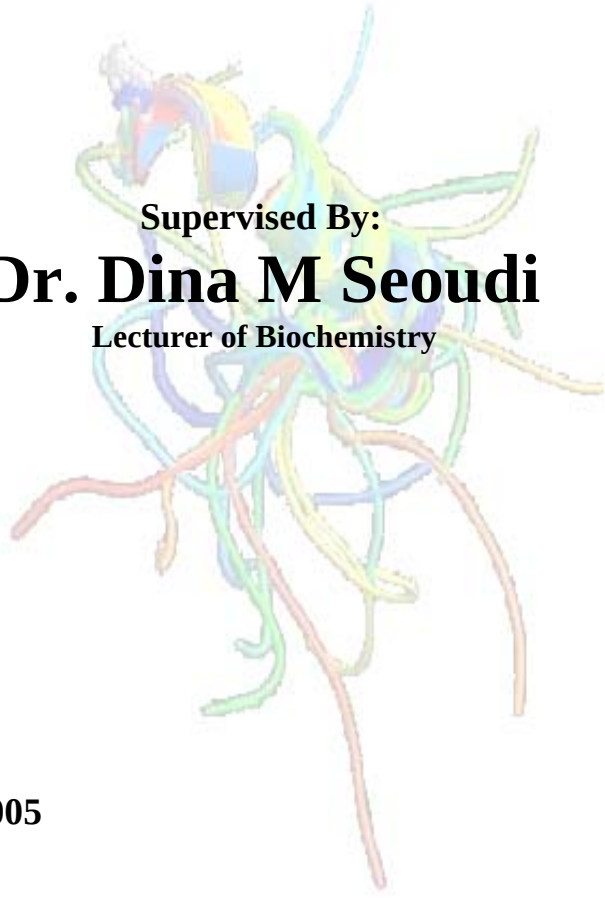
Faculty of Science
Biochemistry Dept.

An Essay About:

Neuropeptide Y

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بِسمِ اللهِ الرحمنِ الرحِيمِ

"و ما أُوتِيتم من الْعِلْمِ إِلا قَلِيلاً"

صدق الله العظِيم

(سورة الإسراء-اية 85)

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In April 29th, 2005

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Glossary

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List of Abbreviations

Adenocorticotropic hormone	:	**ACTH**
Adenine	:	**a**
Adenosine triphosphatase		**ATPase**
Agouti-related protein	:	**AgRP**
Alanine	:	**Ala or A**
Alfa-melanocyte-stimulating hormone	:	**α-MSH**
Alzheimer	:	**AD**
Amino acid terminal of a peptide	:	**-NH_2 or NH^{3+}**
Amphetamine	:	**AMPH**
Arcuate nucleus of the hypothalamus	:	**ARC**
Arginine	:	**Arg or R**
Aspargine	:	**Asn or N**
Aspartate	:	**Asp or D**
Atrial natriuretic peptide	:	**ANF**
Beta	:	**β**
Body mass index	:	**BMI**
Calcitonin gene-related peptide	:	**CGRP**
Calcium	:	**Ca^{2+}**
Carboxypeptidase E	:	**CPH**
Cardiovascular diseases	:	**CVD**
Carboxylic acid terminal of a peptide	:	**H- or COO^-**
Carboxylic terminal of a peptide	:	**C-terminal**
Central nervous system	:	**CNS**
Cholecystokinin	:	**CCK**
Chromosome	:	**q**
Chronic kidney disease	:	**CKD**
Corticotropin-releasing factor	:	**CRF**
Complementary deoxyribonucleic acid	:	**cDNA**
Constitutional delay of puberty	:	**CDP**
Corticotropin releasing hormone	:	**CRH**
C-terminal flanking peptide of NPY	:	**CPON**
Cupper	:	**Cu^{+2}**
Cyclic adenosine monophosphate	:	**cAMP**
Cysteine	:	**Cys or C**
Cytoplasmic domain	:	**CD**
Cytosine	:	**c**
Dipeptidyl peptidase IV	:	**DPP-IV**
Dorsal vagal complex	:	**DVC**

Dorsomdial hypothalamic nucleus	:	**DMH**
Drosophila neuropeptide F	:	**dNPF**
Endoplasmic reticulum	:	**ER**
End-stage renal disease	:	**ESRD**
Figure	:	**Fig.**
Follicle stimulating hormone	:	**FSH**
For example	:	**e.g.**
Gamma	:	**γ**
Gama-aminobutyric acid	:	**GABA**
Gastrin releasing peptide	:	**GRP**
Gastrointestinal	:	**GI**
Growth hormone secretagogue receptor	:	**GHsR**
Glutamate	:	**Glu or E**
Glutamine	:	**Gln or Q**
Glycine	:	**Gly or G**
Gonodotropin releasing hormone	:	**GnRH**
G-protein coupled receptors	:	**GPCRs**
Growth hormone	:	**GH**
Growth hormone releasing hormone	:	**GHRH**
Guanine	:	**g**
Guanidine diphosphate	:	**GDP**
Guanidine triphosphate	:	**GTP**
Histidine	:	**His or H**
Hour	:	**h**
Human neuropeptide Y	:	**hNPY**
Isoleucine	:	**Ile or I**
Hypothalamo-pituitary-adrenocortical	:	**HPA**
Insulin receptor		**INPR**
Interleukin	:	**IL**
Inttracerebroventricular	:	**ICV**
Kilo Dalton (a molecular mass)	:	**kDa**
Kilo gram per meter square	:	**kg/m^2**
Labeled iodine (125)	:	**I^{125}**
Large dense-core vesicles	:	**LDCV**
Leucine	:	**Leu or L**
Leptin receptor	:	**LEPR**
Lithium	:	**Li^+**
Low density lipoprotein-cholesterol	:	**LDL-C**
Luteinzing hormone	:	**LH**
Lysine	:	**Lys or K**
Melanocortin 4 receptor	:	**MC_4R**
Milliliter	:	**ml**

