]. BIBP3226 was also shown to attenuate the vasoconstriction observed during sympathetic nerve stimulation in the kidneys and the nasal mucosa [45]. Similarly, BIBP3226 attenuates the increase in blood pressure observed during cold stress in the rat, implicating the NPY Y1 receptor in the modulation of blood pressure during stress [46]. However, this compound does not seem to affect the elevation of blood pressure observed in spontaneously hypertensive rats or in a model of renovascular hypertension [47]. Another nonpeptidic Y1 antagonist, BIBO3304, was shown to be more soluble and less toxic in vivo [48]. Furthermore, GW1229 (also known as 1229U91 and GR231118), a symmetrical dimeric peptide with high affinity for the Y1 receptor, demonstrated good blocking actions [49]. However, this compound also exhibited agonistic properties at the Y4 receptor [50]. Other antagonists of the Y1 receptor, for instance SR120819A, have been described, but their selectivity, in particular toward Y5 and y6 receptors, is less established [51]. Finally, J-115814, a novel Y1 antagonist with low affinity for other NPY receptors, was recently shown to significantly reduce feeding [52].

The NPY Y2 receptor

A cDNA encoding the human NPY Y2 receptor was initially cloned from SMS-KAN cells and, later, from human brain as well as from the human neuroblastoma cell line KAN-TX [53]. In humans, the Y2 gene maps to chromosome 4q31, close to the Y1 and Y5 receptor locus [54]. This suggests that these receptors arose from a common ancestor by gene duplication. Messenger RNA (mRNA) for the Y2 receptor has been detected in various regions of the central nervous system (CNS) [55, 56]. Postjunctional Y2 receptors seem to be present in a variety of tissues but only at low levels. Interestingly, rabbit kidneys were found to be a good source of this receptor subtype [57]. LN319 and SMS-KAN cells are human cell lines expressing Y2 receptors [58]. Finally, it is noteworthy that the so-called PYY-preferring receptor previously characterized in rat intestinal crypts, where it could mediate the inhibition of fluid secretion, is in fact a peripheral Y2 receptor [59].

The Y2 receptor binds NPY analogs with the following rank order of potency: NPY = PYY = C-terminal fragments > [Leu^{31}, Pro^{34}]NPY > PP [53]. Compared with the prototype Y2 agonist, i.e. NPY_{13-36}, cyclic and truncated fragments of NPY, such as 1–4-Ahx-25–36 NPY and [Leu^{28,31}]NPY_{24-36}, have improved selectivity towards the Y2 receptor [58, 60]. Another NPY analog, the *N*-acetyl [Leu^{28},Leu^{31}]NPY_{24-36} was shown to attenuate cardiac vagal action, a typical Y2-mediated prejunctional action, but was unable to elicit a pressure response following injection [61]. This suggests that this compound had no postjunctional NPY Y1-mediated effects. The first Y2 antagonist, T_4-$[NPY(33-36)]_4$, was created based on the assembly of C-terminal tetrapeptides onto a nonpeptidic template, ensuring three-dimensional stability. This peptidomimetic binds to the Y2 receptor with high affinity and has poor binding capacity to the Y1 receptor. Inhibition of norepinephrine (NE) release from presynaptic rat hypothalamic synaptosomes, a prototypical response mediated by NPY through Y2 receptors, was significantly reduced by T_4-$[NPY(33-36)]_4$ [62]. Recently, BIIEO246, a potent nonpeptidic Y2 antagonist, was synthesized and should prove useful in the future to better delineate the exact physiological role of this receptor [63].

The NPY Y4 receptor

The gene encoding the human NPY Y4 receptor was cloned and originally designated 'PP1' [32]. This gene is located on chromosome 10q11–21. The rat and the murine homologs were also cloned shortly thereafter. The Y4 receptor is mainly expressed in the colon, small intestine and prostate. In the rat, it is also expressed in the testes. In addition, various regions of the CNS seem to display low expression levels.

Among the NPY family, NPY demonstrates high affinity for the Y1, Y2 and Y5 receptors but only moderate affinity for the Y4 subtype [64]. In contrast, PP binds preferentially to Y4 and demonstrates low affinity for the other receptor subtypes. The main characteristic of the Y4 receptor is thus its capacity to bind PP from the corresponding species with very high affinity [64, 65]. Indeed, PP homologs from other species may have 50–100-fold lower affinities. However, the human PP appears to have significant affinities for the human, rat and murine Y4 receptors. PYY and [Pro^{34}]-substituted analogs were also reported to have high affinities for the Y4 subtype [65]. [Leu^{31}, Pro^{34}]NPY was found to be the only NPY analog to interact significantly with the rat Y4 receptor [66]. Interestingly, a recent study shows that high NPY concentrations do not affect forskolin-stimulated cAMP accumulation in cells expressing the human Y4 receptor, whereas nanomolar concentrations of NPY displaced human PP from its receptor [64]. These data suggest that NPY could bind to the Y4 receptor but does not necessarily activate secondary intracellular pathways. Together, these studies indicate that PP is the primary endogenous ligand for the Y4 receptor.

The NPY Y5 receptor

Cloning of rat and human NPY Y5 receptors was reported as identification of the receptor mediating the appetite-stimulating activity of NPY. Therefore, this particular subtype was initially named feeding receptor [67]. As we shall see, whether the Y5 receptor is the main subtype controlling feeding is still debated today [68, 69]. The human gene is located on chromosome 4q32, more precisely at the same locus as the NPY Y1 receptor gene. However, it is transcribed in an opposite direction [67]. Whether expression of these two receptors is controlled by common regulatory elements remains to be demonstrated [70]. mRNA for the Y5 receptor was detected in several brain areas, including those believed to be important for regulation of food intake [71, 72]. In the periphery, it seems mainly expressed in the testis. In transfected cells, this subtype couples to an inhibitory G protein and blocks cAMP accumulation [67].

NPY analogs demonstrate similar rank orders of potency for Y5 binding, inhibition of cAMP accumulation and stimulation of food intake, i.e. NPY = PYY > [Leu^{31}, Pro^{34}] NPY = NPY_{2-36} = PYY_{3-36} > NPY_{13-36} [67]. Interestingly, rat PP has a very low affinity for rat and human Y5 receptors whereas both human and bovine PP have significant affinities for Y5. The first NPY analog with selective binding capacity to Y5 was [D-Trp^{32}]NPY [73]. Described as an antagonist, this substance was later shown to stimulate food intake in rats [74, 75], indicating either that food intake was not mediated by the Y5 subtype or this compound was less selective than expected. Recently, [Ala^{31}, α-amino isobutyric acid^{32}]NPY was described as the first true Y5-selective agonist [76]. This substituted peptide was even more potent than NPY in its capacity to stimulate food intake. Then, a series of peptides was obtained by combination of the [Ala^{31}, α-amino isobutyric acid^{32}]NPY motif with chimeric peptides containing segments of NPY or PP, which display the same selectivity and an even higher affinity for the Y5 receptor. Interestingly, the structures of NPY and [Ala^{31}, α-amino isobutyric acid^{32}]NPY, analyzed by nuclear magnetic resonance, revealed a different conformation in the C-terminal region. Indeed, the α helix of NPY appeared to be substituted for a 3(10)-helical structure in the chimeric compounds. Therefore, the resultant increased peptide flexibility in the C-terminal part of NPY could be crucial for selective binding to the NPY Y5 receptor and its subsequent activation.

The first Y5 receptor antagonist with nanomolar affinity was the CGP71683A compound [77]. This substance displayed very low affinity for the other NPY receptor subtypes, and was reported to inhibit NPY-induced food intake. However, CGP71683A was shown also to bind significantly to muscarinic and serotonin binding sites [78]. Recently, a potent and orally available Y5 antagonist, L-152,804, with binding affinities to the human and rat Y5 receptors in the nanomolar range, was shown to block NPY-mediated increase in intracellular calcium levels in a Y5-transfected cell line [79]. Interestingly, administration of L-152,804 significantly attenuated PP-induced food intake in rats, but not the feeding response elicited by NPY.

The NPY y6 receptor

An additional receptor subtype was cloned recently from different species and designated y6. Fluorescent in situ hybridization has localized the human y6 gene in the chromosome 5q31 region [80]. The tissue distribution of this particular receptor subtype is not very clear. Indeed, expression was observed in the mouse brain by in situ hybridization [81], but not by Northern blot analysis [80]. In rabbits, y6 was detected by reverse transcription-polymerase chain reaction (RT-PCR) in some brain areas, including the hypothalamus and the hippocampus, as well as in the small intestine and the adrenals, but again not seen by Northern blotting. In contrast, Northern blot analysis detected y6 expression in human tissues, including the heart and skeletal muscles [82]. However, the most important finding is that the monkey and the human sequences show a frame shift mutation located in the putative third intracellular loop of the receptor [82]. As compared with other species, this mutation results in an extra stop codon and a truncated protein of only 290 amino acids. Therefore, although mice express a full-length protein, expression in primates does not produce a functional protein [83]. The pharmacological profile of the y6 receptor also remains unclear. For the murine receptor, the order of binding appears to be NPY = PYY = [Leu^{31}, Pro^{34}]NPY = PP > C-terminal fragments {5914}, whereas expression of the rabbit receptor suggests an order of potency of PYY = NPY_{13-36} = NPY > [Leu^{31}, Pro^{34}]NPY > PP [82]. The physiological function of the y6 receptor remains to be discovered.

The NPY y3 receptor

The NPY y3 receptor is a putative receptor that has never been cloned [84]. However, it was pharmacologically and functionally characterized using substituted and truncated NPY analogs. An important characteristic of the y3 receptor is its inability to be activated by PYY. In addition, the potency of NPY analogs on y3 receptors might depend on the species and tissues studied. In this respect, only a small number of tissues seem to express the NPY y3 receptor. For instance, bovine adrenal chromaffin cells [85–87], rat superior cervical ganglia sympathetic neurons [88], rat nucleus tractus solitarii [89], rat cardiac ventricular membranes [90], rat distal colon [91, 92] and differentiated PC12 cells [93] appear to express binding sites with a pharmacological profile consistent with that of the y3 receptor. As an example, the rank order of potency of NPY analogs to stimulate catecholamine secre-

tion in chromaffin cells was NPY > NPY_{13-36} = NPY_{3-36} = PP ⋙ [Leu^{31}, Pro^{34}]NPY = PYY [94]. However, it cannot be excluded that this stimulation pattern reflects the presence of more than one receptor on the surface of the cells, which could then independently bind different ligands or could even form dimeric structures with particular binding affinities for NPY analogs.

The neuropeptide FF receptors

The octapeptide neuropeptide FF, also known as NPFF or F-8-F-amide, and the octadecapeptide neuropeptide AF, also known as NPAF or A-18-F amide, are derived from a common precursor, which was originally isolated from the bovine brain. NPFF has been implicated in nociception and in the modulation of opiate-induced analgesia. Two receptors for NPFF have been identified, namely NPFF1 and NPFF2 [95]. The NPFF1 receptor shares a high degree of homology with the NPY Y2 and Y4 receptors. Although NPY does not appear to bind significantly to either NPFF receptor subtypes, the NPY Y1 receptor antagonist BIBP3226 displays a substantial affinity for NPFF1 and NPFF2, and frog PP, a NPY Y4 receptor agonist, binds NPFF2 [96]. Moreover, NPFF1 and -2 are highly expressed in the hypothalamus, a brain area in which NPY functions are traditionally tested. Along this line, NPFF itself was shown to reduce refeeding in food-deprived rats. Interestingly, NPFF1 maps to human chromosome 10q11–21, a region also bearing the NPY Y4 receptor. Moreover, NPFF2 is located on chromosome 4q31 close to the NPY Y1 Y5 Y2 gene cluster. This raises the interesting possibility that the NPY and NPFF receptors arose from a common ancestor gene by duplication. In addition, it cannot be completely ruled out that some of the functions attributed to NPY receptors, which were initially identified using pharmacological probes, could be ascribed to NPFF receptors.

Signaling

NPY receptors are seven-transmembrane domain receptors that belong to the G-protein-coupled receptor family. After agonist stimulation, the inactivated GDP-bound proteins exchange GDP for GTP on the Gα subunit, which then dissociates from the G$\beta\gamma$ dimer. Both the active GTP-bound Gα subunit and the G$\beta\gamma$ complex can individually regulate the activity of specific downstream effectors. Initial studies demonstrated that NPY mediated some of its physiological effects through the inhibition of the adenylyl cyclase [97, 98]. Therefore, NPY was shown to decrease forskolin-stimulated cAMP accumulation in a variety of cell lines and in tissues. However, NPY had no significant effect on basal cAMP levels. Since this response was pertussis toxin sensitive, it indicated that the receptors were coupled through inhibitory heterotrimeric Gi/o proteins. In addition, NPY appeared also to induce calcium mobilization, and is certainly linked to calcium-signaling pathways [38]. Both intracellular pathways are consistent with the vasoconstrictive properties of NPY and with its capacity to potentiate the actions of other vasoconstrictors. In smooth muscle cells, stimulation of calcium transients did not seem to depend on inositol 1,4,5-trisphosphate (IP_3) production [99, 100]. Interestingly, although NPY had no effect on IP_3 levels alone, it could potentiate the action of angiotensin II on IP_3 production. In addition, NPY had no detectable effect on the activation of protein kinase C (PKC). It is thus unlikely that NPY receptors couple to phospholipase C in smooth muscle cells. However, this view has been challenged, since treatment of smooth muscle cells with a specific phospholipase C inhibitor seemed to block not only NPY-induced IP_3 synthesis but also calcium mobilization [100]. In smooth muscle cells, NPY also induced rapid phosphorylation of myosin light chain, which represents a mandatory step for the initiation of contraction [101]. Using a series of NPY analogs, inhibition of adenylyl cyclase as well as stimulation of intracellular calcium release was shown to be mediated by NPY Y1 receptors. Transfection experiments using NPY Y1 cDNA clones confirm the coupling of this receptor subtype to inhibition of stimulated cAMP accumulation and to an increase in intracellular calcium [102]. In addition, it appeared that NPY could stimulate the mitogen-activated protein kinase (MAPK) pathways [103, 104]. In particular, NPY Y1 receptors induced the phosphorylation of p42/p44 MAPK, also known as extracellularly regulated kinases (ERKs) [105]. This effect appeared sensitive to pertussis toxin and could be dependent on phosphatidylinositol 3 (PI-3)-kinase [103]. These results were confirmed using human erythroleukemia (HEL) cells, in which NPY induced phosphorylation of ERK through NPY Y1 receptors. In addition, NPY was shown to not activate the c-jun N-terminal kinase (JNK), the p38 kinase or PKC in HEL cells [106].

The intracellular signaling pathways associated with Y2 receptors have particularly been studied in rat dorsal root ganglion (DRG) neurons. In these cells, NPY appeared to inhibit calcium influx via the modulation of voltage-dependent calcium channels [107]. However, an NPY-induced increase in intracellular calcium secondary to IP_3 production was also described in these cells. Coupling of NPY Y2 to an inhibitory Gi protein was suggested by the sensitivity of the response to pertussis toxin [108, 109]. In the human SMS-KAN neuroblastoma cell line, NPY was shown to inhibit ω-conotoxin-sensitive calcium influx (N-type channel), as well as angiotensin II-stimulated calcium release from intracellular stores [110]. However, contrary to what was observed in DRG neurons, NPY was not able to trigger intracellular calcium release in SMS-KAN cells. Interestingly, in these cells NPY also inhibits forskolin-induced cAMP accumulation. These

findings were confirmed using Y2-transfected cells, in which receptor activation induced a decrease in cAMP levels and an increase in calcium transients [111]. The human glioblastoma cell line LN319 also proved useful in defining Y2-mediated pathways, since these cells exclusively express this receptor subtype. In these cells, NPY inhibits forskolin-induced cAMP accumulation and stimulates an increase in intracellular calcium via pertussis toxin-sensitive pathways [112]. Interestingly, these pathways could involve the activation of phospholipase C. Accordingly, NPY-induced activation of calcium-independent and diacylglycerol (DAG)-dependent PKC isoforms via NPY Y2 receptors has been demonstrated in human ciliated cells [113]. Finally, the NPY Y2 receptor was also shown to activate MAPK pathways through a Gi protein and via PI-3 kinase [103].

The signaling pathways associated with the other NPY receptor subtypes have mainly been studied in transfected cells. Although the importance of these results is not questioned, functional data obtained in physiological situations are limited. Therefore, the NPY Y4 and Y5 receptors as well as nonprimate y6 receptors have all been shown to couple to inhibition of cAMP accumulation [64, 114]. Whether these receptors stimulate calcium mobilization is still not completely established. The NPY Y4 receptor has been reported to induce intracellular calcium oscillations in rat arcuate neurons [115]. In addition, a Y4 receptor-mediated regulation of calcium and potassium channels was demonstrated following expression in *Xenopus* oocytes or in HEK 293 cells [116]. Stably transfected HEC-1B with a human Y5 receptor cDNA showed no elevation of intracellular calcium upon NPY stimulation [117]. In contrast, NPY dose dependently increases calcium concentrations in LMtk– cells expressing a recombinant human Y5 receptor [77]. This effect was blocked by addition of a selective Y5 antagonist [77]. Finally, other pathways have been reported to be associated with activation of NPY Y5 receptors. In primary mouse cardiomyocytes, NPY induces rapid phosphorylation of ERK, JNK and p38 MAPK as well as activation of PKC [118]. Moreover, NPY potentiates phenylephrine-stimulated MAPK and PKC activation. These stimulatory effects appear to be pertussis toxin sensitive and mediated by NPY Y5 receptors, since Y5 selective-agonists can mimic NPY actions and NPY potentiation of MAPK activation is abolished in NPY Y5-deficient cells. However, these findings were not reproduced in Y5-transfected HEC-1B cells, in which NPY activated neither the MAPK nor the PKC pathways [117]. This observation might reflect distinct NPY intracellular couplings in different cell types.

Besides the direct response elicited by NPY in various cell types, one of its main actions is its capacity to amplify responses induced by other substances. In particular, NPY exerts a synergistic effect on Gq-mediated NE-induced responses. This raises a question about the molecular mechanisms involved in the potentiating actions of NPY [119, 120]. At least three different mechanisms account for the synergistic effects observed in presence of NPY [121]. First, NPY induces the release of intracellular calcium, which must play an important role in the vasoconstrictive response elicited by NPY in smooth muscle cells. In addition, at doses that do not induce a direct vasoconstriction, NPY-induced calcium release could decrease the threshold of activation by other vasoactive substances. Second, a number of molecular events in cells are controlled by a feedback mechanism involving phosphorylation. Relevant to this review is the control by c-AMP-dependent protein kinase (PKA). For instance, the termination of contraction in cardiomyocytes is highly dependent on calcium reuptake by the sarcolemma calcium ATPase (SERCA). SERCA is itself regulated by phospholamban, which inhibits the activity of the pump when present in an unphosphorylated form. Therefore, phosphorylation of phospholamban by PKA activates calcium reuptake and represents an important mechanism for induction of relaxation. It is then expected that NPY, by inhibiting cAMP accumulation, could reduce the activity of PKA and promote contraction. Finally, Gi-coupled NPY receptors could facilitate the effects of receptors associated to a Gq protein by changing the activity of PLC. The production of inositoltriphosphate (IP3) and the subsequent induction of calcium release are important in a number of cellular mechanisms such as contraction or calmodulin-mediated activation of phosphorylation cascades. Moreover, PKC activation could also potentiate some of the effects produced by Gq-coupled receptors. For instance, arachidonic acid generation by phospholipase A is dependent on PKC activity and intracellular calcium release [122]. PLC isoforms can be differently stimulated depending on the types of G protein that are activated in response to agonist stimulation. Indeed, Gq protein stimulates preferentially PLCβ_1 via the Gα subunit, whereas Gi protein stimulates mainly PLCβ_2 through the G$\beta\gamma$ dimer. Interestingly, PLCβ_2 is downregulated by PKA. NPY could therefore facilitate the stimulation of PLCβ_2 activity via direct activation by the G$\beta\gamma$ heterocomplex and indirectly through its inhibitory actions on cAMP production and PKA activation.