Messenger ribonucleic acid	:	**mRNA**
Methionine	:	**Met or M**
Minutes	:	**min**
Manganese	:	**Mn^{+2}**
Monosodium glutamate	:	**MSG**
Mouse	:	**m**
Neuropeptide Y	:	**NPY**
Nitric oxide	:	**NO**
Nor adrenalinergic	:	**NAergic**
Normal course of puberty	:	**NP**
Obstructive sleep apnea syndrome	:	**OSAS**
Pancreatic Polypeptide	:	**PP**
Paraventricular nucleus	:	**PVN**
Parkinson's disease	:	**PD**
Percent	:	**%**
Peptidyl α-hydroxyglycine α-amidating lyase	:	**PAL**
Peptidyl α-hydroxylating mono oxygenase	:	**PHM**
Peptidylglycine α-amidating mono oxygenase	:	**PAM**
Picomole per liter	:	**pmol/L**
Polpeptide YY	:	**PYY**
Potassium	:	**K^{+}**
Prohormone convertase	:	**PC**
Proline	:	**Pro or P**
Prolactin	:	**PRL**
Propiomelanocortin	:	**POMC**
Prostate cancer	:	**CaP**
Renal cell carcinomas	:	**RCCs**
Reverse-transcription polymerase chain reaction	:	**RT-PCR**
Serine	:	**Ser or S**
Short neuropeptide F	:	**sNPF**
Single nucleotide polymorphisms	:	**SNPs**
Sodium	:	**Na^{+}**
Temporal lope epilepsy	:	**TLE**
That is to say	:	**i.e.**
Three dimension	:	**3D**
Threonine	:	**Thr or T**
Thymine	:	**t**
Thyroid stimulating hormone	:	**TSH**
Thyrotropin releasing hormone	:	**TRH**
Transmembrane	:	**TM**
Tryprophan	:	**Trp or W**

Tumour necrosis factor	:	**TNF**
Tyrosine	:	**Tyr or Y**
Valine	:	**Val or V**
Vasoactive intestinal peptide	:	**VIP**
Unknown or nonstandard amino acid	:	**X**
Zinc	:	**Zn^{+2}**
Zona glomerulosa	:	**ZG**

Abstract

This essay focuses on the most abundant bioactive peptide in brain tissue, Neuropeptide Y (NPY), a member of the pancreatic polypeptide family, which has been isolated for the first time by Tatemoto in 1982.

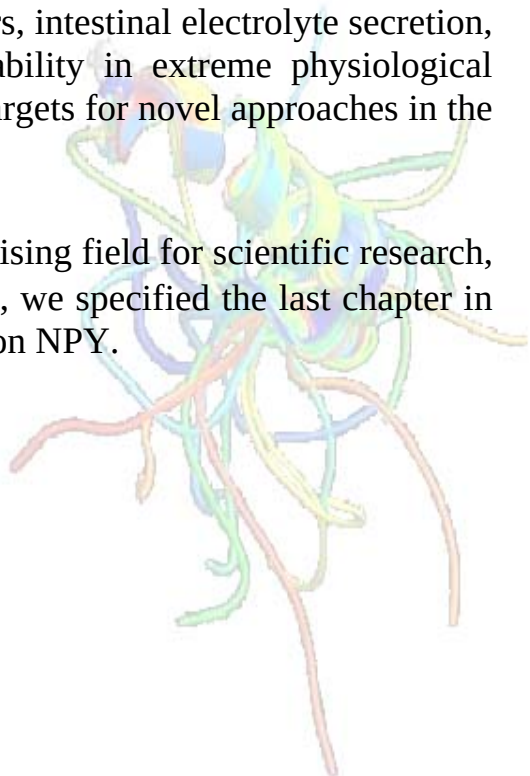
NPY is identified as a 4.3 kDa linear peptide, consisting of 36 amino acids rich in tyrosine. It is mainly biosynthesized in the neurons of the hypothalamic arcuate nucleus (ARC) as a prepro-form, consisting of 97 amino acids.

NPY exhibits a wide spectrum of central and peripheral activities mediated by six G-protein coupled receptor subtypes with seven transmembrane domains, denoted as Y_1, Y_2, y_3, Y_4, Y_5, and y_6.

NPY is implicated in a variety of physiological processes. It is a major regulator of food consumption and energy homeostasis. It has anxiolytic and sedative properties within the central nervous system, and a vast range of effects in the immune system. It has an endocrinal activity and acts as a neurotransmitter. It affects puberty in females as well as lactation. Moreover, dysregulation in NPY signaling causes alterations in bone formation and alcohol consumption.

Evidence suggests that NPY is involved in the pathophysiology of several disorders, such as depression, anxiety, epilepsy, seizures, sleep, learning, memory, circadian rhythm, drug addiction, pain, cardiovascular diseases, rhinitis, endothelial cell dysfunctions, diabetes, hypertension, pheochromocytoma, congestive heart failure, gastrointestinal regulation, intestinal disorders, intestinal electrolyte secretion, urinary sodium excretion and regulation of excitability in extreme physiological circumstances. NPY and its receptors may become targets for novel approaches in the treatment of these diseases.

NPY and its receptors are considered as promising field for scientific research, and to point out the importance of this neuropeptide, we specified the last chapter in this essay to review some of the most recent studies on NPY.



Chapter I

Bioactive peptides are grouped into different families according to their structural similarity or tissue source. They considered among the physiologically important active peptides, which play functional role in biological system.

Bioactive Peptides

Peptides are molecules consisting of several amino acids linked together with peptide bonds. The size of peptides can vary from molecules with only two amino acids to as many as 50. Moreover, peptides do not only exist in natural form but also can be designed synthetically as novel molecules. Thus, their actual number is presently very large (**Reubi, 2003**).

In phylogenetic terms, neuropeptides were established very early as molecules affecting intercellular communication. Although their list is already quite long (**Table 1**), additional neuropeptides are still being identified. With the advent of radioreceptor assays, peptides have been characterized by their ability to bind to a known receptor and thus to displace a ligand or produce a biological response (**Siegel** *et al.*, **1998**).

Peptide neurotransmitters are generally synthesized as larger proteins and are cleaved by proteolysis to produce the neuropeptide molecules. Their synthesis requires the same biochemical machinery as does any protein synthesis and take place in the cell body, not the axon. They travel down the axon to the presynaptic region by one of two generic mechanisms: *fast axonal transport* at a rate of about 400 ml/day and *slow axonal transport* at a rate of 1-5 ml/day (**Devlin, 1997**).

It is significant to note that the number of known neuropeptides far exceeds the number of classical neurotransmitter, and although many neuropeptides have similar structures, they have very different functions (**Siegel** *et al.*, **1998**). They mediate sensory and emotional responses such as hunger, thirst, sex, pleasure and pain. Included to this category are endorphins, and substance P. Substance P is an excitatory neurotransmitter that has a role in pain transmission, whereas endorphins have roles in eliminating the sensation of pain. Some of the peptides found in brain tissue are shown in **Table 2** (**Devlin, 1997**), and the brain anatomy is illustrated in **Figure 1**.

The afferent connections to the histaminergic tuberomamillary neurons seem to contain neuropeptide Y (NPY) or substance P. Amacrine cells (modified nerve cell) also contain various neuropeptides, including substance P, enkephalin, glucagon, somatostatin, neurotensin, NPY, vasoactive intestinal polypeptide and cholecytokinin which co-localize with other neurotransmitter substances and function as neuromodulators. The effects of neuromodulators generally are slower and last longer than those of neurotransmitters (**Siegel** *et al.*, **1998**).



Table 1: Selected bioactive peptide (**Siegel** *et al.*, **1998**).

Hypothalamic releasing factors
CRH: corticotropin releasing hormone
GHRH: growth hormone releasing hormone
GnRH: gonadotropin releasing hormone
Somatostatin
TRH: thyrotropin releasing hormone

Pituitary hormone
ACTH: adenocorticotropic hormone
α-MSH: α-melanocyte stimulating hormone
β-endorphin
GH: growth hormone
PRL: prolactin
FSH: follicle stimulating hormone
LH: luteinzing hormone
TSH: thyrotropin [thyroid stimulating hormone]

GI and brain pepides
CCK: cholecystokinin
Gastrin
GRP: gastrin releasing peptide
Motilin
Neurotensin
Substance K; substance P (tachykinins)

Circulating
Angiotensin
Bradykinin

Frog skin
Bombesin
Caerulein
Ranatensin

Opiate peptides
β-endorphin
Dynorphin
Leu-enkephalin
Met-enkephalin

Neurohypophyseal peptides
Oxytocin
Vasopressin

Neuronal and endocrine
ANF: atrial natriuretic peptide
CGRP: calcitonin gene-related peptide
VIP: vasoactive intestinal peptide

GI and pancreas
Glucagon
PP: pancreatic polypeptide

Neurons
Galanin
Neuromedin K
NPY: neuropeptide Y
PYY: peptide YY

Endocrine
Calcitonin
Insulin
Secretin
Parathyroid hormone

Table 2: Peptides found in brain tissue (**Devlin, 1997**).

Peptide	Structure
β-endorphin	Y G G F M T S E K S Q T P L V T L F K N A I I K N A Y K K G E
Met-enkephalin	Y G G F M
Leu-enkephalin	Y G G F L
Somatostatin	A G G K N F F W I I C S T F T K
Luteinzing hormone releasing hormone	p-E H W S Y G L R P G-NH_2
Thyrotropin releasing hormone	p-E H P-NH_2
Substance P	R P K P E E F F G L M-NH_2
Neurotensin	p-E L Y E N K P R R P Y L L
Angiotensin I	D R V Y I H P F H L
Angiotensin II	D R V Y I H P F
Vasoactive intestinal peptide	H S D A V F T D N Y T R L R K E M A V K K Y L N S I L N-NH_2