Cardiovascular regulation

Although circulating NPY levels are quite low in resting healthy volunteers [23], activation of the sympathetic nervous system substantially stimulates the release of NPY during the course of physical exercise [123, 124], in individuals submitted to cold [123], in stressful situations [125] or during hypoxia [126] from either peripheral

nerves or the adrenal medulla, and then increases plasma concentrations. Elevated NPY concentrations were also reported in hypertension. However, this was again interpreted as an increase in sympathetic activation, which is often found in hypertensive patients [127, 128].

Stimulation of the adrenergic system is a hallmark of bad prognosis in individuals with heart failure. Coronary vasoconstriction elicited by catecholamines participates in myocardial ischemia in patients with angina pectoris. Therefore, NPY has been proposed to be a useful marker for estimating the condition of patients suffering from cardiac diseases. Indeed, plasma NPY immunoreactivity appeared elevated in patients with acute myocardial ischemia [129] and in those with congestive heart failure [130]. The prognostic value of these findings was studied in patients admitted to a coronary care unit [129, 131]. Interestingly, plasma NPY higher than 60 pM was found to be an independent prognostic factor for increased risk of mortality in symptomatic patients without acute myocardial ischemia. More recently, a correlation was also demonstrated in patients with coronary heart disease between plasma NPY levels and the degree of post-exercise ischemia [132]. However, these observations need to be taken with caution. For instance, only 25% of patients with acute heart failure were found to display an increase in circulating NPY levels [131]. In addition, decreased NPY and NE concentrations were measured in myocardial tissues of failing ventricles from patients with idiopathic dilated cardiomyopathy [133]. Furthermore, different studies showed normal plasma NPY concentrations in patients suffering from acute myocardial infarction or chronic heart failure as compared with healthy subjects [134, 135]. It is also noteworthy that exercise does not differentially increase plasma NPY concentrations in healthy volunteers and in patients with congestive heart failure [132]. The discrepancies found in these various situations could be partially explained by the severity of the diseases. NPY release could only play a role during extreme circumstances such as that found in patients with coronary heart disease experiencing acute ischemic conditions during high sympathetic activation.

Recently, an association between a Leu^7-to-Pro^7 polymorphism in the signal peptide of NPY and high serum cholesterol levels was described in a Finnish and a Dutch population of obese subjects [136]. Interestingly, the phenotype was also present in normal-weight Finnish individuals. Furthermore, the Pro^7 substitution was found to be associated with higher serum triglyceride values in young boys, who also had a higher birth weight than homozygous individuals for Leu^7 [137]. The Leu^7/Pro^7 genotype appeared to be associated with carotid atherosclerosis as well as diabetic retinopathy [138]. In addition, the frequency of the Leu^7-to-Pro^7 polymorphism appeared to vary from one population to the other, since the incidence in healthy Japanese was extremely low. It is therefore unlikely that NPY represents a strong genetic factor affecting cholesterol among Japanese people [139]. The exact NPY-mediated mechanisms influencing blood lipid concentrations and deposition in the vasculature are unknown. However, the Leu^7-to-Pro^7 mutation is likely to affect secondary and tertiary structures in the signal peptide. This, in turn, could modify the synthesis, processing and release of the active peptide [140]. Indeed, the distribution of pro-NPY and NPY immunostaining in human umbilical vein endothelial cells (HUVECs) was different according to the genotype. Specifically, cells from individuals with the Leu^7-to-Pro^7 polymorphism seem to synthesize active NPY more efficiently. Along this line, subjects with the Leu^7-to-Pro^7 substitution showed a larger increase in circulating NPY levels during exercise as compared with homozygous controls despite similar basal NPY concentrations. Heart rate was also higher in subjects with the Pro^7 mutation. Together, these results are consistent with more efficient NPY production, which might affect the levels of sympathetic stimulation in target organs.

NPY and blood pressure homeostasis

In the periphery, NPY is mainly expressed in sympathetic nerve endings of NE-producing neurons surrounding blood vessels. The localization of NPY led to the proposal that this peptide could play an important role in modulating the activity of the sympathetic nervous system to control blood pressure [141]. Indeed, NPY was shown to display a potent and long-lasting vasoconstrictor activity in vivo [42]. This direct effect of NPY on the vasculature is insensitive to α-adrenergic blockade [42, 142, 143]. Interestingly, sympathetic nerve stimulation in the presence of adrenoreceptor antagonist evokes a long-lasting vascular response reminiscent of that elicited by NPY. Moreover, at doses that do not affect blood pressure, NPY potentiates the action of various vasoactive substances as well as the contractile response to electrical nerve stimulation [142, 144–146]. In both rats and humans, this synergistic effect is particularly seen in small resistance arterioles [146]. The importance of postsynaptic NPY Y1 receptors in mediating the vasoconstrictive properties of NPY was studied using isolated blood vessels.

The physiological role of the Y1 receptor subtype was eventually demonstrated in mice lacking Y1 expression, which show no blood pressure response to NPY but a normal response to NE. In addition, NPY does not potentiate NE-induced vasoconstriction of blood vessel isolated from NPY Y1-deficient animals. NPY Y1 knockouts have normal blood pressure, suggesting that the NPY Y1 receptor does not play a crucial role in maintaining blood pressure homeostasis in unstimulated conditions [147]. Along these lines, a role for NPY on the control of basal

blood pressure is also not supported by pharmacological studies. Indeed, BIBP3226, a specific Y1 antagonist, does not affect blood pressure in normotensive and in spontaneously hypertensive rats despite increased circulating NPY concentrations in the latter [26]. However, the role of NPY in the activity of the sympathetic system has been suggested by several independent observations. First of all, in vitro, the long-lasting vasoconstriction induced by high-frequency sympathetic stimulation of the guinea pig vena cava can be significantly blunted in the presence of SR120107 [148] or BIBP3226 [45, 149], two NPY Y1 antagonists. In vivo, BIBP3226 can also inhibit the vasoconstrictive response to high-frequency stimulation of sympathetic nerves, indicating that endogenous NPY via the NPY Y1 receptor may play a role in evoking the long-lasting vasoconstriction seen in nasal mucosa, hind limb and skin [45]. In addition, stress-induced hypertension in rats was reversed by BIBP3226, suggesting that NPY could be involved in mediating physiological responses to stress [150]. These findings are substantiated by observations showing an increase in adrenal NPY expression during chronic stress. Moreover, chronic exposure to cold, an extremely stressful stimulus, has a synergistic effect on the pressor response to acute stress. Together, these observations suggest that enhanced NPY secretion could be important in modulating the blood pressure response to chronic stress. Furthermore, the vasopressive properties of NPY as well as its capacity to potentiate adrenergic responsiveness led to the suggestion that this peptide might be beneficial in the management of endotoxic shock. Indeed, in humans experiencing sepsis and septic shock, plasma levels of NPY are significantly increased [151, 152], and NPY-mediated vasoconstriction was shown to be preserved during endotoxemia [153]. NPY infusion is able to prevent the development of hypotension and restore the response to pressor agents in endotoxemic rats at doses that do not affect blood pressure [153]. The fact that the Y1 antagonist BIBP3226 significantly exacerbates the detrimental effects of both endotoxemia and hemorrhage strongly suggests that NPY acts through Y1 receptors to maintain blood pressure during septic and hemorrhagic shocks [154]. It is also indicative of a role of endogenous NPY in controlling blood pressure during shock. Central administration of NPY was shown to restore appetite during endotoxemia, suggesting that downregulation of the hypothalamic pathways regulating food intake in endotoxic shock might also be secondary to modulation of NPY [155–157].

Prejunctional NPY actions

In contrast to its postjunctional effects, NPY exerts also prejunctional actions. In fact, in postganglionic sympathetic neurons [158–160], in parasympathetic neurons [161] as well as in peptidergic neurons [162], NPY inhibits neurotransmitter release, an effect that has been attributed to the activation of Y2 receptors [163, 164]. Therefore, NPY was proposed to regulate inhibition of cholinergic vagal action evoked by the sympathetic nervous system in the heart [165]. Indeed, *N*-acetyl [Leu^{28,31}] NPY_{24-36}, a selective Y2 agonist, mimicked the inhibitory effect of NPY on cardiac vagal activity [166, 167] and on cholinergic mediated vasodilatation [60, 168]. Moreover, the Y2 antagonist BIIE0246 attenuated the inhibitory effect of *N*-acetyl [Leu^{28,31}] NPY_{24-36} on vagal-induced bradycardia, suggesting also the presence of functional Y2 receptors in parasympathetic junctions in the heart. It is important to note that *N*-acetyl [Leu^{28,31}] NPY_{24-36} had no effect on adrenergic receptor-mediated vasoconstriction [168]. In the heart, presynaptic Y2 receptors in parasympathetic nerve terminals could also inhibit the release of acetylcholine. In the trachea, *N*-acetyl [Leu^{28,31}] NPY_{24-36} reduced the size of contraction evoked by a cholinergic stimulation of parasympathetic nerves. Accordingly, *N*-acetyl [Leu^{28,31}] NPY_{24-36} also reduced the amplitude of ATP-induced excitatory junction potentials in the vas deferens [169]. These findings are consistent with early observations indicating that prejunctional NPY receptors inhibited ATP release in the vas deferens [170, 171]. Together these results suggest that Y2 receptors mediate the prejunctional actions of NPY. Finally, NPY was also shown to suppress the release of neurotransmitters from parasympathetic and sensory nerve endings in the airways [44, 162, 172, 173]. NPY inhibits the secretory response to vagal stimulation in the lower airways [174]. Moreover, in humans, NPY appears to reduce sneezing, itching and secretion after an allergen, a capsaicin or a bradykinin challenge [175–177]. These findings suggest that the NPY receptor could represent an interesting target for the treatment of allergic and vasomotor rhinitis.

Role of NPY in catecholamine secretion by chromaffin cells

Because of the interactions between NPY and catecholamines, a possible role of NPY in controlling epinephrine (E) and NE secretion from the adrenals as investigated. Indeed, beside its regulatory action via NPY Y2 receptors on the release of catecholamine in sympathetic nerve endings, NPY was suggested to stimulate catecholamine secretion from intact rat adrenal capsular tissue [178]. NPY was found in adrenals of humans and other species, appeared to be produced by chromaffin cells and was shown to be abundantly secreted by human pheochromocytomas [25, 179–184]. In particular, NPY immunoreactivity can be observed in the adrenal medulla. The vast majority of NPY seems present in E-type chromaffin cells, as demonstrated by the colocalization of NPY and the enzyme phenylethanolamine *N*-

methyltransferase (PNMT) [185]. However, NPY is also present in NE-type cells [186]. The actions of NPY on catecholamine secretion depend in part on experimental conditions. In addition, the type of receptors mediating these NPY effects remains undefined. Nevertheless, the presence of NPY Y1 and y_3 receptors has been reported in bovine chromaffin cells [85–87, 187]. NPY was shown to stimulate the secretion of catecholamines from perfused bovine adrenal glands in the presence of cholinergic agents [187], and to trigger rapid release of catecholamine from chromaffin cells through a NPY receptor subtype whose pharmacological profile is consistent with that described for the y_3 receptor [E. Grouzmann, unpublished]. However, other studies demonstrated a weak inhibitory effect of NPY on NE and E release from bovine chromaffin cells stimulated by cholinergic agonists [86]. In support of these latter observations, antiserum directed to NPY enhanced nicotine-stimulated catecholamine release by rat primary adrenomedulla cells [188]. The NPY receptor subtype, which mediates the inhibitory effect of NPY on nicotine-induced catecholamine secretion, does not appear to be coupled to cAMP (87). NPY Y2 agonists, such as NPY_{13-36}, inhibit catecholamine synthesis stimulated by chronic depolarization in differentiated PC12 pheochromocytoma cells [189]. This effect seems to be dependent on an inhibition of calcium influx through L-type voltage-gated calcium channels, and possibly involves a PKC-dependent pathway [190].

Moreover, NPY itself can be released by chromaffin cells, and appears to exert an autocrine control on catecholamine secretion [94]. Therefore, NPY originating from the adrenal medulla could locally enhance the secretion as well as the downstream effects of catecholamines. Since NPY and catecholamines are colocalized in secretory granules, it is tempting to speculate on a possible cosecretion for maximum actions. In particular, the long-lasting effects of NPY could potentiate the vasoconstrictive actions of catecholamines. Despite these important findings, the exact role of NPY as a modulator of catecholamine release from the adrenals should be further investigated. Nevertheless, these results suggest that NPY antagonists might be useful in the treatment of patients with secreting pheochromocytoma during hypertensive crises. In addition, NPY could serve as a specific and sensitive marker for the diagnosis of these diseases.

Trophic actions of NPY in the cardiovascular system

The trophic effects of sympathetic neurotransmitters on the cardiovascular system have been recognized for a long time. In particular, NE was shown to contribute to cardiovascular remodeling through its growth-promoting activity on smooth muscle and cardiac cells. Because NPY is associated with NE in sympathetic nerve endings, several studies investigated the trophic actions of NPY.

In vascular smooth muscle cells, NPY was shown to induce DNA synthesis in a concentration-dependent manner and to stimulate cell proliferation [191–194]. Using semiselective peptidic agonists, the Y1 receptor was identified as the subtype mediating the mitogenic effects of NPY. This effect was blocked by pertussis toxin, indicating the involvement of a Gi/o protein [191, 194], and an important role for calcium was also proposed [191]. Furthermore, other NPY receptor subtypes could be implicated depending on the concentrations of NPY used to elicit a mitogenic response. Indeed, at concentrations below that evoking a vasopressive response, NPY could induce Y2 and Y5 expression. In turn, these different receptors could mediate the stimulation of intracellular calcium transients and possibly MAPK activation [195]. Parallel experiments using HUVECs indicated that NPY could function as an angiogenic factor [196].

Along these lines, NPY was also reported to exert trophic actions on cardiomyocytes. In adult rat cardiomyocytes, NPY induces an increase in transcription and in protein content [197, 198]. More precisely, NPY appears to activate at least two different intracellular pathways in these cells [198]. First, it stimulates protein synthesis as well as cardiomyocyte hypertrophy via activation of a pertussis toxin-sensitive G-protein-coupled receptor and the phosphatidylinositol (PI) 3-kinase. Second, it induces a shift towards expression of fetal isoforms of cardiac-specific proteins through PKC-dependent pathways and activation of MAPK. Moreover, NPY appears to inhibit a β-adrenergic-mediated attenuation of the hypertrophic response induced by NE [199]. Finally, in neonatal mouse cardiomyocytes, NPY potentiates a phenylephrine-induced activation of PKC and the MAPK pathways. This effect is mediated through Y5 receptors on the surface of cardiac myocytes [118].

Renal effects of NPY

NPY and NE have been found colocalized in the renal vasculature, in the renal cortex and at the corticomedullary interface. In humans, NPY immunoreactivity appears mainly found in the renal tubules, whereas it seems absent in the glomerules [200]. In monkeys, NPY administration into the renal artery resulted in a dose-dependent increase in renal artery resistance, and consequently in a decrease in renal blood flow [201, 202]. In addition, NPY appears to enhance diuresis and natriuresis through direct tubular effects independent of its hemodynamic properties [203]. Direct stimulation of the sympathetic renal nerve causes NE and NPY overflows, which are accompanied by a significant renal vasoconstriction [204, 205].

Together with an activated renin-angiotensin system, chronic stimulation of the sympathetic nervous system contributes to the development of high blood pressure in

hypertensive individuals. Interactions between the adrenergic and the renin-angiotensin system have been demonstrated. For instance, activation of β-adrenergic receptors on juxtaglomerular cells stimulates renin release. Since NPY is closely associated with the sympathetic system, it has been postulated that some of the cardiovascular effects of NPY could be related to modulation of the renin-angiotensin system. Therefore, NPY could participate in maintaining hypertension via its synergistic effects on NE-induced vasoconstriction or by potentially potentiating adrenergic-mediated renin release. In patients demonstrating a stenotic kidney and subsequent activation of the renin-angiotensin system, the concentrations of NPY found in the renal veins were no different between the affected and the normal contralateral kidney [206]. On the other hand, increased sensitivity to NPY was demonstrated in a rat model of renovascular hypertension, namely the two-kidney one-clip (2K1C) hypertension [47]. In this study, the Y1 antagonist BIBP3226 was unable to lower blood pressure in hypertensive rats. However, another Y1 antagonist, BIBO3304TF, was shown to blunt development of high blood pressure in 2K1C rats, indicating that NPY could contribute to the hypertensive response in this model [207]. Nevertheless, the contributing effect of NPY is unlikely to be due to NPY-mediated stimulation of renin release, since NPY failed to potentiate basal or isoproterenol-stimulated renin secretion from rat kidney slices. On the contrary, infusion of exogenous NPY to hypertensive 2K1C rats appeared to suppress renin release during development of renovascular hypertension [208]. These apparent discrepancies could be due to differential effects of NPY during acute and chronic renal response.

Energy homeostasis

Energy homeostasis is the process by which adipose tissue, stored energy, is kept constant over time. Obviously, feeding behavior plays a crucial role in energy homeostasis, because to maintain a constant energy balance, energy (food) intake should match energy expenditure [209–211]. Feeding behavior is a tightly regulated phenomenon, involving several factors for signaling the body needs in energy to the CNS, particularly to the hypothalamus [212]. The hypothalamus integrates neuronal, metabolic and endocrine signals [213], and stimulates different effector pathways for activation of behavioral responses and neuroendocrine axes [214]. It is certainly overly simplified to reduce the regulation of food intake to a series of cross-talks between certain hypothalamic circuitries that would control body fat mass. However, a variety of studies indicate that body weight is indeed controlled by genetic factors. Along these lines, the identification of several genes that are responsible for a change in body weight in rodents suggests that obesity is not solely due to a lack of discipline [215]. In fact, a large number of molecules have been implicated in the control of food intake. In this complex network of pathways, one can distinguish those that provide orexigenic signals from those emitting anorexigenic signals.

Hypothalamic pathways for the control of feeding

NPY is the prototype of appetite-stimulating hormones, inducing particularly carbohydrate intake [216, 217]. In addition, NPY decreases energy expenditure and induces lipogenic enzymes in white adipose tissue [218]. The ability of NPY to induce food intake was reported very early after its first isolation. Intracerebroventricular injection of NPY elicits a strong feeding response [216, 219], even in satiated animals, eventually leading to obesity [220, 221]. Subsequently, a large body of evidence suggested that NPY was a naturally produced neurotransmitter that participates in the hypothalamic integration of energy homeostasis. For instance, NPY expression in the hypothalamus is largely increased in situations of poor metabolic conditions [222, 223]. High hypothalamic NPY levels are found also in obese Zucker rats [224, 225].