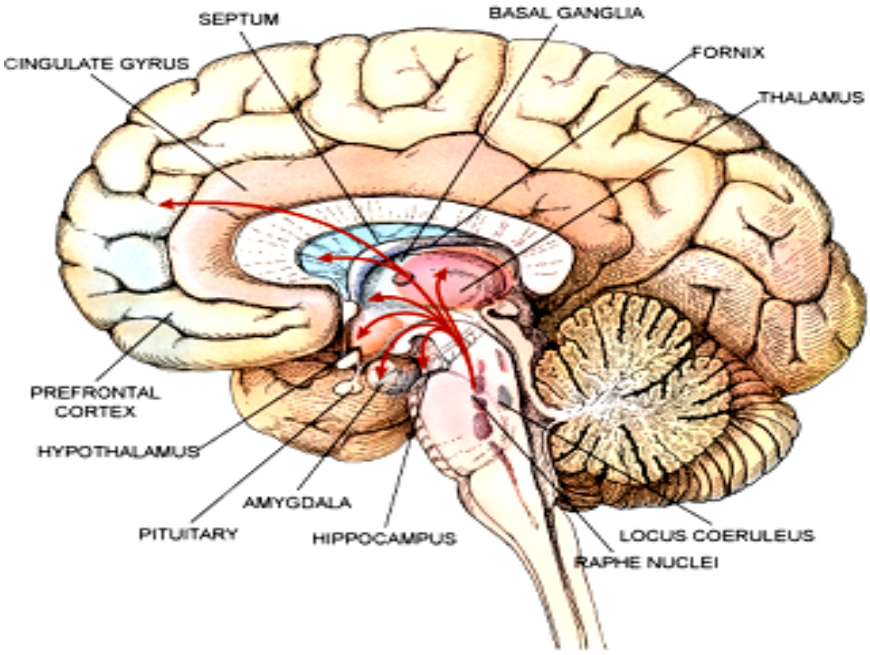


Fig. 1: Anatomy of the human brain.
http://www.sd74.org/Lincoln_Hall/wagner/survivalwebquest/anatomyoffear.htm

Probably the most striking difference between neuropeptides and conventional neurotransmitters is in their biosynthesis. Neuropeptides are derived from larger, inactive precursors that are generally at least 90 amino acids residues in length (**Rouille** *et al.*, **1995**). They present in tissue as much lower concentrations than classical neurotransmitters but are also active at receptors at correspondingly lower concentrations (**Siegel** *et al.*, **1998**).

Neuropeptide Receptors

The first neuropeptide receptors characterized were those for substance P and neurotensin (**Sawada** and **Dickinson**, **1997**), most have the general architecture shown in **Figure 2**. For large glycoprotein ligands, such as thyroid stimulating hormone (TSH) and chorionic gonodotropin, the extracellular domain plays a major role in ligand binding. By contrast, as shown in **Figure 3**, the key components of the binding sites for smaller peptides are the analogous residues, important for binding small non-peptide ligands to their receptors (**Cascieri** *et al.*, **1995**).

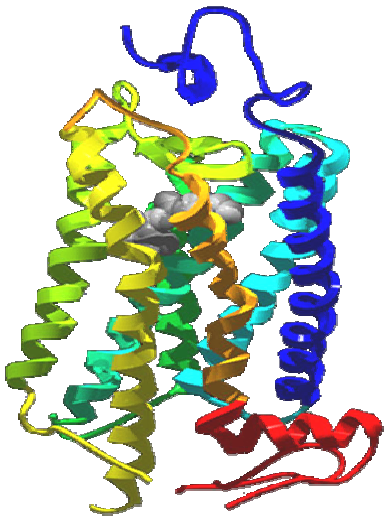


Fig. 2: The general architecture of neuropeptide receptors
www.dartmouth.edu/dms/ pharmtox/gallery/img1.shtmlhttp://

Peptidergic neurotransmission often operates on a slower time scale than conventional neurotransmitters, so it is not surprising that placement of peptide receptors is often not localized to the synapse. Neuropeptides can act at many sites. They may act directly on a postsynaptic target at the synapse; presynaptically on the terminal that released the peptide (autocrine effects); on an immediately adjacent cell (juxtacrine effects); or on a cell a few cell diameters away from the site of release (paracrine effects). In addition, peptides can exert their actions via travel through the circulation to reach their target (endocrine effects) as in case of hypothalamic releasing factors and neurohypophyseal hormones (**Siegel** *et al.*, **1998**).

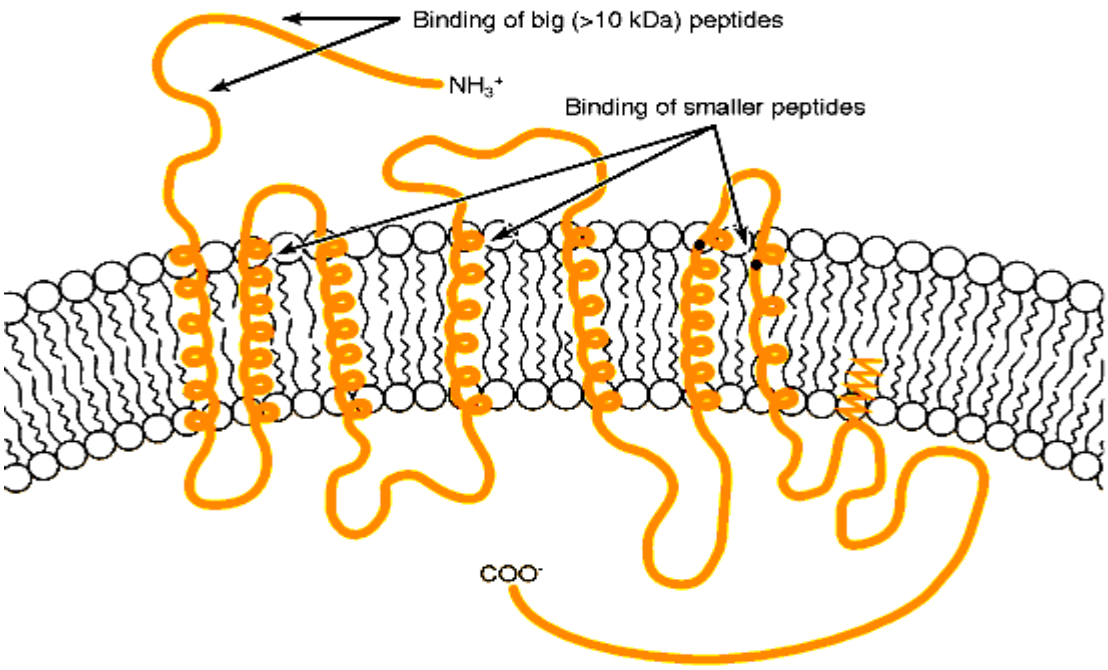


Fig. 3: Neuropeptide receptors usually consist of seven transmembrane domains with binding regions within the membrane and in the NH_2-terminal loop (**Nelson and Cox, 2000**).