The discovery of hormones responsible for adiposity signals provides evidence for a feedback mechanism between peripheral fat stores and the neuronal centers controlling food intake. Insulin and even more so leptin, the product of the ob gene [226], are the only hormones so far identified that are produced in proportion to the amount of body fat, released in the plasma, circulate at levels proportional to fat content, enter the central nervous system and interact with specific receptors present in hypothalamic nuclei involved in the control of food intake [212]. Accordingly, reduced central activation of insulin and/or leptin pathways results in increased NPY expression and secretion in the hypothalamus [214, 223]. For instance, leptin-deficient ob/ob mice as well as rats with insulin-deficient diabetes are characterized by high NPY synthesis and hyperphagia [227–229]. Moreover, leptin administration inhibits hypothalamic NPY expression and secretion, suggesting that NPY is a target of leptin actions in the brain [230]. Similarly, insulin replacement in streptozotocin (STZ)-treated rats normalizes the increase in hypothalamic NPY expression and reduces hyperphagia that develops secondary to insulinopenia [231]. Furthermore, although mice carrying disrupted NPY alleles feed and grow normally, hyperphagia as well as obesity in leptin-deficient ob/ob mice are attenuated in animals lacking NPY expression [232]. NPY-deficient mice are also particularly sensitive to the anorexigenic effects of leptin, indicating that NPY tonically controls leptin actions [233]. Although the physiological relevance remains to be established, it is quite clear that insulin similarly modulates

NPY expression and release in the hypothalamus [234, 235], and could therefore participate in a regulatory feedback mechanism that would prevent hyperphagia in postprandial stage. It is also important to note that NPY itself has been shown to control insulin secretion [236, 237]. Acute intracerebroventricular injections of NPY cause a significant rise in plasma insulin levels. This effect is prevented by adrenalectomy, suggesting that glucocorticoids are necessary for NPY-mediated insulin release [238]. Hyperinsulinemia certainly contributes to obesity by stimulating glucose transport in adipose tissues. In addition, NPY is also produced by insulin secreting β cells, where it could act as a potent autocrine/paracrine inhibitor of insulin secretion [239]. Similarly, NPY appears to exert control on leptin secretion by adipocytes. Indeed, activation of NPY Y1 receptors on the surface of adipocytes inhibits lipolysis and stimulates leptin secretion [240, 241].

Several other pathways involving anabolic transmitters such as the agouti-related protein (AGRP) have been suggested to mediate leptin actions in the hypothalamus. Endogenous AGRP is a strong antagonist at the melanocortin MC3 and MC4 receptors [242]. The MC4 receptor is thought to mediate inhibition of the feeding response when stimulated by the pro-opiomelanocortin (POMC)-derived peptide, the α-melanocyte-stimulating hormone (α-MSH). Since AGRP is expressed by NPY-ergic neurons [243], it was postulated that AGRP could represent a pathway, parallel to NPY, compensating for NPY deficiency in NPY knockouts. However, hypothalamic AGRP expression is not elevated in mice lacking NPY. In addition, the expression of several anorexigenic molecules is stimulated by high leptin concentrations. Among pathways containing possible candidate catabolic effectors, the melanocortins, in particular α-MSH, are indeed stimulated by leptin [244]. Similarly, the cocaine- and amphetamine-regulated transcript (CART) promotes negative energy balance [245]. The emerging picture for the control of energy homeostasis thus involves a series of hypothalamic pathways with opposing effects [212] (fig. 2).

Leptin, and to a lesser degree insulin, activate catabolic pathways while inhibiting neurons containing anabolic effectors. In support of this view, leptin was recently shown to exert a dual effect in the hypothalamus. On the one hand, leptin induces depolarization and stimulates a subset of neurons that release anorexigenic substances, whereas it hyperpolarizes, and then inhibits, neurons containing orexigenic peptides [246]. Therefore, although NPY certainly plays a prominent role in these different regulatory loops, it is by no means the only regulatory substance controlling appetite [212]. In addition, leptin is not the sole modulator of hypothalamic NPY expression. For instance, fatty acid synthase (FAS) inhibitors were shown to dramatically reduce food intake and adiposity in mice, resulting in weight loss [247–249]. NPY mRNA levels in the hypothalamus of mice treated with FAS inhibitors were even lower than those measured in fed animals, despite the fact that circulating leptin concentrations were reduced. This suggests the existence of leptin-independent pathways for the regulation of hypothalamic NPY gene expression. Nevertheless, fasted mice receiving FAS inhibitors responded normally to NPY intracerebroventricular injection. These substances appear to target some neuronal networks lying upstream the NPY pathways.

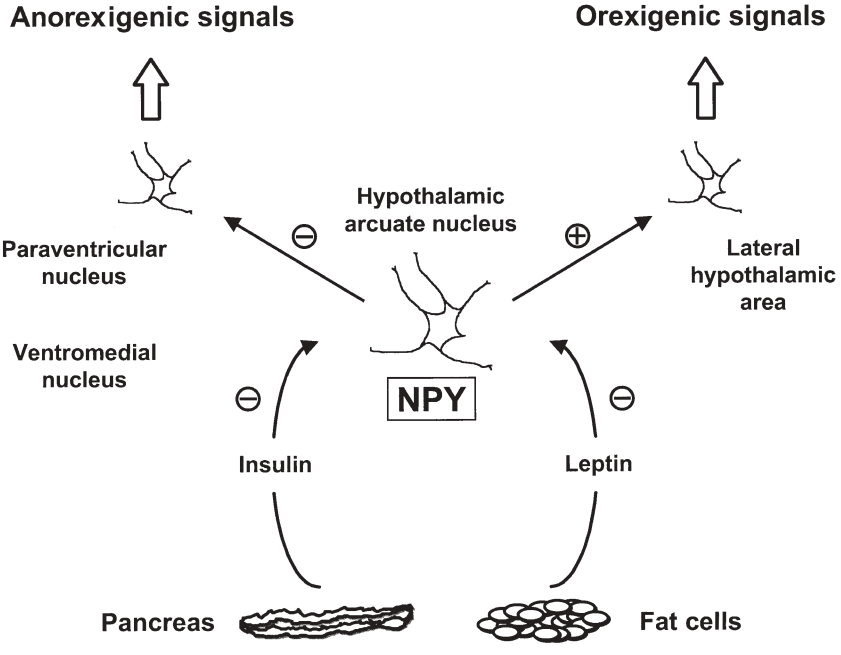

Figure 2. Schematic view of the feedback loops controlling food intake in the hypothalamus. See text for details.

Anatomically, the satiety and the hunger centers appear localized within nuclei of the basal hypothalamus. The arcuate nucleus (ARC) is the first integrator of peripheral signals because of the absence of the blood brain barrier around the median eminence. Neurons that express the two orexigenic peptides NPY and AGRP are present in the ARC [243]. POMC, the precursor molecule of α-MSH, as well as CART, are colocalized in a separate set of arcuate neurons. Both subsets of neurons appear sensitive to leptin actions [230, 250]. Similarly, insulin receptors are highly expressed in the ARC. Thus, high leptin levels inhibit NPY/AGRP neurons and activate POMC/CART neurons. Conversely, in poor metabolic conditions, low leptin and insulin levels result in inhibition of arcuate POMC and CART expression, and stimulate the NPY/AGRP anabolic pathway.

Neurons from the ARC project to two relevant hypothalamic sites, namely the paraventricular nucleus (PVN) [222, 251], and the LHA. The PVN is crucial in integrating orexigenic signals. Approximately 20% of NPY-producing neurons innervate the PVN. Destruction of the PVN produces hyperphagia, whereas its stimulation inhibits food intake. These experiments suggest that anorexigenic molecules may be produced in the PVN. Nevertheless, specific microinjections in the PVN of orexigenic signaling substances, in particular of NPY, stimulate feeding. And most important, this site is the only hypothalamic area in which the release of NPY in response to fasting was demonstrated [222]. Therefore, it is hypothesized that NPY/AGRP neurons originating from the ARC control food intake by inhibiting catabolic effector signaling in the PVN. Such molecules are indeed present in the PVN. For example, the corticotropin-releasing hormone (CRH), the prime regulator of the corticotrope axis, is synthesized in the PVN and decreases feeding when injected centrally [252]. Accordingly, POMC/CART neurons should stimulate PVN neurons containing catabolic effectors. This, however, has not been demonstrated.

The hypothesis that the LHA plays an opposite role in the control of food intake is first supported by observations of bilateral lesioning of the LHA. Destruction of these sites causes anorexia [253]. This indicates that orexigenic neurotransmitters could be synthesized in that area. Indeed, the melanin-concentrating hormone (MCH), as well as two recently discovered peptides with anabolic properties named orexin A and B, is present in the LHA or the adjacent region, the perifornical area (PFA) [254]. Thus, they are potentially involved in the induction of feeding behavior. NPY-containing neurons from the ARC are detected in the LHA/PFA regions. Therefore, the integrated model would predict that NPY/AGRP input in the LHA/PFA could stimu-late orexigenic effector signaling in these areas of the brain.

The model for first- and second-order neuronal control of feeding behavior also predicts that POMC/CART neurons projecting form the ARC stimulate anorexic peptide signaling in the PVN and, conversely, inhibit anabolic effector-containing neurons in the LHA/PFA areas. In addition, it should be noted that these different hypothalamic neuronal systems are highly interconnected. Along this line, neurons of the PVN and of the LHA/PFA project to the ARC, and could then exert feedback controls on ARC neurons. Whether NPY is expressed in specific subsets of PVN or LHA/PFA neurons remains to be established. However, it is likely that leptin plays a direct role in the activity of PVN and LHA/PFA, since leptin receptors have been found in these different areas [255]. Finally, extrahypothalamic sites in the brain contain NPY-expressing neurons, which could play a role in the control of feeding. In particular, a neuronal population in the brain stem coexpresses NPY, NE and E. NE and E in the hypothalamus come from these particular neuron subsets. Therefore, coordinate regulation of the activity of specific areas in the hypothalamus by NPY and catecholamines for the control of food intake has been suggested [256].

NPY receptors involved in the control of feeding

Because of the prominent role of NPY in the hypothalamic network controlling feeding behavior, the identification of the NPY receptors involved in the orexigenic actions of this peptide has been the center of great attention [68, 257, 258]. Indeed, these receptors could constitute potential targets of antiobesity treatment. As already mentioned, NPY activates at least five receptor subtypes [30, 31]. Among these different receptors, the Y1 and Y5 receptors appear to represent the most likely candidates for mediating the appetite stimulatory capacity of NPY [68]. As expected, both receptors are expressed in hypothalamic regions involved in the control of feeding [72].

Initial studies used the different order of potency of truncated or substituted peptides of the NPY family to address the role of each NPY receptor in the control of feeding [259]. Early experiments therefore suggested that NPY regulated food intake through the Y1 receptor since [Leu^{31}, Pro^{34}]NPY, a Y1 receptor agonist, stimulated feeding, whereas NPY_{13-36}, a Y2 receptor agonist, did not [260]. However, in the same series of experiments, NPY_{2-36}, a truncated peptide with reduced affinity for the Y1 receptor was shown to be a highly potent inducer of eating [260]. Moreover, the subsequent cloning of the Y5 receptor demonstrated that the affinity profile of peptidic agonists for this receptor subtype correlates with their capacity to stimulate feeding in rats. [Leu^{31}, Pro^{34}]NPY as well as NPY_{2-36} have high affinity for the Y5 receptor. Recently, the first selective agonist for the Y5 subtype was described. This peptidic compound binds to Y5 in the nanomolar range and is able to stimulate feeding when injected centrally [76].

A number of selective Y1 and Y5 receptor antagonists have also been used to assess the importance of these receptor subtypes in feeding behavior. First, BIBP3226 and BIBO3304, two Y1 receptor antagonists, were shown to block the feeding response to intracerebroventricular NPY injection [48, 261]. Similarly, 1229U91 is a high-affinity Y1 receptor antagonist that blocks daily as well as NPY-induced food intake [262]. However, this latter compound also has agonistic properties for the Y4 receptor. More recently, a Y1 antagonist (J-115814) that reduces spontaneous as well as NPY-stimulated feeding was described [52]. This drug was also active after intravenous and intraperitoneal injections. The fact that J-115814 was completely devoid of activity in NPY Y1-deficient mice, whereas it significantly reduced food intake in wild-types and in NPY Y5 knockouts, demonstrated the high selectivity of the compound and represented strong evidence for a role of the Y1 receptor in mediating feeding responses. On the other hand, the efficacy of the Y5 receptor antagonist CGP71683A in reducing food intake in fasted rats suggested that the Y5 subtype could play a role in eating responses [263]. It should be noted that CGP71683A does not affect daily food intake, indicating that the implication of the Y5 receptor could be restricted to stimulated conditions. However, a recent paper provided evidence that this compound also displays a certain degree of affinity for serotonin or muscarinic sites [78].

The NPY Y1 and Y5 receptors were investigated by genetic studies to identify a possible linkage between obesity and highly polymorphic markers in these genes. No linkage was found in a French Caucasian population, suggesting that these loci do not play a major role in human obesity [264]. Moreover, the attempt to detect genetic variants by single strand conformation polymorphism (SSCP) analysis was also negative. These results were confirmed in a study investigating extremely obese children. Indeed, an association could not be demonstrated between a nonconservative mutation affecting the first extracellular domain of the NPY Y5 receptor and the variability in body weight in this cohort [265]. In contrast, three single nucleotide polymorphisms in the promoter of the NPY Y5 receptor gene as well as in the 3′-untranslated region of the gene were in strong linkage desequilibrium among lean and obese Pima Indians [266]. This would indicate that the Y5 receptor subtype or a locus in the close vicinity, possibly the NPY Y1 receptor gene, could contribute to obesity in this selected population.

Since no clear picture emerged from pharmacological interventions, various attempts to downregulate or to invalidate NPY receptors in vivo were used to address the physiological importance of the different subtypes in feeding behavior. Intracerebroventricular injection of antisense oligonucleotides directed against the Y5 receptor attenuated fast-induced feeding in rats [267, 268]. Results from experiments using the same approach to block the Y1 subtype showed either a decrease or a paradoxical stimulation of food intake [269–271], and are not particularly informative. Knockout studies aimed at disrupting the Y1 and Y5 receptors raised many hopes to obtain definite answers on the respective importance of each subtype [147, 272, 273]. Surprisingly, both strains showed a higher body weight due to fat accumulation as compared with wild types. However, the reasons for the development of obesity are different in each strain. Mice lacking Y1 appear to have slightly decreased daily food intake, while fast-induced refeeding is severely affected. These animals also demonstrate decreased metabolic rate, secondary to decreased locomotor activity and movement-associated thermogenesis. Therefore, low-energy expenditure rather than high-energy intake appears responsible for the increase in body weight observed in Y1 knockouts. In contrast, Y5-deficient mice are hyperphagic. Accordingly, compensatory regulation of NPY receptors in the hypothalamus of NPY knockouts involves the upregulation of NPY Y1 receptor expression but not that of the NPY Y5 subtype. Finally, to further complicate our understanding of NPY-mediated control of food intake, mice lacking the Y2 receptor subtype also demonstrate hyperphagia, increased body weight and fat deposition, [274], and hypothalamic-specific deletion of the Y2 gene results in increased food consumption but decreased body weight [275]. Together, these data are indicative of a prominent role for the Y1 receptor. Nevertheless, both the Y1 and Y5 receptors could control redundant pathways within the hypothalamus. Obviously, this does not rule out the possible involvement of other subtypes, such as the Y2 receptor. In addition, several authors have postulated the existence of yet unidentified NPY receptors, which would be distinct from either the Y1 or Y5 subtype. Today, it is not absolutely clear how many NPY receptors constitute the family [30]. More definite answers on the identification of the NPY feeding receptors should be obtained using a combination of approaches, including generation of mice lacking more than one NPY receptor, production of tissue-specific knockouts as well as development of highly selective antagonists and their injection at precise hypothalamic sites.

Role of NPY in neuroendocrine regulation

The role of the hypothalamus in neuroendocrine regulation, and the discovery of NPY as a central modulator of hypothalamic functions, raised the question of its possible implication in controlling the activity of different neuroendocrine axes. Indeed, it has long been known that the activity of the neuroendocrine system is closely associated with the energy status of the body, and dysregulations in energy homeostasis affect neuroendocrine functions (fig. 3).

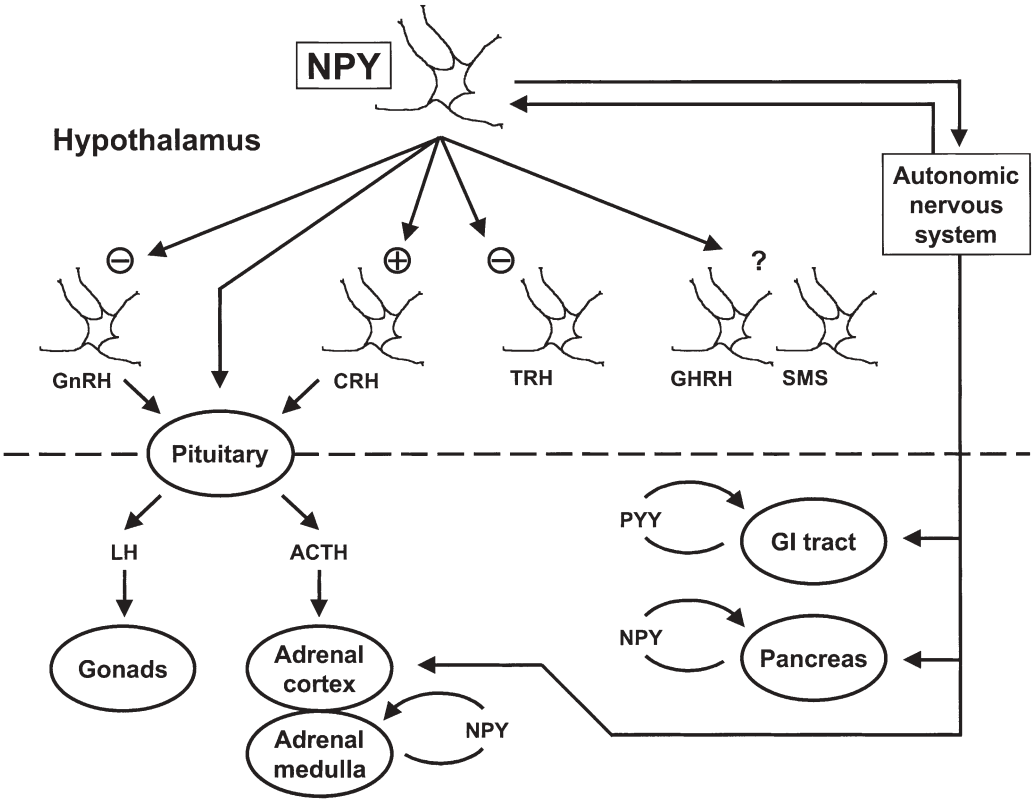

Figure 3. Schematic view of the NPY-mediated regulatory pathways controlling the activity of neuroendocrine axes. See text for details.

NPY and the corticotrope axis

The hypothalamo-pituitary-adrenal (HPA) axis is chronically activated in obese individuals, occasionally to the point of inducing hypercorticism. Similarly, rodent models with genetically determined obesity, such as ob/ob and db/db mice [276] as well as fa/fa Zucker rats [277, 278] demonstrate increased corticotrope axis activity. Obesity in these different models develops secondary to a defect in the leptin pathway. Although leptin deficiency does not seem to be the prime cause of human obesity, it could involve leptin resistance [279]. It is also relevant that leptin inhibits activation of the corticotrope axis in response to stress [280]. As mentioned earlier, low leptin levels or a defective leptin pathway result in stimulated hypothalamic NPY expression [227]. Therefore, obese individuals are expected to show enhanced NPY expression in the hypothalamus. In addition, high hypothalamic NPY concentrations also occur in normal animals, under conditions of low-energy intake such as fasting [222]. Starvation is a strong activator of the stress axis [281], a situation in which secretion of corticotropin-releasing hormone (CRH) is thought to prevent hypoglycemia. In the hypothalamus, the main site of CRH synthesis and the source of CRH-containing neurons controlling ACTH release is the PVN, an area in which NPY-ergic nerve terminals are abundant [282]. Accordingly, it was reported initially that infusion of NPY in the lateral ventricle of rats increased CRH concentrations in the hypothalamus and stimulated the release of ACTH and corticosterone [282–284]. Subsequently, this finding was confirmed by demonstration of dose-dependent activation of the HPA axis by NPY following its injection into the third ventricle, the lateral ventricle [283], the PVN [282] or even after intravenous administration. NPY thus appears to stimulate directly CRH synthesis and release by neurons of the PVN.

In an attempt to identify the receptor involved, it was recently suggested using a series of NPY analogs that the pharmacological profile of such receptor was not consistent with the Y1, y3 or y6 subtypes [285]. Moreover, the NPY receptor that controls the HPA axis does not seem to regulate food intake. Therefore, the receptor subtype responsible for stimulation of the corticotrope axis remains to be discovered. Finally, it is important to note that despite all the evidence listed above, the significance of these findings should be questioned in the face of data obtained in NPY-deficient mice which do not show reduced HPA axis activity under either fed or fasted conditions [286].

NPY could regulate the activity of the corticotrope axis at different locations outside the hypothalamus. Although NPY-containing neurons do not seem to project

to the pituitary, NPY can be found in the gland after its release in the hypothalamo-hypophysial portal vessels. In contrast, NPY-ergic neurons are found in the adrenals [287]. Nevertheless, studies assessing NPY actions on the adrenals provided conflicting results, and the exact physiological role of NPY at these sites has not been established. On the one hand, NPY appears to inhibit ACTH-stimulated corticosterone release from adrenal cells. On the other hand, NPY was reported to increase corticosterone secretion. Therefore, in the adrenals, modulation by NPY of HPA axis activity appears rather marginal. However, it is noteworthy that NPY seems to have more striking effects on aldosterone secretion [288]. Indeed, NPY was found to increase aldosterone concentrations, particularly under conditions of low ACTH levels [289]. Similarly, in the absence of a functional renin-angiotensin system, NPY could stimulate the growth of the zona glomerulosa.

Glucocorticoids, through their type II receptor, affect NPY expression and concentrations in the PVN as well as the ARC. For instance, dexamethasone increases NPY levels, whereas the type II selective antagonist RU486 blocks this effect [290]. RU486 was shown to prevent the orexigenic actions of NPY [291]. In adrenalectomized rats, central NPY levels are either decreased or unchanged [292, 293]. However, infusion of glucocorticoids in these animals increases hypothalamic NPY expression and synthesis [294], and upregulates NPY Y1 receptor expression [295]. Whether or not corticosterone is essential for the activation of NPY production in the hypothalamus in response to fasting is controversial. But it is noteworthy that the anorexic effects of leptin are greatly enhanced in adrenalectomized rats and that glucocorticoids abolish leptin actions in this model [296]. It is also relevant that adrenalectomy reduces the number of NPY Y1 and Y5 receptors in the VMH and simultaneously blunts acute NPY-induced insulin release [297], suggesting that glucocorticoids could control NPY-mediated insulin secretion via modulation of NPY Y1 and Y5 receptor expression. Therefore, a glucocorticoid-mediated increase in hypothalamic NPY levels as well as a simultaneous decrease in CRH concentrations could by themselves represent significant components of the dysregulations leading to the development of obesity.

Finally, the inhibitory effects of CRH on NPY expression provide additional evidence for regulatory cross-talks between the HPA axis and hypothalamic NPY. Along this line, it is relevant that CRH was shown to decrease food intake [252, 298]. Therefore, the anorexigenic actions of CRH could partially result from a blockade of the feeding-stimulatory activity of NPY. In contrast, corticosterone, via a feedback regulatory loop, prevents hypothalamic CRH secretion and could in turn stimulate NPY synthesis by abolishing the inhibitory actions of CRH on NPY-containing neurons [299].

NPY and the gonadotrope axis

Beside stimulation of the corticotrope axis, starvation also affects the activity of the gonadotrope axis [281]. Therefore, reproductive functions can be severely impaired in these circumstances, leading to gradual hypogonadism of hypothalamic origin. Indeed, fasting or food restriction induces suppression of the pituitary-gonadal functions, which can be restored by gonadotropin-releasing hormone (GnRH) substitution [300]. As already discussed, hypothalamic NPY expression and release are greatly enhanced in adverse metabolic conditions. In addition, it has been demonstrated that chronic NPY infusion in the third cerebral ventricle prevents sexual maturation in female rats [301], and produces hypogonadism in adult male and female animals [302, 303]. Therefore, NPY could represent a crucial modulator of the gonadotrope axis and in particular of GnRH secretion in the hypothalamus [304]. Indeed, central NPY injection results in remarkable decreases in the number of luteinizing hormone (LH) pulses [305] and in plasma testosterone levels [306]. The inhibitory effects of fasting on the gonadotrope axis as well as the increase in hypothalamic NPY levels were blunted by leptin administration [281]. It is therefore likely that the beneficial effect of leptin on GnRH secretion was at least in part associated with its capacity to prevent NPY synthesis and release from neurons of the ARC [307, 308]. Leptin has also been reported to advance the onset of puberty in intact rodents [309, 310]. Given the inhibitory role of NPY on sexual maturation, leptin-mediated suppression of NPY expression in the ARC is probably important in controlling pubertal development, an effect that could be mediated by the Y1 receptor [311, 312]. The emerging picture favors a role of leptin in centrally signaling the adequacy of energy stores for reproduction, and therefore in setting the progression towards puberty [313–315].

In addition to its inhibitory action on the reproductive function of intact rats, NPY has also a marked inhibitory effect on LH release in castrated animals [316–318]. The NPY-induced inhibition of the gonadotrope axis was initially thought to be mediated by the NPY Y1 receptor, based on pharmacological studies using the NPY Y1 agonist [Leu^{31}, Pro^{34}]NPY [319]. However, this NPY analog was shown later to bind with a significant affinity to the newly identified NPY Y5 and Y4 receptors. Therefore, using a panel of more defined semiselective agonists, it was subsequently suggested that the most likely candidate for mediating NPY actions on the gonadotrope axis was the NPY Y5 receptor [320]. Although involvement of the NPY Y1 receptor could not be completely excluded, these conclusions were based on observations showing that PYY_{3-36} (with Y2 and Y5 selectivity), hPP (with Y4 and Y5 selectivity) and [D-Trp^{32}]NPY (a selective Y5 agonist) were as potent as NPY to inhibit LH surge.

However, the effects of NPY on the reproductive function are very complex [321–323]. In fact, early studies identified a dual action of NPY on LH release, since an acute NPY injection seems to briefly stimulate LH secretion in both intact males and ovariectomized sex steroid-primed females. Therefore, under certain conditions, NPY also exhibits stimulatory activity on basal and cyclic secretion of GnRH. Consistently, antisense probes directed against NPY mRNA can block the triggering actions of NPY on GnRH release. Likewise, passive immunization against NPY also reveals a stimulatory role of endogenous NPY synthesis in the regulation of GnRH secretion during the pre-ovulatory LH surge [324]. Because NPY and [Leu^{31}, Pro^{34}]NPY similarly stimulate LH release in ovarian steroid-primed ovariectomized rats, it was assumed that NPY Y1 receptors in the hypothalamus could mediate these actions of NPY. The involvement of the Y1 receptor was also confirmed using the specific Y1 antagonist BIBP3224 [325]. However, [Leu^{31}, Pro^{34}]NPY was reported to have mixed actions on several NPY receptors, including the Y4 subtype. Unexpectedly, experiments using the NPY Y1 antagonist 1229U91 provide the first evidence for the involvement of another type of receptor. Indeed, this compound also displays substantial agonistic activity at the Y4 receptor. Therefore, 1229U91 efficiently blocks LH surge in ovariectomized rats, possibly via the Y1 receptor, and induces LH release in unstimulated conditions through Y4 receptors [326, 327]. These findings represent compelling evidence for a role of the Y4 receptor in stimulation of LH secretion. Y4 deficiency was recently shown to improve fertility in ob/ob mice, suggesting that the Y4 receptor controls at least in part inhibition of the gonadotrope axis under conditions of high hypothalamic NPY expression [328]. Finally, since the [Leu^{31}, Pro^{34}]NPY analog as well as [D-Trp^{32}]NPY both elicit stimulation of LH secretion, participation of the NPY Y5 receptor cannot formally be ruled out. Indeed, studies performed in vitro using rat hypothalamic explants suggest that the NPY Y5 receptor is responsible for stimulation of the GnRH pulse generator in prepubertal animals [329]. To conclude, NPY is almost certainly involved in modulating the activity of the gonadotrope axis. However, data obtained in the NPY-knockout mouse also definitely demonstrate that animals develop normal reproductive functions in the absence of NPY, and therefore that this neuropeptide is not mandatory for the normal activity of the reproductive axis [233, 286].

NPY and the thyrotrope and somatotrope axes

Finally, central NPY injection can also suppress the thyrotrope [330] and somatotrope axes [305]. Similar to what was observed for the corticotropic and gonadotropic axes, this NPY effect mimics normal neuroendocrine responses to starvation. Indeed, decreased thyroid hormone is expected to limit energy expenditure in periods of low-energy intake. Therefore, thyrotropin-releasing hormone (TRH) mRNA levels in the paraventricular nucleus are reduced in fasted animals, resulting in a fall in plasma thyroxine levels. Administration of leptin appears to prevent fasting-induced suppression of TRH expression, and to partially restore thyroxine concentrations during starvation [281, 331]. Leptin could directly regulate TRH-containing cells in the PVN, since this population expresses the leptin receptor [332]. However, the effects of leptin on the thyrotrope axis might also result from indirect regulation of TRH neurons by arcuate NPY and POMC-expressing cells. Indeed, NPY-ergic neurons innervate TRH neurons in the PVN and could therefore exert an inhibitory effect on the thyroid axis. The reduction of TRH expression observed in the PVN of NPY-infused rats, and the concomitant decrease in circulating T_3 and T_4 levels are consistent with this observation [330]. In the same way, NPY blunts the release of pro-TRH by primary hypothalamic neurons in vitro. However, fasted NPY knockouts display a similar fall in thyroxine as that seen in wild type, suggesting that NPY is not mandatory to modulate the thyrotrope axis.

Pulsatile growth hormone (GH) secretion is controlled by neurons producing GH-releasing hormone (GHRH) and those secreting somatostatin. In addition, GH could regulate its own secretion via a feedback control of GH and somatostatin-containing neurons. NPY neurons in the hypothalamus appear to express GH receptors [333]. Using intraventricular injections of rather large doses of NPY, an inhibitory effect was demonstrated on GH secretion in ovariectomized rats. This effect seems to result from direct regulation of hypothalamic GHRH-containing neurons. Indeed, NPY-containing neurons are present in hypothalamic areas that control GH release. However, inhibition could equally be the result of stimulation of somatostatin production. In any case, single injection of NPY produces a long-lasting reduction of plasma GH [334]. It is therefore relevant that leptin-deficient ob/ob mice with increased hypothalamic NPY expression demonstrate significant reduction in hepatic IGF-1 expression, which can be partially corrected by NPY inactivation. Leptin seems to modulate the activity of the somatotrope axis, since leptin treatment prevents suppression of GH secretion during fasting [335]. In this instance as well, leptin could control both GHRH and somatostatin synthesis and release. It is also noteworthy that the leptin receptor is expressed in the pituitary, indicating that control at this level might regulate GH secretion. Because of the effects of leptin on hypothalamic NPY expression, it is likely that part of its action results from modulation of the NPY pathways in the hypothalamus. Finally, contrasting with its inhibitory effect, NPY was also suggested to exert a stimulatory action on GH secretion via direct activation of pituitary cells.

Therapeutic potentials

NPY is widely distributed in the central and peripheral nervous systems, and elicits a number of important physiological responses. NPY is thus likely to play a major role in several organ systems and represents a desirable target for therapeutic purposes. For example, its emerging role as a prominent integrator of energy homeostasis suggests that modulation of NPY-mediated pathways could be relevant for the management of obesity. Along these lines, it is tempting to speculate that an orally active Y1 antagonist could have strong beneficial effects in metabolic syndromes by inhibiting food intake, increasing thermogenesis, activating lipolysis and at the same time reducing blood pressure while lowering the threshold of activation of the gonadotrope axis. On the other hand, this ideal picture is challenged by the numerous effects which have been assigned to NPY and could complicate the design of highly specific drugs. For instance, blockade of feeding pathways for the treatment of obesity is likely to significantly affect some other important regulatory functions.

In summary, numerous observations suggest that selective antagonists or agonists of NPY receptors could represent useful compounds for the treatment of feeding and metabolic disorders, obesity, diabetes, hypertension as well as heart failure. However, because of the large tissue distribution of NPY receptors and since each particular NPY receptor appears to control several different pathways, one should use great caution in designing drugs that might affect distinct physiological activities.

Future directions

NPY has also been involved in anxiety, memory retention, seizure and drug addiction. Several reviews have addressed a possible regulatory role for NPY in the central nervous system [336–343], and we will not discuss these points in great detail. The anxiolytic properties of NPY appear to be mediated by Y1 receptors [344]. Interestingly, cerebrospinal fluid from suicidal patients contains either diminished or unchanged NPY concentrations [345–347], whereas NPY levels are increased in the brain after antidepressant therapy [348]. NPY was also reported to exert antidepressant-like activity via Y1 receptors in the mouse swimming test [349]. Moreover, NPY receptors, in particular the Y1, Y2 and Y5 subtypes, are present in the hippocampus [350–352], an area of the brain that has been implicated in the modulation of cognition. A NPY-mediated anticonvulsant action has been proposed because hippocampal NPY expression was shown to be upregulated in rodent models of epilepsy [338, 339]. The beneficial effect of NPY could result from its capacity to inhibit glutamate release. The Y2 and Y5 receptor subtypes are the most likely candidates for mediating the antiepileptic properties of NPY [353, 354]. Finally, voluntary alcohol consumption and resistance to the intoxicating effect of ethanol appear to be in part regulated by NPY [355, 356] via a Y1-mediated mechanism [357]. NPY administration modifies behavior towards ethanol consumption in rats [358, 359], and more important, evidence suggests that NPY could play a role in modulating ethanol consumption and seizure during alcohol withdrawal [360–362]. In the future, it will be important to determine whether common NPY pathways modulate anxiety, alcohol consumption and seizure.

1 Groop L. (2000) Genetics of the metabolic syndrome. Br. J. Nutr. **83** Suppl. **1:** S39–S48
2 Good D. J. (2000) How tight are your genes? Transcriptional and posttranscriptional regulation of the leptin receptor, NPY and POMC genes. Horm.Behav. **37:** 284–298
3 Bjorntorp P. and Rosmond R. (2000) Neuroendocrine abnormalities in visceral obesity. Int. J. Obes. Relat. Metab. Disord. **24** Suppl. **2:** S80–S85
4 Tatemoto K., Carlquist M. and Mutt V. (1982) Neuropeptide Y – a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature **296:** 659–660
5 Ekblad E., Edvinsson L., Wahlestedt C., Uddman R., Hakanson R., and Sundler F. (1984) Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. Regul. Pept. **8:** 225–235
6 Tatemoto K. (1982) Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine secretion. Proc. Natl. Acad. Sci. USA **79:** 2514–2518
7 Larhammar D. (1996) Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. Regul. Pept. **62:** 1–11
8 Schwartz T. W., Fuhlendorff J., Kjems L. L., Kristensen M. S., Vervelde M., O'Hare M. et al. (1990) Signal epitopes in the three-dimensional structure of neuropeptide Y. Interaction with Y1, Y2, and pancreatic polypeptide receptors. Ann. N.Y. Acad. Sci. **611:** 35–47
9 Minth C. D., Bloom S. R., Polak J. M. and Dixon J. E. (1984) Cloning, characterization and DNA sequence of a human cDNA encoding neuropeptide tyrosine. Proc. Natl. Acad. Sci. USA **81:** 4577–4581
10 Higuchi H., Yang H. Y. and Sabol S. L. (1988) 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
11 Brakch N., Rist B., Beck-Sickinger A. G., Goenaga J., Wittek R., Burger E. et al. (1997). Role of prohormone convertases in pro-neuropeptide Y processing: coexpression and in vitro kinetic investigations. Biochem. **36**: 16309–16320
12 Hopsu-Havu V. K. and Glenner G. G. (1966) A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-beta- naphthylamide. Histochemie **7:** 197–201
13 Grandt D., Dahms P., Schimiczek M., Eysselein V. E., Reeve J. R. Jr and Mentlein R. (1993) [Proteolytic processing by dipeptidyl aminopeptidase IV generates receptor selectivity for peptide YY (PYY)]. Med. Klin. **88:** 143–145
14 Stange T., Kettmann U. and Holzhausen H. J. (1996) Immunoelectron microscopic single and double labelling of aminopeptidase N (CD 13) and dipeptidyl peptidase IV (CD 26). Acta Histochem. **98:** 323–331
15 Shibuya-Saruta H., Kasahara Y. and Hashimoto Y. (1996) Human serum dipeptidyl peptidase IV (DPPIV) and its unique properties. J. Clin. Lab Anal. **10:** 435–440