Pancreatic Polypeptide Family

The pancreatic polypeptide-fold family of peptides consists of three 36-amino acid peptides, namely peptide YY (PYY), pancreatic polypeptide (PP) and neuropeptide Y (NPY) (**Berglund** *et al.*, **2003**). NPY and PYY act similarly in a majority of biological models, but exceptions have been noted. Within this family, NPY is most conserved among species because it subserves many important functions, binds to multiple distinct receptors, and most of its receptor subtypes are structurally well conserved. On the other hand, PP is least conserved, it evolves very rapidly along with its receptor, suggesting that PP may have less crucial functions that may differ between species (**Bard** *et al.*, **1995**, **Larhammar, 1996a**, **Larhammar, 1996b** and **Wraith** *et al.*, **2000**). All members of this family are with a large number of tyrosine residues, including both ends of the molecule, sharing a high amino acid homology (**Fig. 4**), and characterized by a hairpin tertiary structure (**Simonneaux and Ribelayga, 2003**). The tertiary structure of turkey PP has been elucidated by crystallography (**Larhammar, 1996a**). This tertiary structure has been termed the "PP-fold", which has U-shaped and consists of an extended polyproline helix and an α helix connected by a β turn (**Michel** *et al.*, **1998**).

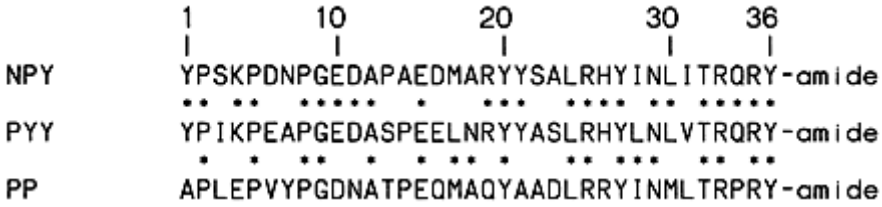


Fig. 4: Alignment of human neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP). Amino acid identities between peptides are indicated by a star (**Michel** *et al.*, **1998**).

Human pancreatic polypeptides are shown to regulate important functions, including food intake, circadian rhythms, mood, blood pressure, intestinal secretion, and gut motility, through four receptors: Y_1, Y_2, Y_4, and Y_5. Additional receptor subtypes have been proposed based on pharmacology observed in native tissues (**Berglund** *et al.*, **2003**). Because of the large number of tyrosine residues, which are abbreviated by the letter Y in the single letter amino acid code, receptors of these peptides are designated by a capital Y (**Michel** *et al.*, **1998**).

Peptide YY (PYY)

The gut hormone peptide YY (PYY) is expressed in PP cells and in somatostatin cells (**Batterham& Bloom, 2003** and **Lundell** *et al.*, **1995**). Also, it is biosynthesized and secreted from endocrine ileal L-cells of the gastrointestinal tract (GI) tract in response to caloric ingestion (**Batterham& Bloom, 2003** and **Hung** *et al.*, **2004**) and can also coexist with glucagon in pancreatic acini and enteroglucagon in endocrine cells of the lower bowel (**Michel** *et al.*, **1998**).

PYY exists in two endogenous forms: PYY (1-36) and PYY (3-36). The latter is produced by the action of the enzyme dipeptidyl peptidase IV (DPP-IV). PYY (1-36) binds to and activates at least three Y receptor subtypes (Y_1, Y_2, and Y_5), whereas PYY (3-36) is more selective for Y_2 receptor. In response to food ingestion, plasma PYY (3-36) concentrations rise within 15 min and plateau by approximately 90 min. The peak PYY (3-36) level achieved is proportional to the calories ingested, suggesting that PYY (3-36) may signal food ingestion from the gut to appetite-regulating circuits within the brain. It may also constitute the majority of circulating PYY-like immunoreactivity in the hypothalamic arcuate nucleus (ARC), a key brain area regulating appetite that has access to nutrients and hormones within the peripheral circulation (**Batterham& Bloom, 2003**, **Pittner** *et al.*, **2004** and **Mondal** *et al.*, **2005**).

Peripheral administration of PYY (3-36) is known to inhibit food intake, body weight gain and glycemic indices in diverse rodent models of metabolic disease of both sexes. These findings justify further exploration of the potential physiologic and therapeutic roles of PYY (3-36). This anorexigenic effect is masked by stress inhibition of appetite, and it is therefore important for animals to be thoroughly acclimatized for PYY (3-36) to be effective (**McGowan** *et al.*, **2004** and **Pittner** *et al.*, **2004**).

In humans, peripheral infusion of PYY (3-36), at a dose which produced normal postprandial concentrations, significantly decreased appetite and reduced food intake by 33% over 24 h. These findings suggest that PYY (3-36) released in response to a meal acts to physiologically regulate food intake (**Batterham and Bloom, 2003**).