16 Yaron A. and Mlynar D. (1968) Aminopeptidase-P. Biochem. Biophys. Res. Commun. **32:** 658–663
17 Medeiros M. D. and Turner A. J. (1994) Processing and metabolism of peptide-YY: pivotal roles of dipeptidylpeptidase-IV, aminopeptidase-P and endopeptidase-24.11. Endocrinology **134:** 2088–2094
18 Ryan J. W., Denslow N. D., Greenwald J. A. and Rogoff M. A. (1994) Immunoaffinity purifications of aminopeptidase P from guinea pig lungs, kidney and serum. Biochem. Biophys. Res. Commun. **205:** 1796–1802
19 Mentlein R., von Kolszynski M., Sprang R. and Lucius R. (1990) Proline-specific proteases in cultivated neuronal and glial cells. Brain Res. **527:** 159–162
20 Medeiros M. S. and Turner A. J. (1996) Metabolism and functions of neuropeptide Y. Neurochem. Res. **21:** 1125–1132
21 Allen J. M., Yeats J. C., Adrian T. E. and Bloom S. R. (1984) Radioimmunoassay of neuropeptide Y. Regul. Pept. **8:** 61–70
22 Corder R. and Lowry P. J. (1985). An immunoradiometric assay for the measurement of neuropeptide Y in plasma. Peptides **6:** 1195–1200
23 Lundberg J. M., Martinsson A., Hemsen A., Theodorsson-Norheim E., Svedenhag J., Ekblom B. et al. (1985) Co-release of neuropeptide Y and catecholamines during physical exercise in man. Biochem. Biophys. Res. Commun. **133:** 30–36
24 Theodorsson-Norheim E., Hemsen A. and Lundberg J. M. (1985) Radioimmunoassay for neuropeptide Y (NPY): chromatographic characterization of immunoreactivity in plasma and tissue extracts. Scand. J. Clin. Lab. Invest. **45:** 355–365
25 Grouzmann E., Comoy E. and Bohuon C. (1989) Plasma neuropeptide Y concentrations in patients with neuroendocrine tumors. J. Clin. Endocrinol. Metab. **68:** 808–813
26 Grouzmann E., Aubert J. F., Waeber B. and Brunner H. R. (1992) A sensitive and specific two-site, sandwich-amplified enzyme immunoassay for neuropeptide Y. Peptides **13:** 1049–1054
27 Wahlestedt C., Grundemar L., Hakanson R., Heilig M., Shen G. H., Zukowska-Grojec Z. et al. (1990) Neuropeptide Y receptor subtypes, Y1 and Y2. Ann. N.Y. Acad. Sci. **611:** 7–26
28 Wahlestedt C., Yanaihara N. and Hakanson R. (1986) Evience for different pre- and post-junctional receptors for neuropeptide Y and related peptides. Regul. Pept. **13:** 307–318
29 Fuhlendorff J., Gether U., Aakerlund L., Langeland-Johansen N., Thogersen H., Melberg S. G. et al. (1990) [Leu31,Pro34] Neuropeptide Y: a specific Y1 receptor agonist. Proc. Natl. Acad. Sci. USA **87:** 182–186
30 Blomqvist A. and Herzog H. (1997) Y-receptor subtypes – how many more? Trends Neurosci. **20:** 294–298
31 Gehlert D. R. (1998) Multiple receptors for the pancreatic polypeptide (PP-fold) family: physiological implications. Proc. Soc. Exp. Biol. Med. **218:** 7–22
32 Larhammar D., Wraith A., Berglund M. M., Holmberg S. K. and Lundell I. (2001) Origins of the many NPY-family receptors in mammals. Peptides **22:** 295–307
33 Wraith A., Tornsten A., Chardon P., Harbitz I., Chowdhary B. P., Andersson L. et al. (2000) Evolution of the neuropeptide Y receptor family: gene and chromosome duplications deduced from the cloning and mapping of the five receptor subtype genes in pig. Genome Res. **10:** 302–310
34 Eva C., Keinänen K., Monyer H., Seeburg P. and Sprengel R. (1992) Molecular cloning of a novel G protein-coupled receptor that may belong to the neuropeptide receptor family. FEBS Lett. **271:** 81–84
35 Eva C., Oberto A., Sprengel R. and Genazzani E. (1992) The murine NPY-1 receptor gene. Structure and delineation of tissue-specific expression. FEBS Lett. **314:** 285–288
36 Ball H. J., Shine J. and Herzog H. (1995) Multiple promoters regulate tissue-specific expression of the human NPY-Y1 receptor gene. J. Biol. Chem. **270:** 27272–27276
37 Aakerlund L., Gether U., Fuhlendorff J., Schwartz T. W. and Thastrup O. (1990) Y1 receptors for neuropeptide Y are coupled to mobilization of intracellular calcium and inhibition of adenylate cyclase. FEBS Lett. **260:** 73–78
38 Motulsky H. J. and Michel M. C. (1988) Neuropeptide Y mobilizes Ca2+ and inhibits adenylate cyclase in human erythroleukemia cells. Am. J. Physiol. **255:** E880–E885
39 Krause J., Eva C., Seeburg P. and Sprengel R. (1992) Neuropeptide Y1 subtype pharmacology of a recombinantly expressed neuropeptide receptor. Mol. Pharmacol. **41:** 817–821
40 Mullins D., Kirby D., Hwa J., Guzzi M., Rivier J. and Parker E. (2001) Identification of potent and selective neuropeptide Y Y(1) receptor agonists with orexigenic activity in vivo. Mol. Pharmacol. **60:** 534–540
41 Soll R. M., Dinger M. C., Lundell I., Larhammer D. and Beck-Sickinger A. G. (2001) Novel analogues of neuropeptide Y with a preference for the Y1-receptor. Eur. J. Biochem. **268:** 2828–2837
42 Lundberg J. M. and Tatemoto K. (1982) Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to alpha-adrenoceptor blockade. Acta Physiol. Scand. **116:** 393–402
43 Rudolf K., Eberlein W., Engel W., Wieland H. A., Willim K. D., Entzeroth M., Wienen W. et al. (1994) The first highly potent and selective non-peptide neuropeptide Y Y1 receptor antagonist: BIBP3226. Eur. J. Pharmacol. **271:** R11–R13
44 Cervin A., Lindberg S., Mercke U. and Uddman R. (1992) Neuropeptide Y in the rabbit maxillary sinus modulates cholinergic acceleration of mucociliary activity. Acta Otolaryngol. **112:** 872–881
45 Lundberg J. M. and Modin A. (1995) Inhibition of sympathetic vasoconstriction in pigs in vivo by the neuropeptide Y-Y1 receptor antagonist BIBP 3226. Br. J. Pharmacol. **116:** 2971–2982
46 Zukowska-Grojec Z., Dayao E. K., Karwatowska-Prokopczuk E., Hauser G. J. and Doods H. N. (1996) Stress-induced mesenteric vasoconstriction in rats is mediated by neuropeptide Y Y1 receptors. Am. J. Physiol. **270:** H796–H800
47 Mezzano V., Donoso V., Capurro D. and Huidobro-Toro J. P. (1998) Increased neuropeptide Y pressor activity in Goldblatt hypertensive rats: in vivo studies with BIBP 3226. Peptides **19:** 1227–1232
48 Wieland H. A., Engel W., Eberlein W., Rudolf K. and Doods H. N. (1998) Subtype selectivity of the novel nonpeptide neuropeptide Y Y1 receptor antagonist BIBO 3304 and its effect on feeding in rodents. Br. J. Pharmacol. **125:** 549–555
49 Leban J. J., Heyer D., Landavazo A., Matthews J., Aulabaugh A. and Daniels A. J. (1995) Novel modified carboxy terminal fragments of neuropeptide Y with high affinity for Y2-type receptors and potent functional antagonism at a Y1- type receptor. J. Med. Chem. **38:** 1150–1157
50 Gehlert D. R., Schober D. A., Beavers L., Gadski R., Hoffman J. A., Smiley D. L. et al. (1996) Characterization of the peptide binding requirements for the cloned human pancreatic polypeptide-preferring receptor. Mol. Pharmacol. **50:** 112–118
51 Serradeil-Le Gal C., Valette G., Rouby P. E., Pellet A., Oury-Donat F., Brossard G. et al. (1995) SR 120819A, an orally-active and selective neuropeptide Y Y1 receptor antagonist. FEBS Lett. **362**: 192–196
52 Kanatani A., Hata M., Mashiko S., Ishihara A., Okamoto O., Haga Y. et al. (2001) A typical Y1 receptor regulates feeding behaviors: effects of a potent and selective Y1 antagonist, J-115814. Mol. Pharmacol. **59:** 501–505
53 Gerald C., Walker M. W., Vaysse P. J., He C., Branchek T. A. and Weinshank R. L. (1995) Expression cloning and pharmacological characterization of a human hippocampal neuropeptide Y/peptide YY Y2 receptor subtype. J. Biol. Chem. **270:** 26758–26761

54 Lutz C. M., Frankel W. N., Richards J. E. and Thompson D. A. (1997) Neuropeptide Y receptor genes on human chromosome 4q31-q32 map to conserved linkage groups on mouse chromosomes 3 and 8. Genomics **41:** 498–500

55 Gehlert D. R., Beavers L. S., Johnson D., Gackenheimer S. L., Schober D. A. and Gadski R. A. (1996) Expression cloning of a human brain neuropeptide Y Y2 receptor. Mol. Pharmacol. **49:** 224–228

56 Zhang X., Shi T., Holmberg K., Landry M., Huang W., Xiao H. et al. (1997) Expression and regulation of the neuropeptide Y Y2 receptor in sensory and autonomic ganglia. Proc. Natl. Acad. Sci. USA **94:** 729–734

57 Wieland H. A., Willim K. and Doods H. N. (1995) Receptor binding profiles of NPY analogues and fragments in different tissues and cell lines. Peptides **16:** 1389–1394

58 Beck-Sickinger A. G., Grouzmann E., Hoffmann E., Gaida W., van Mei E. G., Waeber B. et al. (1992) A novel cyclic analog of neuropeptide Y specific for the Y2 receptor. Eur. J. Biochem. **206:** 957–964

59 Goumain M., Voisin T., Lorinet A. M., Ducroc R., Tsocas A., Roze C. et al. (2001) The peptide YY-preferring receptor mediating inhibition of small intestinal secretion is a peripheral Y(2) receptor: pharmacological evidence and molecular cloning. Mol. Pharmacol. **60:** 124–134

60 Lacroix J. S., Ulman L. G. and Potter E. K. (1994) Modulation by neuropeptide Y of parasympathetic nerve-evoked nasal vasodilatation via Y2 prejunctional receptor. Br. J. Pharmacol. **113:** 479–484

61 Mahns D. A., Lacroix J. S. and Potter E. K. (1998) Inhibition of vagal vasodilatation by a selective neuropeptide Y Y2 receptor agonist in the bronchial circulation of anaesthetised dogs. J.Auton. Nerv. Syst. **73:** 80–85

62 Grouzmann E., Buclin T., Martire M., Cannizzaro C., Dorner B., Razaname A. et al. (1997) Characterization of a selective antagonist of neuropeptide Y at the Y2 receptor. Synthesis and pharmacological evaluation of a Y2 antagonist. J. Biol. Chem. **272:** 7699–7706

63 Dumont Y., Cadieux A., Doods H., Pheng L. H., Abounader R., Hamel E. et al. (2000) BIIE0246, a potent and highly selective non-peptide neuropeptide Y Y(2) receptor antagonist. Br. J. Pharmacol. **129:** 1075–1088

64 Voisin T., Goumain M., Lorinet A. M., Maoret J. J. and Laburthe M. (2000) Functional and molecular properties of the human recombinant Y4 receptor: resistance to agonist-promoted desensitization. J. Pharmacol. Exp. Ther. **292:** 638–646

65 Bard J. A., Walker M. W., Branchek T. A. and Weinshank R. L. (1995) Cloning and functional expression of a human Y4 subtype receptor for pancreatic polypeptide, neuropeptide Y, and peptide YY. J. Biol. Chem. **270:** 26762–26765

66 Lundell I., Statnick M. A., Johnson D., Schober D. A., Starback P., Gehlert D. R. et al. (1996) The cloned rat pancreatic polypeptide receptor exhibits profound differences to the orthologous receptor. Proc. Natl. Acad. Sci. USA **93:** 5111– 5115

67 Gerald C., Walker M. W., Criscione L., Gustafson E. L., Batzl-Hartmann C., Smith K. E. et al. (1996) A receptor subtype involved in neuropeptide-Y-induced food intake. Nature **382:** 168–171

68 Inui A. (1999). Neuropeptide Y feeding receptors: are multiple subtypes involved? Trends Pharmacol. Sci. **20:** 43–46

69 Bischoff A. and Michel M. C. (1999) Emerging functions for neuropeptide Y5 receptors. Trends Pharmacol. Sci. **20:** 104–106

70 Naveilhan P., Neveu I., Arenas E. and Ernfors P. (1998) Complementary and overlapping expression of Y1, Y2 and Y5 receptors in the developing and adult mouse nervous system. Neurosci. **87:** 289–302

71 Dumont Y., Fournier A. and Quirion R. (1998) Expression and characterization of the neuropeptide Y Y5 receptor subtype in the rat brain. J. Neurosci. **18:** 5565–5574

72 Dumont Y., Jacques D., Bouchard P. and Quirion R. (1998) Species differences in the expression and distribution of the neuropeptide Y Y1, Y2, Y4 and Y5 receptors in rodents, guinea pig and primate brains. J. Comp. Neurol. **402:** 372–384

73 Balasubramaniam A., Sheriff S., Johnson M. E., Prabhakaran M., Huang Y., Fischer J. E. et al. (1994) [D-TRP32]neuropeptide Y: a competitive antagonist of NPY in rat hypothalamus. J. Med. Chem. **37:** 811–815

74 Hwa J. J., Witten M. B., Williams P., Ghibaudi L., Gao J., Salisbury B. G. et al. (1999) Activation of the NPY Y5 receptor regulates both feeding and energy expenditure. Am. J. Physiol. **277:** R1428–R1434

75 Haynes A. C., Arch J. R., Wilson S., McClue S. and Buckingham R. E. (1998) Characterisation of the neuropeptide Y receptor that mediates feeding in the rat: a role for the Y5 receptor? Regul. Pept. **75–76:** 355–361

76 Cabrele C., Langer M., Bader R., Wieland H. A., Doods H. N., Zerbe O. et al. (2000) The first selective agonist for the neuropeptide YY5 receptor increases food intake in rats. J. Biol. Chem. **275:** 36043–36048

77 Criscione L., Rigollier P., Batzl-Hartmann C., Rueger H., Stricker-Krongrad A., Wyss P. et al. (1998) Food intake in free-feeding and energy-deprived lean rats is mediated by the neuropeptide Y5 receptor. J. Clin. Invest. **102:** 2136–2145

78 Della Zuana O., Sadlo M., Germain M., Feletou M., Chamorro S., Tisserand F. et al. (2001) Reduced food intake in response to CGP 71683A may be due to mechanisms other than NPY Y5 receptor blockade. Int. J. Obes. Relat. Metab. Disord. **25:** 84–94

79 Kanatani A., Ishihara A., Iwaasa H., Nakamura K., Okamoto O., Hidaka M. et al. (2000) L-152,804: orally active and selective neuropeptide Y Y5 receptor antagonist. Biochem. Biophys. Res. Commun. **272:** 169–173

80 Gregor P., Feng Y., DeCarr L. B., Cornfield L. J. and McCaleb M. L. (1996) Molecular characterization of a second mouse pancreatic polypeptide receptor and its inactivated human homologue. J. Biol. Chem. **271:** 27776–27781

81 Weinberg D. H., Sirinathsinghji D. J., Tan C. P., Shiao L. L., Morin N., Rigby M. R. et al. (1996) Cloning and expression of a novel neuropeptide Y receptor. J. Biol. Chem. **271:** 16435–16438

82 Matsumoto M., Nomura T., Momose K., Ikeda Y., Kondou Y., Akiho H. et al. (1996) Inactivation of a novel neuropeptide Y/peptide YY receptor gene in primate species. J. Biol. Chem. **271:** 27217–27220

83 Starback P., Wraith A., Eriksson H. and Larhammar D. (2000) Neuropeptide Y receptor gene y6: multiple deaths or resurrections? Biochem. Biophys. Res. Commun. **277:** 264–269

84 Lee C. C. and Miller R. J. (1998) Is there really an NPY Y3 receptor? Regul. Pept. **75–76:** 71–78

85 Wahlestedt C., Regunathan S. and Reis D. J. (1992) Identification of cultured cells selectively expressing Y1-, Y2- or Y3-type receptors for neuropeptide Y/peptide YY. Life Sci. **50:** L7–12

86 Higuchi H., Costa E. and Yang H. Y. (1988) Neuropeptide Y inhibits the nicotine-mediated release of catecholamines from bovine adrenal chromaffin cells. J. Pharmacol. Exp. Ther. **244:** 468–474

87 Norenberg W., Bek M., Limberger N., Takeda K. and Illes P. (1995) Inhibition of nicotinic acetylcholine receptor channels in bovine adrenal chromaffin cells by Y3-type neuropeptide Y receptors via the adenylate cyclase/protein kinase A system. Naunyn Schmiedebergs Arch. Pharmacol. **351:** 337–347

88 Foucart S., Bleakman D., Bindokas V. P. and Miller R. J. (1993) Neuropeptide Y and pancreatic polypeptide reduce calcium currents in acutely dissociated neurons from adult rat superior cervical ganglia. J. Pharmacol. Exp. Ther. **265:** 903–909

89 Grundemar L., Wahlestedt C. and Reis D. J. (1991) Neuropeptide Y acts at an atypical receptor to evoke cardiovascu-

lar depression and to inhibit glutamate responsiveness in the brainstem. J. Pharmacol. Exp. Ther. **258:** 633–638
90 Balasubramaniam A., Sheriff S., Rigel D. F. and Fischer J. E. (1990) Characterization of neuropeptide Y binding sites in rat cardiac ventricular membranes. Peptides **11:** 545–550
91 Dumont Y., Satoh H., Cadieux A., Taoudi-Benchekroun M., Pheng L. H., St Pierre S. et al. (1993) Evaluation of truncated neuropeptide Y analogues with modifications of the tyrosine residue in position 1 on Y1, Y2 and Y3 receptor sub-types. Eur. J. Pharmacol. **238:** 37–45
92 Jacques D., Cadieux A., Dumont Y. and Quirion R. (1995) Apparent affinity and potency of BIBP3226, a non-peptide neuropeptide Y receptor antagonist, on purported neuropeptide Y Y1, Y2 and Y3 receptors. Eur. J. Pharmacol. **278:** R3–R5
93 McCullough L. A. and Westfall T. C. (1996) Mechanism of catecholamine synthesis inhibition by neuropeptide Y: role of Ca2+ channels and protein kinases. J. Neurochem. **67:** 1090–1099
94 Cavadas C., Silva A. P., Mosimann F., Cotrim M. D., Ribeiro C. A., Brunner H. R. et al. (2001) NPY regulates catecholamine secretion from human adrenal chromaffin cells. J. Clin. Endocrinol. Metab. **86:** 5956–5963
95 Bonini J. A., Jones K. A., Adham N., Forray C., Artymyshyn R., Durkin M. et al. (2000) Identification and characterization of two G protein-coupled receptors for neuropeptide FF. J. Biol. Chem. **275:** 39324–39331
96 Mollereau C., Gouarderes C., Dumont Y., Kotani M., Detheux M., Doods H. et al. (2001) Agonist and antagonist activities on human NPFF(2) receptors of the NPY ligands GR231118 and BIBP3226. Br. J. Pharmacol. **133:** 1–4
97 Kassis S., Olasmaa M., Terenius L. and Fishman P. H. (1987) Neuropeptide Y inhibits cardiac adenylate cyclase through a pertussis toxin-sensitive G protein. J. Biol. Chem. **262:** 3429–3431
98 Westlind-Danielsson A., Unden A., Abens J., Andell S. and Bartfai T. (1987) Neuropeptide Y receptors and the inhibition of adenylate cyclase in the human frontal and temporal cortex. Neurosci. Lett. **74:** 237–242
99 Reynolds E. E. and Yokota S. (1988) Neuropeptide Y receptor-effector coupling mechanisms in cultured vascular smooth muscle cells. Biochem. Biophys. Res. Commun. **151:** 919–925
100 Shigeri Y., Nakajima S. and Fujimoto M. (1995) Neuropeptide YY1 receptors-mediated increase in intracellular Ca2+ concentration via phospholipase C-dependent pathway in porcine aortic smooth muscle cells. J. Biochem. **118:** 515–520
101 Lobaugh L. A. and Blackshear P. J. (1990) Neuropeptide Y stimulation of myosin light chain phosphorylation in cultured aortic smooth muscle cells. J. Biol. Chem. **265:** 18393–18399
102 Herzog H., Hort Y. J., Ball H. J., Hayes G., Shine J. and Selbie L. A. (1992) Cloned human neuropeptide Y receptor couples to two different second messenger systems. Proc. Natl. Acad. Sci. USA **89:** 5794–5798
103 Nie M. and Selbie L. A. (1998) Neuropeptide Y Y1 and Y2 receptor-mediated stimulation of mitogen-activated protein kinase activity. Regul. Pept. **75–76**: 207–213
104 Mannon P. J. and Mele J. M. (2000) Peptide YY Y1 receptor activates mitogen-activated protein kinase and proliferation in gut epithelial cells via the epidermal growth factor receptor. Biochem. J. **350 Pt 3:** 655–661
105 Nakamura M., Sakanaka C., Aoki Y., Ogasawara H., Tsuji T., Kodama H. et al. (1995) Identification of two isoforms of mouse neuropeptide Y-Y1 receptor generated by alternative splicing. Isolation, genomic structure and functional expression of the receptors. J. Biol. Chem. **270:** 30102–30110
106 Keffel S., Schmidt M., Bischoff A. and Michel M. C. (1999) Neuropeptide-Y stimulation of extracellular signal-regulated kinases in human erythroleukemia cells. J. Pharmacol. Exp. Ther. **291:** 1172–1178
107 Bleakman D., Colmers W. F., Fournier A. and Miller R. J. (1991) Neuropeptide Y inhibits Ca2+ influx into cultured dorsal root ganglion neurones of the rat via a Y2 receptor. Br. J. Pharmacol. **103:** 1781–1789
108 Lynch J. W., Lemos V. S., Bucher B., Stoclet J. C. and Takeda K. (1994) A pertussis toxin-insensitive calcium influx mediated by neuropeptide Y2 receptors in a human neuroblastoma cell line. J. Biol. Chem. **269:** 8226–8233
109 Freitag C., Svendsen A. B., Feldthus N., Lossl K. and Sheikh S. P. (1995) Coupling of the human Y2 receptor for neuropeptide Y and peptide YY to guanine nucleotide inhibitory proteins in permeabilized SMS-KAN cells. J. Neurochem. **64:** 643–650
110 Shigeri Y. and Fujimoto M. (1994) Y2 receptors for neuropeptide Y are coupled to three intracellular signal transduction pathways in a human neuroblastoma cell line. J. Biol. Chem. **269**: 8842–8848
111 Nakamura M., Aoki Y. and Hirano D. (1996) Cloning and functional expression of a cDNA encoding a mouse type 2 neuropeptide Y receptor. Biochim. Biophys. Acta **1284:** 134–137
112 Grouzmann E., Meyer C., Burki E. and Brunner H. (2001) Neuropeptide Y Y2 receptor signalling mechanisms in the human glioblastoma cell line LN319. Peptides **22:** 379–386
113 Wong L. B., Park C. L. and Yeates D. B. (1998) Neuropeptide Y inhibits ciliary beat frequency in human ciliated cells via nPKC, independently of PKA. Am. J. Physiol. **275:** C440–C448
114 Walker M. W., Smith K. E., Bard J., Vaysse P. J., Gerald C., Daouti S. et al. (1997) A structure-activity analysis of the cloned rat and human Y4 receptors for pancreatic polypeptide. Peptides **18:** 609–612
115 Sun L. and Miller R. J. (1999) Multiple neuropeptide Y receptors regulate K+ and Ca2+ channels in acutely isolated neurons from the rat arcuate nucleus. J. Neurophysiol. **81:** 1391–1403
116 Sun L., Philipson L. H. and Miller R. J. (1998) Regulation of K+ and Ca++ channels by a family of neuropeptide Y receptors. J. Pharmacol. Exp. Ther. **284:** 625–632
117 Bischoff A., Puttmann K., Kotting A., Moser C., Buschauer A. and Michel M. C. (2001) Limited signal transduction repertoire of human Y(5) neuropeptide Y receptors expressed in HEC-1B cells. Peptides **22:** 387–394
118 Pellieux C., Sauthier T., Domenighetti A., Marsh D. J., Palmiter R. D., Brunner H. R. et al. (2000) Neuropeptide Y (NPY) potentiates phenylephrine-induced mitogen-activated protein kinase activation in primary cardiomyocytes via NPY Y5 receptors. Proc. Natl. Acad. Sci. USA **97:** 1595–1600
119 Haynes J. M., Selbie L. A. and Hill S. J. (1999) Gi-Protein alpha-subunit mRNA antisense oligonucleotide inhibition of Gi-coupled receptor contractile activity in the epididymis of the guinea-pig. Br. J. Pharmacol. **127:** 85–90
120 Selbie L. A., King N. V., Dickenson J. M. and Hill S. J. (1997) Role of G-protein beta gamma subunits in the augmentation of P2Y2 (P2U)receptor-stimulated responses by neuropeptide Y Y1 Gi/o-coupled receptors. Biochem. J. **328 (Pt 1):** 153–158
121 Selbie L. A. and Hill S. J. (1998). G protein-coupled-receptor cross-talk: the fine-tuning of multiple receptor-signalling pathways. Trends Pharmacol. Sci. **19:** 87–93
122 Selbie L. A., Darby K., Schmitz-Peiffer C., Browne C. L., Herzog H., Shine J. et al. (1995) Synergistic interaction of Y1-neuropeptide Y and alpha 1b-adrenergic receptors in the regulation of phospholipase C, protein kinase C, and arachidonic acid production. J. Biol. Chem. **270:** 11789–11796
123 Morris M. J., Russell A. E., Kapoor V., Cain M. D., Elliott J. M., West M. J. et al. (1986) Increases in plasma neuropeptide Y concentrations during sympathetic activation in man. J. Auton. Nerv. Syst. **17:** 143–149

124 Morris M. J., Elliott J. M., Cain M. D., Kapoor V., West M. J. and Chalmers J. P. (1986) Plasma neuropeptide y levels rise in patients undergoing exercise tests for the investigation of chest pain. Clin. Exp. Pharmacol. Physiol. **13:** 437–440

125 Russell A. E., Cain M. D., Kapoor V., Morris M. J. and Chalmers J. P. (1989) Neuropeptide Y-like immunoreactivity of plasma during hypoglycaemia in man. J. Auton. Nerv. Syst. **26:** 85–88

126 Kaijser L., Pernow J., Berglund B. and Lundberg J. M. (1990) Neuropeptide Y is released together with noradrenaline from the human heart during exercise and hypoxia. Clin. Physiol. **10:** 179–188

127 Solt V. B., Brown M. R., Kennedy B., Kolterman O. G. and Ziegler M. G. (1990) Elevated insulin, norepinephrine and neuropeptide Y in hypertension. Am. J. Hypertens. **3:** 823–828

128 Chalmers J., Morris M., Kapoor V., Cain M., Elliott J., Russell A. et al. (1989) Neuropeptide Y in the sympathetic control of blood pressure in hypertensive subjects. Clin. Exp. Hypertens. A **11** Suppl. **1:** 59–66

129 Ullman B., Hulting J. and Lundberg J. M. (1994) Prognostic value of plasma neuropeptide-Y in coronary care unit patients with and without acute myocardial infarction. Eur. Heart J. **15:** 454–461

130 Ullman B., Lindvall K., Lundberg J. M., Sigurdsson A. and Swedberg K. (1994) Response of plasma neuropeptide Y and noradrenaline to dynamic exercise and ramipril treatment in patients with congestive heart failure. Clin. Physiol. **14:** 123–134

131 Hulting J., Sollevi A., Ullman B., Franco-Cereceda A. and Lundberg J. M. (1990) Plasma neuropeptide Y on admission to a coronary care unit: raised levels in patients with left heart failure. Cardiovasc. Res. **24:** 102–108

132 Madsen B. K., Husum D., Videbaek R., Stokholm K. H., Saelsen L. and Christensen N. J. (1993) Plasma immunoreactive neuropeptide Y in congestive heart failure at rest and during exercise. Scand. J. Clin. Lab Invest **53:** 569–576

133 Anderson F. L., Port J. D., Reid B. B., Hanson G., Kralios A. C., Hershberger R. E. et al. (1992) Effect of therapeutic dopamine administration on myocardial catecholamine and neuropeptide Y concentrations in the failing ventricles of patients with idiopathic dilated cardiomyopathy. J. Cardiovasc. Pharmacol. **20:** 800–806

134 Missouris C. G., Grouzmann E., Buckley M. G., Barron J., MacGregor G. A. and Singer D. R. (1998) How does treatment influence endocrine mechanisms in acute severe heart failure? Effects on cardiac natriuretic peptides, the renin system, neuropeptide Y and catecholamines. Clin. Sci. **94:** 591–599

135 Dubois-Rande J. L., Como E., Merlet P., Benvenuti C., Carville C., Hittinger L. et al. (1992) Relationship among neuropeptide Y, catecholamines and haemodynamics in congestive heart failure. Eur. Heart J. **13:** 1233–1238

136 Karvonen M. K., Pesonen U., Koulu M., Niskanen L., Laakso M., Rissanen A. et al. (1998) Association of a leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y with high serum cholesterol and LDL cholesterol levels. Nat. Med. **4:** 1434–1437

137 Karvonen M. K., Koulu M., Pesonen U., Uusitupa M. I., Tammi A., Viikari J. et al. (2000) Leucine 7 to proline 7 polymorphism in the preproneuropeptide Y is associated with birth weight and serum triglyceride concentration in preschool aged children. J.Clin. Endocrinol. Metab. **85:** 1455–1460

138 Niskanen L., Voutilainen-Kaunisto R., Terasvirta M., Karvonen M. K., Valve R., Pesonen U. et al. (2000) Leucine 7 to proline 7 polymorphism in the neuropeptide y gene is associated with retinopathy in type 2 diabetes. Exp. Clin. Endocrinol. Diabetes **108:** 235–236

139 Makino K., Kataoka Y., Hirakawa Y., Ikeda A., Yamauchi A. and Oishi A. R. (2001) A leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y was not identified in the Japanese population. J. Clin. Pharm. Ther. **26:** 201–203

140 Kallio J., Pesonen U., Kaipio K., Karvonen M. K., Jaakkola U., Heinonen O. J. et al. (2001) Altered intracellular processing and release of neuropeptide Y due to leucine 7 to proline 7 polymorphism in the signal peptide of preproneuropeptide Y in humans. FASEB J. **15:** 1242–1244

141 Walker P., Grouzmann E., Burnier M. and Waeber B. (1991) The role of neuropeptide Y in cardiovascular regulation. Trends Pharmacol. Sci. **12:** 111–115

142 Wahlestedt C., Edvinsson L., Ekblad E. and Hakanson R. (1985) Neuropeptide Y potentiates noradrenaline-evoked vasoconstriction: mode of action. J. Pharmacol. Exp. Ther. **234:** 735–741

143 Edvinsson L., Hakanson R., Steen S., Sundler F., Uddman R. and Wahlestedt C. (1985) Innervation of human omental arteries and veins and vasomotor response to noradrenaline, neuropeptide Y, substance P and vasoactive intestinal peptide. Regul. Pept. **12:** 67–79

144 Edvinsson L., Ekblad E., Hakanson R. and Wahlestedt C. (1984) Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. Br. J. Pharmacol. **83:** 519–525

145 Han C. and Abel P. W. (1987) Neuropeptide Y potentiates contraction and inhibits relaxation of rabbit coronary arteries. J. Cardiovasc. Pharmacol. **9:** 675–681

146 Schuerch L. V., Linder L. M., Grouzmann E. and Haefeli W. E. (1998) Human neuropeptide Y potentiates alpha1-adrenergic blood pressure responses in vivo. Am. J. Physiol. **275:** H760–H766

147 Pedrazzini T., Seydoux J., Kustner P., Aubert J.-F., Grouzmann E., Beermann F. et al. (1998) Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. Nat. Med. **4:** 722–726

148 Malmstrom R. E. and Lundberg J. M. (1995) Endogenous NPY acting on the Y1 receptor accounts for the long-lasting part of the sympathetic contraction in guinea-pig vena cava: evidence using SR 120107A. Acta Physiol. Scand. **155:** 329–330

149 Malmstrom R. E. and Lundberg J. M. (1995) Neuropeptide Y accounts for sympathetic vasoconstriction in guinea-pig vena cava: evidence using BIBP 3226 and 3435. Eur. J. Pharmacol. **294:** 661–668

150 Han S., Chen X., Cox B., Yang C. L., Wu Y. M., Naes L. et al. (1998) Role of neuropeptide Y in cold stress-induced hypertension. Peptides **19:** 351–358

151 Watson J. D., Sury M. R., Corder R., Carson R., Bouloux P. M., Lowry P. J. et al. (1988) Plasma levels of neuropeptide tyrosine Y (NPY) are increased in human sepsis but are unchanged during canine endotoxin shock despite raised catecholamine concentrations. J. Endocrinol. **116:** 421–426

152 Arnalich F., Sanchez J. F., Martinez M., Jimenez M., Lopez J., Vazquez J. J. et al. (1995) Changes in plasma concentrations of vasoactive neuropeptides in patients with sepsis and septic shock. Life Sci. **56:** 75–81

153 Hauser G. J., Myers A. K., Dayao E. K. and Zukowska-Grojec Z. (1993) Neuropeptide Y infusion improves hemodynamics and survival in rat endotoxic shock. Am. J. Physiol. **265:** H1416–H1423.

154 Qureshi N. U., Dayao E. K., Shirali S., Zukowska-Grojec Z. and Hauser G. J. (1998) Endogenous neuropeptide Y mediates vasoconstriction during endotoxic and hemorrhagic shock. Regul. Pept**. 75–76:** 215–220

155 McMahon C. D., Buxton D. F., Elsasser T. H., Gunter D. R., Sanders L. G., Steele B. P. et al. (1999) Neuropeptide Y restores appetite and alters concentrations of GH after central administration to endotoxic sheep. J. Endocrinol. **161:** 333–339

156 Gayle D., Ilyin S. E. and Plata-Salaman C. R. (1999) Feeding status and bacterial LPS-induced cytokine and neuropeptide gene expression in hypothalamus. Am. J. Physiol. **277:** R1188–R1195
157 Sergeyev V., Broberger C. and Hokfelt T. (2001) Effect of LPS administration on the expression of POMC, NPY, galanin, CART and MCH mRNAs in the rat hypothalamus. Brain Res. Mol. Brain Res. **90:** 93–100
158 Lundberg J. M., Pernow J., Tatemoto K. and Dahlof C. (1985) Pre- and postjunctional effects of NPY on sympathetic control of rat femoral artery. Acta Physiol. Scand. **123:** 511–513
159 Pernow J., Lundberg J. M., Kaijser L., Hjemdahl P., Theodorsson-Norheim E., Martinsson A. et al. (1986) Plasma neuropeptide Y-like immunoreactivity and catecholamines during various degrees of sympathetic activation in man. Clin. Physiol. **6:** 561–578
160 Lundberg J. M. and Stjarne L. (1984) Neuropeptide Y (NPY) depresses the secretion of 3H-noradrenaline and the contractile response evoked by field stimulation, in rat vas deferens. Acta Physiol. Scand. **120:** 477–479
161 Stjernquist M., Emson P., Owman C., Sjoberg N. O., Sundler F. and Tatemoto K. (1983) Neuropeptide Y in the female reproductive tract of the rat. Distribution of nerve fibres and motor effects. Neurosci. Lett. **39:** 279–284
162 Grundemar L., Grundstrom N., Johansson I. G., Andersson R. G. and Hakanson R. (1990) Suppression by neuropeptide Y of capsaicin-sensitive sensory nerve-mediated contraction in guinea-pig airways. Br. J. Pharmacol. **99:** 473–476
163 Wahlestedt C. and Hakanson R. (1986) Effects of neuropeptide Y (NPY) at the sympathetic neuroeffector junction. Can pre- and postjunctional receptors be distinguished? Med. Biol. **64**: 85–88
164 Westfall T. C., Han S. P., Chen X. L., del Valle K., Curfman M., Ciarleglio A. et al. (1990) Presynaptic peptide receptors and hypertension. Ann. N.Y. Acad. Sci. **604:** 372–388
165 Potter E. K. (1985) Prolonged non-adrenergic inhibition of cardiac vagal action following sympathetic stimulation: Neuromodulation by neuropeptide Y? Neurosci. Lett. **54:** 117– 121
166 Potter E. K., Barden J. A., McCloskey M. J., Selbie L. A., Tseng A., Herzog H. et al. (1994) A novel neuropeptide Y analog, N-acetyl [Leu28,Leu31]neuropeptide Y-(24- 36), with functional specificity for the presynaptic (Y2) receptor. Eur. J. Pharmacol. **267:** 253–262
167 Smith-White M. A., Herzog H. and Potter E. K. (2002) Role of neuropeptide Y Y(2) receptors in modulation of cardiac parasympathetic neurotransmission. Regul. Pept. **103:** 105–111
168 Mahns D. A., Revington M. L., Runcie M. J., McCloskey D. I. and Potter E. K. (1998) Inhibition of sympathetic cholinergic vasodilatation by a selective NPY Y2 receptor agonist in the gracilis muscle of anaesthetised dogs. J. Auton. Nerv. Syst. **68:** 14–20
169 Smith-White M. A., Hardy T. A., Brock J. A. and Potter E.K. (2001) Effects of a selective neuropeptide Y Y2 receptor antagonist, BIIE0246, on Y2 receptors at peripheral neuroeffector junctions. Br. J. Pharmacol. **132:** 861–868
170 Ellis J. L. and Burnstock G. (1990) Neuropeptide Y neuromodulation of sympathetic co-transmission in the guinea-pig vas deferens. Br. J. Pharmacol. **100:** 457–462
171 Cheung D. W. and Dukkipati R. (1991) An electrophysiological study on the effect of neuropeptide Y on sympathetic neurotransmission in guinea pig vas deferens. J. Pharmacol. Exp. Ther. **257:** 979–983
172 Grundemar L., Widmark E., Waldeck B. and Hakanson R. (1988) Neuropeptide Y: prejunctional inhibition of vagally induced contractions in the guinea pig trachea. Regul. Pept. **23:** 309–313
173 Grundemar L. (1998) Suppression of sensory C fiber-mediated contractions by neuropeptide Y Y1 receptors in the guinea pig bronchi. Regul. Pept. **75–76:** 181–184
174 Takahashi T., Ichinose M., Yamauchi H., Miura M., Nakajima N., Ishikawa J. et al. (1993) Neuropeptide Y inhibits neurogenic inflammation in guinea pig airways. J. Appl. Physiol. **75:** 103–107
175 Lacroix J. S., Ricchetti A. P., Morel P., Mossimann B., Waeber B. and Grouzmann E. (1996) Intranasal administration of neuropeptide Y in man: systemic absorption and functional effects. Br. J. Pharmacol. **118:** 2079–2084
176 Baraniuk J. N., Silver P. B., Kaliner M. A. and Barnes P. J. (1992) Neuropeptide Y is a vasoconstrictor in human nasal mucosa. J. Appl. Physiol. **73:** 1867–1872
177 Lacroix J. S. and Mosimann B. L. (1996) Attenuation of allergen-evoked nasal responses by local pretreatment with exogenous neuropeptide Y in atopic patients. J. Allergy Clin. Immunol. **98:** 611–616
178 Renshaw D., Thomson L. M., Carroll M., Kapas S. and Hinson J. P. (2000) Actions of neuropeptide Y on the rat adrenal cortex. Endocrinology **141:** 169–173
179 Takiyyuddin M. A., Brown M. R., Dinh T. Q., Cervenka J. H., Braun S. D., Parmer R. J. et al. (1994) Sympatho-adrenal secretion in humans: factors governing catecholamine and storage vesicle peptide co-release. J. Auton. Pharmacol. **14:** 187– 200
180 Majane E. A., Alho H., Kataoka Y., Lee C. H. and Yang T. (1986) Neuropeptide Y in bovine adrenal glands and characterization. Endocrinology **117:** 1162–1168
181 de Quidt M. E. and Emson P. C. (1986) Neuropeptide Y in the adrenal gland: characterization, distribution and drug effects. Neurosci. **19:** 1011–1022
182 Damase-Michel C., Tavernier G., Giraud P., Montastruc J. L., Montastruc P. and Tran M. A. (1993) Effects of clonidine, dihydralazine and splanchnic nerve stimulation on the release of neuropeptide Y, MET-enkephalin and catecholamines from dog adrenal medulla. Naunyn Schmiedebergs Arch. Pharmacol. **348:** 379–384
183 Wikstrom L. M., Meister B., Franck J. and Fried G. (1996) Changes in enkephalin and neuropeptide Y-like immunoreactivity in rabbit chromaffin tissues during perinatal development. Regul. Pept. **61:** 37–44
184 Allen J. M., Adrian T. E., Polak J. M. and Bloom S. R. (1983) Neuropeptide Y (NPY) in the adrenal gland. J. Auton. Nerv. Syst. **9:** 559–563
185 Lundberg J. M., Fried G., Pernow J. and Theodorsson-Norheim E. (1986) Co-release of neuropeptide Y and catecholamines upon adrenal activation in the cat. Acta Physiol. Scand. **126**: 231–238
186 Gulbenkian S., Wharton J., Hacker G. W., Varndell I. M., Bloom S. R. and Polak J. M. (1985) JM Co-localization of neuropeptide tyrosine (NPY) and its C-terminal flanking peptide (C-PON). Peptides **6:** 1237–1243
187 Hexum T. D. and Russett L. R. (1989) Stimulation of cholinergic receptor mediated secretion from the bovine adrenal medulla by neuropeptide Y. Neuropeptides **13:** 35–41
188 Shimoda K., Shen G. H., Pfeiffer R. F., McComb R. D. and Yang H. Y. (1993) Antiserum against neuropeptide Y enhances the nicotine-mediated release of catecholamines from cultured rat adrenal chromaffin cells. Neurochem. Int. **23:** 71–77
189 DiMaggio D. A., Farah J. M. Jr and Westfall T. C. (1994) Effects of differentiation on neuropeptide-Y receptors and responses in rat pheochromocytoma cells. Endocrinology **134:** 719–727
190 McCullough L. A., Egan T. M. and Westfall T. C. (1998) Neuropeptide Y inhibition of calcium channels in PC-12 pheochromocytoma cells. Am. J. Physiol. **274:** C1290–C1297
191 Shigeri Y. and Fujimoto M. (1993) Neuropeptide Y stimulates DNA synthesis in vascular smooth muscle cells. Neurosci. Lett. **149:** 19–22
192 Zukowska-Grojec Z., Pruszczyk P., Colton C., Yao J., Shen G. H., Myers A. K. et al. (1993) Mitogenic effect of neuropeptide