Levels of the anorexigenic hormone PYY (3-36) are low in overweight individuals and could provide an important therapeutic avenue in the quest to combat the obesity epidemic (**McGowan** *et al.*, **2004**). Mutations in PYY and Y_2 are not commonly found in humans with severe early-onset obesity. The relationship between common variants in Y_2 and obesity-related traits deserves further exploration in other populations (**Hung** *et al.*, **2004**).

Pancreatic Polypeptide (PP)

Pancreatic polypeptide (PP) is mainly found in pancreatic cells distinct from those storing insulin, glucagon, or somatostatin (**Michel** *et al.*, **1998**). It is released into the circulation from pancreatic endocrine cells post-prandially (**Bard** *et al.*, **1995**).

PP receptors have been characterized by binding or functional assays in liver, intestine, vas deferens, spinal cord, adrenal gland, superior cervical ganglia, brainstem nuclei, and PC-12 cells (**Bard** *et al.*, **1995**).

PP is a hormone. It exerts regulatory effects on pancreatic exocrine secretion, gall bladder contraction, gastrointestinal motility, gastric acid secretion, pancreatic secretion, and corticosterone secretion (**Bard** *et al.*, **1995** and **Lundell** *et al.*, **1995**). It has similar action to PYY, and in addition, it redistributes blood flow in gut vessels (**Lundell** *et al.*, **1995**).

Although PP was discovered first and NPY last, evolutionary analysis shows that PP is actually the newest member of the family (**Larhammar, 1996a**). PYY and NPY presumably evolved by duplication from a common ancestral gene in an early vertebrate ancestor. The corresponding genes are

located on different chromosomes. The PP gene probably arose by duplication of the PYY gene; both genes are located close to one another in the same chromosomal segment (**Larhammar, 1996a**). Based on these evolutionary considerations, it is recommended that the family is denoted by the NPY family.

Neuropeptide Y (NPY)

This bioactive peptide will be discussed in much more details in the rest of this essay.

Chapter II

Neuropeptide Y, a member of the pancreatic polypeptide family, is a main brain neurotransmitter, which expressed in neurons of the hypothalamic arcuate nucleus that project mainly to paraventricular nucleus an important site of NPY release.

Neuropeptide Y

In 1982, Tatemoto succeeded for the first time in isolating NPY from porcine brain (**Tatemoto, 1982**). NPY is subsequently found in all mammals as well as in a wide variety of animal species including birds, reptiles, amphibians and fishes (**Nordmann *et al.*, 1999**), and a number of rodent species including Syrian hamsters "*Mesocricetus auratus*" (**Corp *et al.*, 2001**). The human variant (hNPY) is found in rats, guinea pigs and rabbits (**Nordmann *et al.*, 1999**).

Structure

NPY is identified as a 4.3 kDa linear peptide (**Mohring *et al.*, 2005**), consisting of 36 amino acids rich in tyrosine (**Cavadas *et al.*, 2001**). It is biosynthesized in a prepro-form consisting of 97 amino acids (**Fig. 5**). hNPY has the following amino acid sequence: H-Met-Leu-Gly-Asn-Lys-Arg-Leu-Gly-Leu-Ser-Gly-Leu-Thr-Leu-Ala-Leu-Ser-Leu-Leu-Val-Cys-Leu-Gly-Ala-Leu-Ala-Glu-Ala-**Tyr**-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Met-Ala-Arg-**Tyr**-**Tyr**-Ser-Ala-Leu-Arg-His-**Tyr**-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-**Tyr**-Gly-Lys-Arg-Ser-Ser-Pro-Glu-Tyr-Leu-Ile-Ser-Asp-Leu-Leu-Met-Arg-Glu-Ser-Tyr-Glu-Asn-Val-Pro-Arg-Tyr-Arg-Leu-Glu-Asp-Pro-Ala-Met-Trp-NH_2 (**Reubi *et al.*, 2001**). The porcine variant has a leucine residue instead of methionine at position 17 of the active form (**Nordmann *et al.*, 1999**) (**Fig. 6**). Neuropeptide Y has a high degree of sequence homology with peptide YY (70%), and pancreatic polypeptide (50%) (**Fig. 4**) (**Tatemoto, 1982**).

Note: The amino acid sequence written in black is the active form of NPY (36 amino acids).