Y in rat vascular smooth muscle cells. Peptides **14:** 263–268
193 Erlinge D., Yoo H., Edvinsson L., Reis D. J. and Wahlestedt C. (1993) Mitogenic effects of ATP on vascular smooth muscle cells vs. other growth factors and sympathetic cotransmitters. Am. J. Physiol. **265:** H1089–H1097
194 Erlinge D., Brunkwall J. and Edvinsson L. (1994) Neuropeptide Y stimulates proliferation of human vascular smooth muscle cells: cooperation with noradrenaline and ATP. Regul. Pept. **50:** 259–265
195 Zukowska-Grojec Z., Karwatowska-Prokopczuk E., Fisher T. A. and Ji H. (1998) Mechanisms of vascular growth-promoting effects of neuropeptide Y: role of its inducible receptors. Regul. Pept. **75–76:** 231–238
196 Zukowska-Grojec Z., Karwatowska-Prokopczuk E., Rose W., Rone J., Movafagh S., Ji H. et al. (1998) Neuropeptide Y: a novel angiogenic factor from the sympathetic nerves and endothelium. Circ. Res. **83:** 187–195
197 Millar B. C., Schluter K. D., Zhou X. J., McDermott B. J. and Piper H. M. (1994) Neuropeptide Y stimulates hypertrophy of adult ventricular cardiomyocytes. Am. J. Physiol. **266:** C1271–C1277
198 Goldberg Y., Taimor G., Piper H. M. and Schluter K. D. (1998) Intracellular signaling leads to the hypertrophic effect of neuropeptide Y. Am. J. Physiol. **275:** C1207–C1215
199 Kanevskij M., Taimor G., Schafer M., Piper H. M. and Schluter K. D. (2002) Neuropeptide Y modifies the hypertrophic response of adult ventricular cardiomyocytes to norepinephrine. Cardiovasc. Res. **53:** 879–887
200 Grouzmann E., Alvarez-Bolado G., Meyer C., Osterheld M. C., Burnier M., Brunner H. R. et al. (1994) Localization of neuropeptide Y and its C-terminal flanking peptide in human renal tissue. Peptides **15:** 1377–1382
201 Echtenkamp S. F. and Dandridge P. F. (1989) Influence of renal sympathetic nerve stimulation on renal function in the primate. Am. J. Physiol. **257:** F204–F209
202 Echtenkamp S. F. and Dandridge P. F. (1989) Renal actions of neuropeptide Y in the primate. Am. J. Physiol. **256:** F524–F531
203 Bischoff A. and Michel M. C. (1998) Renal effects of neuropeptide Y. Pflugers Arch. **435:** 443–453
204 Malmstrom R. E. and Lundberg J. M. (1997) Time-dependent effects of ischaemia on neuropeptide Y mechanisms in pig renal vascular control in vivo. Acta Physiol Scand. **161**:327-338.
205 Malmstrom,R.E. (1997). Neuropeptide Y Y1 receptor mechanisms in sympathetic vascular control. Acta Physiol. Scand. Suppl. **636:** 1–55
206 Januszewicz A., Symonides B., Lapinski M., Lewandowski J., Januszewicz M., Rowinski O. et al. (1995) Endothelin-1 and neuropeptide Y plasma concentrations in renal venous blood of hypertensive patients with unilateral renal artery stenosis. J. Hum. Hypertens. **9:** 815–820
207 Shin L. H., Dovgan P. S., Nypaver T. J., Carretero O. A. and Beierwaltes W. H. (2000) Role of neuropeptide Y in the development of two-kidney, one-clip renovascular hypertension in the rat. J. Vasc. Surg. **32:** 1015–1021
208 Waeber B., Evequoz D., Aubert J. F., Fluckiger J. P., Juillerat L., Nussberger J. et al. (1990) Prevention of renal hypertension in the rat by neuropeptide Y. J. Hypertens. **8:** 21–25
209 Bray G. A. and York D. A. (1998) The MONA LISA hypothesis in the time of leptin. Recent Prog. Horm. Res. **53:** 95–117
210 Bray G. A. (1991) Obesity, a disorder of nutrient partitioning: the MONA LISA hypothesis. J. Nutr. **121:** 1146–1162
211 Edholm O. G. (1977) Energy balance in man studies carried out by the Division of Human Physiology, National Institute for Medical Research. J. Hum. Nutr. **31:** 413–431
212 Schwartz M.W., Woods S. C., Porte D. Jr, Seeley R. J. and Baskin D. G. (2000) Central nervous system control of food intake. Nature **404:** 661–671
213 Mystkowski P. and Schwartz M. W. (2000) Gonadal steroids and energy homeostasis in the leptin era. Nutrition **16:** 937–946
214 Elmquist J. K., Maratos-Flier E., Saper C. B. and Flier J. S. (1998) Unraveling the central nervous system pathways underlying responses to leptin. Nat. Neurosci. **1:** 445–450
215 Barsh G. S., Farooqi I. S. and O'Rahilly S. (2000) Genetics of body-weight regulation. Nature **404:** 644–651
216 Stanley B. G., Daniel D. R., Chin A. S. and Leibowitz S. F. (1985) Paraventricular nucleus injections of peptide YY and neuropeptide Y preferentially enhance carbohydrate ingestion. Peptides **6:** 1205–1211
217 Welch C. C., Grace M. K., Billington C. J. and Levine A. S. (1994) Preference and diet type affect macronutrient selection after morphine, NPY, norepinephrine and deprivation. Am. J. Physiol. **266:** R426–R433
218 Rohner-Jeanrenaud F. (1999) Neuroendocrine regulation of nutrient partitioning. Ann. N.Y. Acad. Sci. **892:** 261–271
219 Kalra S. P. (1997) Appetite and body weight regulation: is it all in the brain? Neuron **19:** 227–230
220 Vettor R., Zarjevski N., Cusin I., Rohner-Jeanrenaud F. and Jeanrenaud B. (1994) Induction and reversibility of an obesity syndrome by intracerebroventricular neuropeptide Y administration to normal rats. Diabetologia **37:** 1202–1208
221 Zarjevski N., Cusin I., Vettor R., Rohner-Jeanrenaud F. and Jeanrenaud B. (1993) Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. Endocrinology **133:** 1753–1758
222 Kalra S. P., Dube M. G., Sahu A., Phelps C. P. and Kalra P. S. (1991) Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proc. Natl. Acad. Sci. USA **88:** 10931–10935
223 Williams G., Steel J. H., Cardoso H., Ghatei M. A., Lee Y. C., Gill J. S. et al. (1988) Increased hypothalamic neuropeptide Y concentrations in diabetic rat. Diabetes **37:** 763–772
224 Beck B., Burlet A., Bazin R., Nicolas J. P. and Burlet C. (1993) 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
225 McKibbin P. E., Cotton S. J., McMillan S., Holloway B., Mayers R., McCarthy H. D. et al. (1991) 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
226 Zhang Y., Proenca R., Maffei M., Barone M., Leopold L. and Friedman J. M. (1994) Positional cloning of the mouse obese gene and its human homologue. Nature **372:** 425–432
227 Schwartz M. W., Baskin D. G., Bukowski T. R., Kuijper J. L., Foster D., Lasser G. et al. (1996) Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes **45:** 531–535
228 White J. D., Olchovsky D., Kershaw M. and Berelowitz M. (1990) Increased hypothalamic content of preproneuropeptide-Y messenger ribonucleic acid in streptozotocin-diabetic rats. Endocrinology **126:** 765–772
229 Beck B. (2000) Neuropeptides and obesity. Nutrition **16:** 916–923
230 Schwartz M. W., Seeley R. J., Campfield L. A., Burn P. and Baskin D. G. (1996) Identification of targets of leptin action in rat hypothalamus. J. Clin. Invest. **98:** 1101–1106
231 McKibbin P. E., McCarthy H. D., Shaw P. and Williams G. (1992) Insulin deficiency is a specific stimulus to hypothalamic neuropeptide Y: a comparison of the effects of insulin replacement and food restriction in streptozocin-diabetic rats. Peptides **13:** 721–727
232 Erickson J. C., Hollopeter G. and Palmiter R. D. (1996) Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. Science **274:** 1704–1707

233 Erickson J. C., Clegg K. E. and Palmiter R. D. (1996) Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature **381:** 415–421
234 Sahu A., Dube M. G., Phelps C. P., Sninsky C. A., Kalra P. S. and Kalra S. P. (1995) Insulin and insulin-like growth factor II suppress neuropeptide Y release from the nerve terminals in the paraventricular nucleus: a putative hypothalamic site for energy homeostasis. Endocrinology **136:** 5718–5724
235 Sipols A. J., Baskin D. G. and Schwartz M. W. (1995) Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. Diabetes **44:** 147–151
236 Vettor R., Pagano C., Granzotto M., Englaro P., Angeli P., Blum W.F. et al. (1998) Effects of intravenous neuropeptide Y on insulin secretion and insulin sensitivity in skeletal muscle in normal rats. Diabetologia **41:** 1361–1367
237 Sainsbury A., Rohner-Jeanrenaud F., Cusin I., Zakrzewska K. E., Halban P. A., Gaillard R. C. et al. (1997) Chronic central neuropeptide Y infusion in normal rats: status of the hypothalamo-pituitary-adrenal axis, and vagal mediation of hyperinsulinaemia. Diabetologia **40:** 1269–1277
238 Zakrzewska K. E., Sainsbury A., Cusin I., Rouru J., Jeanrenaud B. and Rohner-Jeanrenaud F. (1999) Selective dependence of intracerebroventricular neuropeptide Y-elicited effects on central glucocorticoids. Endocrinology **140**: 3183–3187
239 Adeghate E., Ponery A. S., Pallot D. J. and Singh J. (2001) Distribution of vasoactive intestinal polypeptide, neuropeptide-Y and substance P and their effects on insulin secretion from the in vitro pancreas of normal and diabetic rats. Peptides **22:** 99–107
240 Labelle M., Boulanger Y., Fournier A., St Pierre S. and Savard R. (1997) Tissue-specific regulation of fat cell lipolysis by NPY in 6-OHDA-treated rats. Peptides **18:** 801–808
241 Dyer C. J., Simmons J. M., Matteri R. L. and Keisler D. H. (1997) Effects of an intravenous injection of NPY on leptin and NPY-Y1 receptor mRNA expression in ovine adipose tissue. Domest. Anim Endocrinol. **14:** 325–333
242 Ollmann M. M., Wilson B. D., Yang Y. K., Kerns J. A., Chen Y., Gantz I. et al. (1997) Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science **278:** 135–138
243 Broberger C., Johansen J., Johansson C., Schalling M. and Hokfelt T. (1998) The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. Proc. Natl. Acad. Sci. USA **95:** 15043–15048.
244 Mizuno T. M., Kleopoulos S. P., Bergen H. T., Roberts J. L., Priest C. A. and Mobbs C. V. (1998) Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin. Diabetes **47:** 294–297
245 Kristensen P., Judge M. E., Thim L., Ribel U., Christjansen K. N., Wulff B. S. et al. (1998) Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature **393:** 72–76
246 Spanswick D., Smith M. A., Groppi V. E., Logan S. D. and Ashford M. L. (1997) Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. Nature **390:** 521–525
247 Loftus T. M., Jaworsky D. E., Frehywot G. L., Townsend C. A., Ronnett G. V., Lane M. D. et al. (2000) Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. Science **288:** 2379–2381
248 Kumar M. V., Shimokawa T., Nagy T. R. and Lane M. D. (2002) Differential effects of a centrally acting fatty acid synthase inhibitor in lean and obese mice. Proc. Natl. Acad. Sci. USA **99:** 1921–1925
249 Shimokawa T., Kumar M. V. and Lane M. D. (2002) Effect of a fatty acid synthase inhibitor on food intake and expression of hypothalamic neuropeptides. Proc. Natl. Acad. Sci. USA **99:** 66–71
250 Elias C. F., Lee C., Kelly J., Aschkenasi C., Ahima R. S., Couceyro P. R. et al. (1998) Leptin activates hypothalamic CART neurons projecting to the spinal cord. Neur **21:** 1375–1385
251 Jhanwar-Uniyal M., Beck B., Jhanwar Y. S., Burlet C. and Leibowitz S. F. (1993) Neuropeptide Y projection from arcuate nucleus to parvocellular division of paraventricular nucleus: specific relation to the ingestion of carbohydrate. Brain Res. **631:** 97–106
252 Richard D., Huang Q. and Timofeeva E. (2000) The corticotropin-releasing hormone system in the regulation of energy balance in obesity. Int. J. Obes. Relat. Metab. Disord. **24** Suppl. **2:** S36–S39
253 Bernardis L. L. and Bellinger L. L. (1993) The lateral hypothalamic area revisited: neuroanatomy, body weight regulation, neuroendocrinology and metabolism. Neurosci. Biobehav. Rev. **17:** 141–193
254 Broberger C., De Lecea L., Sutcliffe J. G. and Hokfelt T. (1998) Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. J. Comp. Neurol. **402**: 460–474
255 De Matteis R. and Cinti S. (1998) Ultrastructural immunolocalization of leptin receptor in mouse brain. Neuroendocrinology **68:** 412–419
256 Wellman P. J. (2000) Norepinephrine and the control of food intake. Nutrition **16:** 837–842
257 Iyengar S., Li D. L. and Simmons R. M. (1999) Characterization of neuropeptide Y-induced feeding in mice: do Y1-Y6 receptor subtypes mediate feeding? J. Pharmacol. Exp. Ther. **289:** 1031–1040
258 Parker R. M. and Herzog H. (1999) Regional distribution of Y-receptor subtype mRNAs in rat brain. Eur. J. Neurosci. **11:** 1431–1448
259 Stanley B. G., Magdalin W., Seirafi A., Nguyen M. M. and Leibowitz S. F. (1992) Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the Y1 receptor mediating this peptide's effect. Peptides **13:** 581–587
260 Leibowitz S. F. and Alexander J. T. (1991) Analysis of neuropeptide Y-induced feeding: dissociation of Y1 and Y2 receptor effects on natural meal patterns. Peptides **12:** 1251–1260
261 Kask A., Rago L. and Harro J. (1998) Evidence for involvement of neuropeptide Y receptors in the regulation of food intake: studies with Y1-selective antagonist BIBP3226. Br. J. Pharmacol. **124:** 1507–1515
262 Kanatani A., Ishihara A., Asahi S., Tanaka T., Ozaki S. and Ihara M. (1996) Potent neuropeptide Y Y1 receptor antagonist, 1229U91: blockade of neuropeptide Y-induced and physiological food intake. Endocrinology **137:** 3177–3182
263 Yokosuka M., Dube M. G., Kalra P. S. and Kalra S. P. (2001) The mPVN mediates blockade of NPY-induced feeding by a Y5 receptor antagonist: a c-FOS analysis. Peptides **22:** 507–514
264 Roche C., Boutin P., Dina C., Gyapay G., Basdevant A., Hager J. et al. (1997) Genetic studies of neuropeptide Y and neuropeptide Y receptors Y1 and Y5 regions in morbid obesity. Diabetologia **40:** 671–675
265 Rosenkranz K., Hinney A., Ziegler A., von Prittwitz S., Barth N., Roth H. et al. (1998) Screening for mutations in the neuropeptide Y Y5 receptor gene in cohorts belonging to different weight extremes. Int.J.Obes.Relat Metab Disord. **22:** 157– 163
266 Jenkinson C. P., Cray K., Walder K., Herzog H., Hanson and Ravussin E. (2000) Novel polymorphisms in the neuropeptide-Y Y5 receptor associated with obesity in Pima Indians. Int. J. Obes. Relat. Metab. Disord. **24:** 580–584

267 Flynn M. C., Turrin N. P., Plata-Salaman C. R. and French-Mullen J. M. (1999) Feeding response to neuropeptide Y-related compounds in rats treated with Y5 receptor antisense or sense phosphothio-oligodeoxynucleotide. Physiol. Behav. **66:** 881–884

268 Schaffhauser A. O., Stricker-Krongrad A., Brunner L., Cumin F., Gerald C., Whitebread S. et al. (1997) Inhibition of food intake by neuropeptide Y Y5 receptor antisense oligodeoxynucleotides. Diabetes **46:** 1792–1798

269 Lopez-Valpuesta F. J., Nyce J. W., Griffin-Biggs T. A., Ice J. C. and Myers R. D. (1996) Antisense to NPY-Y1 demonstrates that Y1 receptors in the hypothalamus underlie NPY hypothermia and feeding in rats. Proc. R. Soc. Lond. B Biol. Sci. **263:** 881–886

270 Lopez-Valpuesta F. J., Nyce J. W. and Myers R. D. (1996) NPY-Y1 receptor antisense injected centrally in rats causes hyperthermia and feeding. Neuroreport **7:** 2781–2784

271 Schaffhauser A. O., Whitebread S., Haener R., Hofbauer K. G. and Stricker-Krongrad A. (1998) Neuropeptide Y Y1 receptor antisense oligodeoxynucleotides enhance food intake in energy-deprived rats. Regul. Pept. **75–76:** 417–423

272 Marsh D. J., Hollopeter G., Kafer K. E. and Palmiter R. D. (1998) Role of the Y5 neuropeptide Y receptor in feeding and obesity. Nat. Med. **4:** 718–721

273 Kanatani A., Mashiko S., Murai N., Sugimoto N., Ito J., Fukuroda T. et al. (2000) Role of the Y1 receptor in the regulation of neuropeptide Y-mediated feeding: comparison of wild-type, Y1 receptor-deficient, and Y5 receptor-deficient mice. Endocrinology **141:** 1011–1016

274 Naveilhan P., Hassani H., Canals J. M., Ekstrand A. J., Larefalk A., Chhajlani V. et al. (1999) Normal feeding behavior, body weight and leptin response require the neuropeptide Y Y2 receptor. Nat. Med. **5:** 1188–1193

275 Sainsbury A., Schwarzer C., Couzens M., Fetissov S., Furtinger S., Jenkins A. et al. (2002) Important role of hypothalamic Y2 receptors in body weight regulation revealed in conditional knockout mice. Proc. Natl. Acad. Sci. USA **99:** 8938–8943

276 Edwardson J. A. and Hough C. A. (1975) The pituitary-adrenal system of the genetically obese (ob/ob) mouse. J. Endocrinol. **65:** 99–107