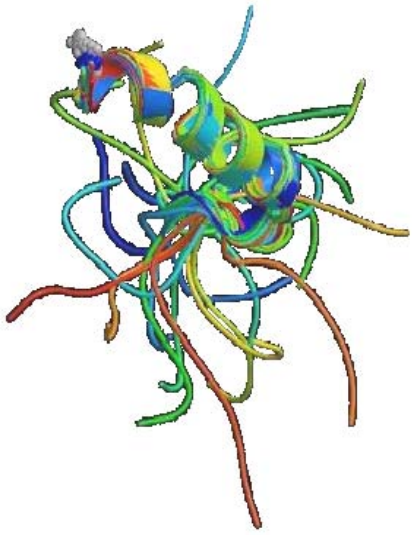


Fig. 5: 3D structure of human prepro-NPY
http://www.astrazeneca.com

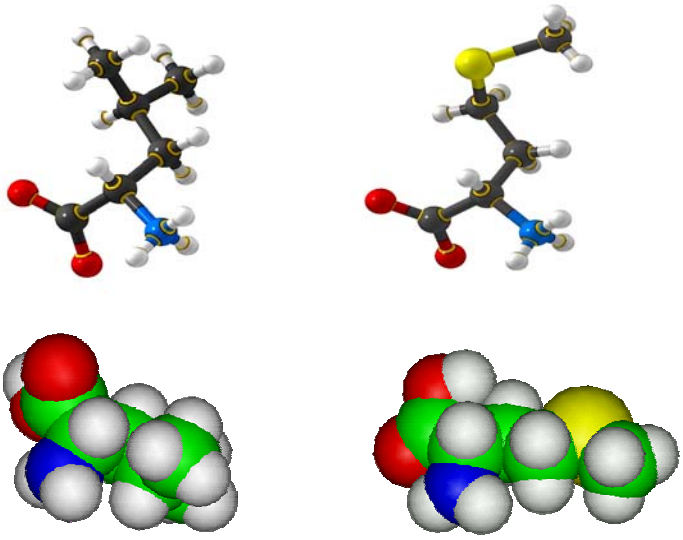


Fig. 6: Chemical structure of leucine (on the left side) and methionine (on the right side)
(Note: Carbon is in green or grey, Oxygen is in red, Hydrogen is in white, Nitrogen is in blue and Sulfur is in yellow)

Gene Expression

NPY gene expression can be positively regulated by synergistic actions of glucocorticoids, cAMP elevation, and protein kinase C activation (**Higuchi** *et al.*, **1988**). Three possible exones encode hNPY, they are represented as follows:

Exon I

(ACCESSION NUMBER M14296)

```
ORIGIN      Chromosome 7pter-q22; 965 bp downstream of segment 1.
  1 cccgtccgtt gagccttctg tgcctgcaga tgctaggtaa caagcgactg gggctgtccg
 61 gactgaccct cgccctgtcc ctgctcgtgt gcctgggtgc gctggccgag gcgtacccct
121 ccaagccgga caacccgggc gaggacgcac cagcggagga catggccaga tactactcag
181 cgctgggaca ctacatcaac ctcatcacca ggcagaggtg ggtgggaccg cgggaccgat
241 tccggga
//
```

Exon II

(ACCESSION NUMBER M14297)

```
ORIGIN      Chromosome 7pter-q22; 4300 bp downstream of segment 2.
  1 acttgcttta aaagactttt ttttttccag atatggaaaa cgatctagcc cagagacact
 61 gatttcagac ctcttgatga gagaaagcac agaaaatgtt cccagaactc ggtatgacaa
121 ggcttgtgat ggggacattg tt
//
```

Exon III

(ACCESSION NUMBER M14298)

```
ORIGIN      Chromosome 7pter-q22; 2300 bp downstream of segment 3.
  1 ccttacatgc tttgcttctt atgttttaca ggcttgaaga ccctgcaatg tggtgatggg
 61 aaatgagact tgctctctgg ccttttccta ttttcagccc atatttcatc gtgtaaaacg
121 agaatccacc catcctacca atgcatgcag ccactgtgct gaattctgca atgttttcct
181 ttgtcatcat tgtatatatg tgtgtttaaa taaagtatca tgcattcaaa agtgtatcct
241 cctcaatgaa aaatctatta caatagtgag gattattttc gttaaactta ttattaacaa
//
```

Site of Production

NPY was reported as an abundant peptide in brain tissue in 1982, and latter in the same year, it was described for the first time by **Tatemoto** (**1982**), as one of the neuropeptides whose concentration is the highest in the central (**Malendowicz** *et al.*, **1996**) and peripheral nervous systems (**Larhammar, 1996a**). It is involved in cardiovascular regulation, particularly through the sympathetic nerve fibers innervating arteries, veins and the heart (**Michalkiewicz** *et al.*, **2001**).

NPY comes from preproneuropeptide Y after signal peptide removal, cleavage between NPY and the C-terminal flanking peptide of NPY (CPON) (**Fig. 7**). It is made by neurons throughout the brain and by other secretory cells of the body (**William** *et al.*, **2003**), and it is released during sympathetic stimulation (**Zoccali** *et al.*, **2003**). It presents in the limbic structure, cortex, cerebral trunk, spinal cord, chromaffin cells of the adrenal medulla and vascular bed of many organs (**Simonneause and Ribelayga, 2003**). It is produced mainly in neurons of the hypothalamic arcuate nucleus (ARC), that projects mainly to paraventricular nucleus (PVN), an important site of NPY release (**Havel** *et al.*, **2000**) (**Fig. 1** and **8**).