277 Cunningham J. J., Calles-Escandon J., Garrido F., Carr D. B. and Bode H. H. (1986) Hypercorticosteronuria and diminished pituitary responsiveness to corticotropin-releasing factor in obese Zucker rats. Endocrinology **118:** 98–101

278 Guillaume-Gentil C., Rohner-Jeanrenaud F., Abramo F., Bestetti G. E., Rossi G. L. and Jeanrenaud B. (1990) Abnormal regulation of the hypothalamo-pituitary-adrenal axis in the genetically obese fa/fa rat. Endocrinology **126:** 1873–1879

279 Considine R. V., Sinha M. K., Heiman M. L., Kriauciunas A., Stephens T. W., Nyce M. R. et al. (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. **334:** 292–295

280 Heiman M. L., Ahima R. S., Craft L. S., Schoner B., Stephens T. W. and Flier J. S. (1997) Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinology **138:** 3859–3863

281 Ahima R. S., Prabakaran D., Mantzoros C., Qu D., Lowell B., Maratos-Flier E. et al. (1996) Role of leptin in the neuroendocrine response to fasting. Nature **382:** 250–252

282 Wahlestedt C., Skagerberg G., Ekman R., Heilig M., Sundler F. and Hakanson R. (1987) Neuropeptide Y (NPY) in the area of the hypothalamic paraventricular nucleus activates the pituitary-adrenocortical axis in the rat. Brain Res. **417:** 33–38

283 Haas D. A. and George S. R. (1987) Neuropeptide Y administration acutely increases hypothalamic corticotropin-releasing factor immunoreactivity: lack of effect in other rat brain regions. Life Sci. **41:** 2725–2731

284 Harfstrand A., Eneroth P., Agnati L. and Fuxe K. (1987) 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:** 167–179

285 Small C. J., Morgan D. G., Meeran K., Heath M. M., Gunn I., Edwards C. M. et al. (1997) Peptide analogue studies of the hypothalamic neuropeptide Y receptor mediating pituitary adrenocorticotrophic hormone release. Proc. Natl. Acad. Sci. USA **94:** 11686–11691

286 Erickson J. C., Ahima R. S., Hollopeter G., Flier J. S. and Palmiter R. D. (1997) Endocrine function of neuropeptide Y knockout mice. Regul. Pept. **70:** 199–202

287 Steiner H. J., Schmid K. W., Fischer-Colbrie R., Sperk G. and Winkler H. (1989) Co-localization of chromogranin A and B, secretogranin II and neuropeptide Y in chromaffin granules of rat adrenal medulla studied by electron microscopic immunocytochemistry. Histochemistry **91:** 473–477

288 Mazzocchi G. and Nussdorfer G. G. (1987) Neuropeptide-Y acutely stimulates rat zona glomerulosa in vivo. Neuropeptides **9:** 257–262

289 Bernet F., Bernard J., Laborie C., Montel V., Maubert E. and Dupouy J. P. (1994) Neuropeptide Y (NPY)- and vasoactive intestinal peptide (VIP)-induced aldosterone secretion by rat capsule/glomerular zone could be mediated by catecholamines via beta 1 adrenergic receptors. Neurosci. Lett. **166:** 109–112

290 Barnea A., Cho G., Hajibeigi A., Aguila M. C. and Magni P. (1991) 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

291 Tempel D. L. and Leibowitz S. F. (1993) Glucocorticoid receptors in PVN: interactions with NE, NPY, and Gal in relation to feeding. Am. J. Physiol. **265:** E794–E800

292 White B. D., Dean R. G. and Martin R. J. (1990) Adrenalectomy decreases neuropeptide Y mRNA levels in the arcuate nucleus. Brain Res. Bull. **25:** 711–715

293 Akabayashi A., Watanabe Y., Wahlestedt C., McEwen B. S., Paez X. and Leibowitz S. F. (1994) Hypothalamic neuropeptide Y, its gene expression and receptor activity: relation to circulating corticosterone in adrenalectomized rats. Brain Res. **665:** 201–212

294 Dean R. G. and White B. D. (1990) Neuropeptide Y expression in rat brain: effects of adrenalectomy. Neurosci. Lett. **114:** 339–344

295 Larsen P. J., Jessop D. S., Chowdrey H. S., Lightman S. L. and Mikkelsen J. D. (1994) 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

296 Solano J. M. and Jacobson L. (1999) Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA. Am. J. Physiol. **277:** E708–E716

297 Wisialowski T., Parker R., Preston E., Sainsbury A., Kraegen E., Herzog H. et al. (2000) Adrenalectomy reduces neuropeptide Y-induced insulin release and NPY receptor expression in the rat ventromedial hypothalamus. J. Clin. Invest. **105:** 1253–1259

298 Cone R. D. (2000) The corticotropin-releasing hormone system and feeding behavior – a complex web begins to unravel. Endocrinology **141:** 2713–2714

299 Jeanrenaud B. and Rohner-Jeanrenaud F. (2000) CNS-periphery relationships and body weight homeostasis: influence of the glucocorticoid status. Int. J. Obes. Relat. Metab. Disord. **24** Suppl. **2:** S74–S76

300 Marshall J. C. and Kelch R. P. (1979) Low dose pulsatile gonadotropin-releasing hormone in anorexia nervosa: a model of human pubertal development. J. Clin. Endocrinol. Metab. **49:** 712–718

301 Pierroz D. D., Gruaz N. M., d'Alieves V. and Aubert M. L. (1995) Chronic administration of neuropeptide Y into the lateral ventricle starting at 30 days of life delays sexual maturation in the female rat. Neuroendocrinologyy **61:** 293–300

302 Pierroz D. D., Catzeflis C., Aebi A. C., Rivier J. E. and Aubert M. L. (1996) Chronic administration of neuropeptide Y into the lateral ventricle inhibits both the pituitary-testicular axis and growth hormone and insulin-like growth factor I secretion in intact adult male rats. Endocrinology **137:** 3–12

303 Catzeflis C., Pierroz D. D., Rohner-Jeanrenaud F., Rivier J. E., Sizonenko P. C. and Aubert M. L. (1993) Neuropeptide Y administered chronically into the lateral ventricle profoundly inhibits both the gonadotropic and the somatotropic axis in intact adult female rats. Endocrinology **132:** 224–234

304 Gruaz N. M., Pierroz D. D., Rohner-Jeanrenaud F., Sizonenko P. C. and Aubert M. L. (1993) Evidence that neuropeptide Y could represent a neuroendocrine inhibitor of sexual maturation in unfavorable metabolic conditions in the rat. Endocrinology **133:** 1891–1894

305 McDonald J. K., Lumpkin M. D., Samson W. K. and McCann S. M. (1985) Neuropeptide Y affects secretion of luteinizing hormone and growth hormone in ovariectomized rats. Proc. Natl. Acad. Sci. USA **82:** 561–564

306 Pierroz D. D., Aebi A. C., Huhtaniemi I. T. and Aubert M. L. (1999) Many LH peaks are needed to physiologically stimulate testosterone secretion: modulation by fasting and NPY. Am. J. Physiol. **276:** E603–E610

307 Bergonzelli G. E., Pralong F. P., Glauser M., Cavadas C., Grouzmann E. and Gaillard R. C. (2001) Interplay between galanin and leptin in the hypothalamic control of feeding via corticotropin-releasing hormone and neuropeptide Y. Diabetes **50:** 2666–2672

308 Stephens T. W., Basinski M., Bristow P. K., Bue-Valleskey J. M., Burgett S. G., Craft L. et al. (1995) The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature **377:** 530–532

309 Chehab F. F., Mounzih K., Lu R. and Lim M. E. (1997) Early onset of reproductive function in normal female mice treated with leptin. Science **275:** 88–90

310 Ahima R. S., Dushay J., Flier S. N., Prabakaran D. and Flier J. S. (1997) Leptin accelerates the onset of puberty in normal female mice. J. Clin. Invest. **99:** 391–395

311 Pralong F. P., Voirol M., Giacomini M., Gaillard R. C. and Grouzmann E. (2000) Acceleration of pubertal development following central blockade of the Y1 subtype of neuropeptide Y receptors. Regul. Pept. **95:** 47–52

312 Pralong F. P., Gonzales C., Voirol M. J., Palmiter R. D., Brunner H. R., Gaillard R. C. et al. (2002) The neuropeptide Y Y1 receptor regulates leptin-mediated control of energy homeostasis and reproductive functions. FASEB J. **16:** 712–714

313 Kiess W., Muller G., Galler A., Reich A., Deutscher J., Klammt J. et al. (2000) Body fat mass, leptin and puberty. J. Pediatr. Endocrinol. Metab. **13** Suppl. **1:** 717–722

314 Cunningham M. J., Clifton D. K. and Steiner R. A. (1999) Leptin's actions on the reproductive axis: perspectives and mechanisms. Biol. Reprod. **60:** 216–222

315 Hileman S. M., Pierroz D. D. and Flier J. S. (2000) Leptin, nutrition, and reproduction: timing is everything. J. Clin. Endocrinol. Metab. **85:** 804–807

316 McDonald J. K., Lumpkin M. D. and DePaolo L. V. (1989) Neuropeptide-Y suppresses pulsatile secretion of luteinizing hormone in ovariectomized rats: possible site of action. Endocrinology **125:** 186–191

317 Khorram O., Pau K. Y. and Spies H. G. (1988) Release of hypothalamic neuropeptide Y and effects of exogenous NPY on the release of hypothalamic GnRH and pituitary gonadotropins in intact and ovariectomized does in vitro. Peptides **9:** 411–417

318 Kalra S. P. and Crowley W. R. (1984) Differential effects of pancreatic polypeptide on luteinizing hormone release in female rats. Neuroendocrinology **38:** 511–513

319 Kalra S. P., Fuentes M., Fournier A., Parker S. L. and Crowley W. R. (1992) Involvement of the Y-1 receptor subtype in the regulation of luteinizing hormone secretion by neuropeptide Y in rats. Endocrinology **130:** 3323–3330

320 Raposinho P. D., Broqua P., Pierroz D. D., Hayward A., Dumont Y., Quirion R. et al. (1999) Evidence that the inhibition of luteinizing hormone secretion exerted by central administration of neuropeptide Y (NPY) in the rat is predominantly mediated by the NPY-Y5 receptor subtype. Endocrinology **140:** 4046–4055

321 Kalra S. P. and Crowley W. R. (1984) Norepinephrine-like effects of neuropeptide Y on LH release in the rat. Life Sci. **35:** 1173–1176

322 Bauer-Dantoin A. C., McDonald J. K. and Levine J. E. (1991) Neuropeptide Y potentiates luteinizing hormone (LH)-releasing hormone-stimulated LH surges in pentobarbital-blocked proestrous rats. Endocrinology **129:** 402–408

323 Kalra S. P. and Crowley W. R. (1992) Neuropeptide Y: a novel neuroendocrine peptide in the control of pituitary hormone secretion, and its relation to luteinizing hormone. Front Neuroendocrinol. **13:** 1–46

324 Minami S., Frautschy S. A., Plotsky P. M., Sutton S. W. and Sarkar D. K. (1990) Facilitatory role of neuropeptide Y on the onset of puberty: effect of immunoneutralization of neuropeptide Y on the release of luteinizing hormone and luteinizing-hormone-releasing hormone. Neuroendocrinology **52:** 112–115

325 Leupen S. M., Besecke L. M. and Levine J. E. (1997) Neuropeptide Y Y1-receptor stimulation is required for physiological amplification of preovulatory luteinizing hormone surges. Endocrinology **138:** 2735–2739

326 Raposinho P. D., Broqua P., Hayward A., Akinsanya K., Galyean R., Schteingart C. et al. (2000) Stimulation of the gonadotropic axis by the neuropeptide Y receptor Y1 antagonist/Y4 agonist 1229U91 in the male rat. Neuroendocrinology **71:** 2–7

327 Jain M. R., Pu S., Kalra P. S. and Kalra S. P. (1999) Evidence that stimulation of two modalities of pituitary luteinizing hormone release in ovarian steroid-primed ovariectomized rats may involve neuropeptide Y Y1 and Y4 receptors. Endocrinology **140:** 5171–5177

328 Sainsbury A., Schwarzer C., Couzens M., Jenkins A., Oakes S. R., Ormandy C. J. et al. (2002) Y4 receptor knockout rescues fertility in ob/ob mice. Genes Dev. **16:** 1077–1088

329 Lebrethon M. C., Vandersmissen E., Gerard A., Parent A. S., Junien J. L. and Bourguignon J. P. (2000) In vitro stimulation of the prepubertal rat gonadotropin-releasing hormone pulse generator by leptin and neuropeptide Y through distinct mechanisms. Endocrinology **141:** 1464–1469

330 Fekete C., Kelly J., Mihaly E., Sarkar S., Rand W. M., Legradi G. et al. (2001) Neuropeptide Y has a central inhibitory action on the hypothalamic-pituitary-thyroid axis. Endocrinology **142:** 2606–2613

331 Seoane L. M., Carro E., Tovar S., Casanueva F. F. and Dieguez C. (2000) Regulation of in vivo TSH secretion by leptin. Regul. Pept. **92:** 25–29

332 Nillni E. A., Vaslet C., Harris M., Hollenberg A., Bjorbak C. and Flier J. S. (2000) Leptin regulates prothyrotropin-releasing hormone biosynthesis. Evidence for direct and indirect pathways. J. Biol. Chem. **275:** 36124–36133

333 Chan Y. Y., Clifton D. K. and Steiner R. A. (1996) Role of NPY neurones in GH-dependent feedback signalling to the brain. Horm. Res. **45** Suppl. **1:** 12–14

334 Rettori V., Milenkovic L., Aguila M. C. and McCann S. M. (1990) Physiologically significant effect of neuropeptide Y to suppress growth hormone release by stimulating somatostatin discharge. Endocrinology **126:** 2296–2301

335 Vuagnat B. A., Pierroz D. D., Lalaoui M., Englaro P., Pralong F. P., Blum W. F. et al. (1998) Evidence for a leptin-neuropeptide Y axis for the regulation of growth hormone secretion in the rat. Neuroendocrinology **67:** 291–300
336 Heilig M. and Widerlov E. (1995) Neurobiology and clinical aspects of neuropeptide Y. Crit. Rev. Neurobiol. **9:** 115–136
337 Heilig M. and Thorsell A. (2002) Brain neuropeptide Y (NPY) in stress and alcohol dependence. Rev. Neurosci. **13:** 85–94.
338 Vezzani A., Sperk G. and Colmers W. F. (1999) Neuropeptide Y: emerging evidence for a functional role in seizure modulation. Trends Neurosci. **22:** 25–30
339 Baraban S. C. (1998) Neuropeptide Y and limbic seizures. Rev. Neurosci. **9:** 117–128
340 Tecott L. H. and Heberlein U. (1998) Y do we drink? Cell **95:** 733–735
341 Kosten T. R., Markou A. and Koob G. F. (1998) Depression and stimulant dependence: neurobiology and pharmacotherapy. J. Nerv. Ment. Dis. **186:** 737–745
342 Markou A., Kosten T. R. and Koob G. F. (1998) Neurobiological similarities in depression and drug dependence: a self- medication hypothesis. Neuropsychopharmacology **18:** 135–174
343 Li T. K. (2000) Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. J. Stud. Alcohol **61:** 5–12
344 Wahlestedt C., Merlo Pich E., Koob G. F., Yee F. and Heilig M. (1993) Modulation of anxiety and neuropeptide Y-Y1 receptors by antisense oligodeoxynucleotides. Science **259:** 528–531
345 Widerlov E., Lindstrom L. H., Wahlestedt C. and Ekman R. (1988) Neuropeptide Y and peptide YY as possible cerebrospinal fluid markers for major depression and schizophrenia, respectively. J. Psychiatr. Res. **22:** 69–79
346 Traskman-Bendz L., Ekman R., Regnell G. and Ohman R. (1992) HPA-related CSF neuropeptides in suicide attempters. Eur. Neuropsychopharmacol. **2:** 99–106
347 Roy A. (1993) Neuropeptides in relation to suicidal behavior in depression. Neuropsychobiology **28:** 184–186
348 Obuchowicz E. (1996) Long-term treatment with chlorpromazine and haloperidol but not with sulpiride and clozapine markedly elevates neuropeptide Y-like immunoreactivity in the rat hypothalamus. Neuropeptides **30:** 471–478
349 Redrobe J. P., Dumont Y., Fournier A. and Quirion R. (2002) The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. Neuropsychopharmacology **26:** 615–624
350 St Pierre J. A., Nouel D., Dumont Y., Beaudet A. and Quirion R. (2000) Sub-population of cultured hippocampal astrocytes expresses neuropeptide Y Y(1) receptors. Glia **30:** 82–91
351 St Pierre J. A., Dumont Y., Nouel D., Herzog H., Hamel E. and Quirion R. (1998) Preferential expression of the neuropeptide Y Y1 over the Y2 receptor subtype in cultured hippocampal neurons and cloning of the rat Y2 receptor. Br. J. Pharmacol. **123:** 183–194
352 Redrobe J. P., Dumont Y., St Pierre J. A. and Quirion R. (1999) Multiple receptors for neuropeptide Y in the hippocampus: putative roles in seizures and cognition. Brain Res. **848:** 153–166
353 Palmiter R. D., Erickson J. C., Hollopeter G., Baraban S. C. and Schwartz M. W. (1998) Life without neuropeptide Y. Recent Prog. Horm. Res. **53:** 163–199
354 Marsh D. J., Baraban S. C., Hollopeter G. and Palmiter R. D. (1999) Role of the Y5 neuropeptide Y receptor in limbic seizures. Proc. Natl. Acad. Sci. USA **96:** 13518–13523
355 Thiele T. E., Marsh D. J., Ste M. L., Bernstein I. L. and Palmiter R. D. (1998) Ethanol consumption and resistance are inversely related to neuropeptide Y levels. Nature **396:** 366–369
356 Thiele T. E., Miura G. I., Marsh D. J., Bernstein I. L. and Palmiter R. D. (2000) Neurobiological responses to ethanol in mutant mice lacking neuropeptide Y or the Y5 receptor. Pharmacol. Biochem. Behav. **67:** 683–691
357 Thiele T. E., Koh M. T. and Pedrazzini T. (2002) Voluntary alcohol consumption is controlled via the neuropeptide Y Y1 receptor. J. Neurosci. **22:** RC208
358 Badia-Elder N. E., Stewart R. B., Powrozek T. A., Roy K. F., Murphy J. M. and Li T. K. (2001) Effect of neuropeptide Y (NPY) on oral ethanol intake in Wistar, alcohol-preferring (P), and -nonpreferring (NP) rats. Alcohol Clin. Exp. Res. **25:** 386–390
359 Kelley S. P., Nannini M. A., Bratt A. M. and Hodge C. W. (2001) Neuropeptide-Y in the paraventricular nucleus increases ethanol self- administration. Peptides **22**: 515–522
360 Kauhanen J., Karvonen M. K., Pesonen U., Koulu M., Tuomainen T. P., Uusitupa M. I. et al. (2000) Neuropeptide Y polymorphism and alcohol consumption in middle-aged men. Am. J. Med. Genet. **93:** 117–121
361 Ilveskoski E., Kajander O. A., Lehtimaki T., Kunnas T., Karhunen P. J., Heinala P. et al. (2001) Association of neuropeptide y polymorphism with the occurrence of type 1 and type 2 alcoholism. Alcohol Clin. Exp. Res. **25:** 1420–1422
362 Okubo T. and Harada S. (2001) Polymorphism of the neuropeptide Y gene: an association study with alcohol withdrawal. Alcohol Clin. Exp. Res. **25:** 59S–62S