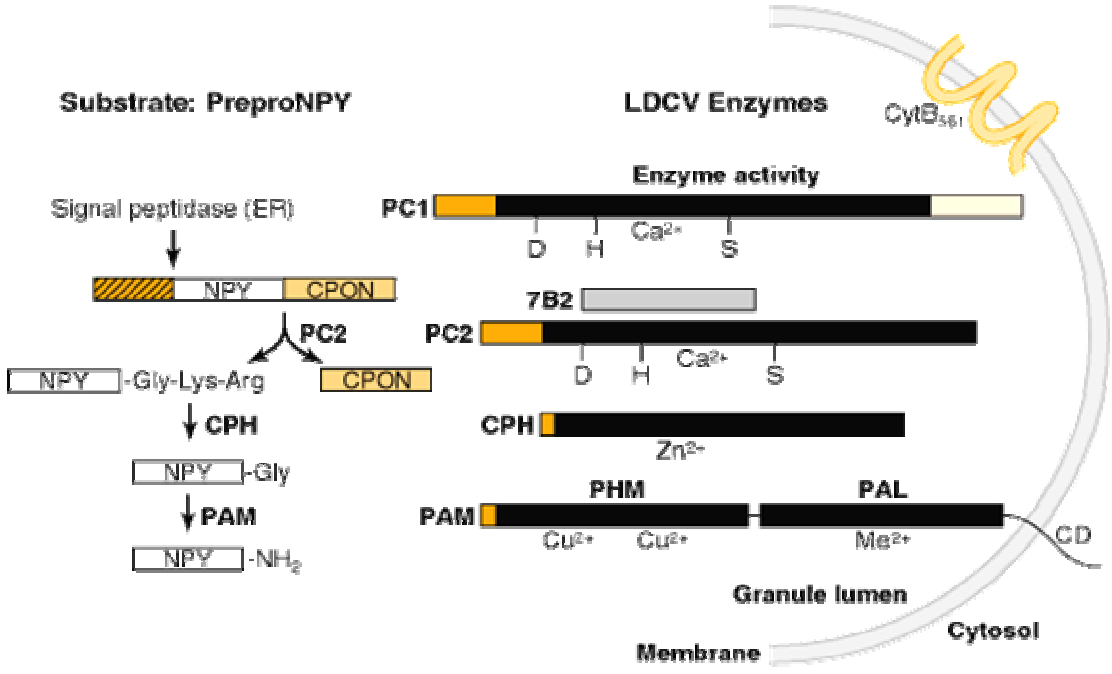


Fig. 7: Sequential enzymatic steps lead from the peptide precursor to bioactive precursor shown at the left is processed NPY peptides. The neuropeptide Y (NPY) precursor shown at the left is processed sequentially by the enzymes of the large dense-core vesicles (LDCV) shown at the right. ER, endoplasmic reticulum; PC, prohormone convertase; CPON, C-terminal flanking peptide of NPY; CPH, carboxypeptidase E; PAM, peptidylglycine α-amidating mono-oxygenase; PHM, peptidylglycine α-hydroxylating mono-oxygenase; PAL, peptidyl-α-hydroxyglycine α-amidating lyase; CD, cytoplasmic domain (**Siegel** *et al.*, **1998**).

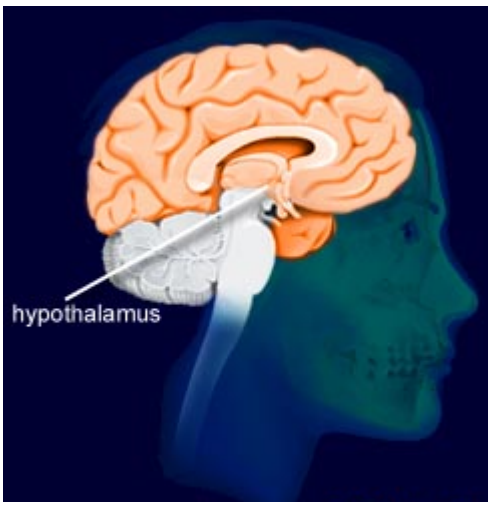


Fig. 8: Hypothalamus, the main site of NPY production.

Site of Action

Central NPY can influence peripheral tissues by alterations in autonomic neuronal activity. This is probably mediated by NPY projections from the hypothalamus to brain stem areas where sympathetic or parasympathetic neuronal activity is modulated (**Baldock** *et al.*, **2002**). Unlike that of many inhibitory transmitters, the action of NPY is restricted to presynaptic sites in Ammon's horn and is specific to terminals releasing excitatory amino acids onto principal neurons in the hippocampus. The plasticity of the NPY system in response to elevated activity can generally be considered adaptive (**William** *et al.*, **2003**).

Biochemical Assay

Serum NPY can be assayed by competitive radioimmunoassay using an antiserum raised against synthetic NPY conjugated to bovine thyroglobulin. NPY in serum samples and standard compete with ^{125}I-labelled NPY in binding to the antibodies.^{125}I-NPY binds in a reverse proportion to the concentration of NPY in standards and samples. Antibody-bound ^{125}I-NPY is separated from the unbound fraction by using double antibody coupled to solid phase. The radioactivity of the antibody-bound ^{125}I-NPY is measured. The lowest detectable concentration is 6pmol/L (**Theodorsson-Norheim** *et al.*, **1985**).

Chapter III

The effect of NPY can be mediated by several NPY receptor subtypes, named Y_1-Y_6, among which Y_1, Y_2, Y_4, and Y_5 have been well characterized and shown to be physiologically expressed. All known NPY receptors belong to the large superfamily of G-protein coupled heptahelical receptors.

NPY Receptors

NPY neurons abundantly innervate the hypothalamus, where NPY is involved in the regulation and integration of a broad range of homeostatic functions. In order to understand NPY mediated behavioral, autonomic and neuroendocrine effects, it is important to characterize in detail the distribution of the hypothalamic NPY receptors. These receptors are found with individual distribution patterns in many hypothalamic neurons including neuroendocrine motoneurons, magnocellular neurosecretory neurons and numerous neurons connecting the hypothalamus with the limbic and the autonomic nervous systems (**Fetissov** *et al.*, **2004**).

Pancreatic polypeptide family exhibits a wide spectrum of central and peripheral activities mediated by six G-protein coupled receptor subtypes with seven transmembrane domains (**Nordmann** *et al.*, **1999**), denoted as Y_1, Y_2, y_3, Y_4, Y_5, and y_6 (**Balasubramaniam, 2002**) note that they are designated by subscript numbers (**Michel** *et al.*, **1998**). They mediate either inhibition of adenylate cyclase or increases in intracellular calcium (**Gehlert, 2004**).

Recent studies with GPCRs have shown that homo- and heterodimerization may be important in determining receptor function and pharmacology (**Berglund** *et al.*, **2003**). Researchers have shown the occurrence of heterodimerization between different members of the GPCRs family. Two receptors can interact with each other to make a dimer through their extracellular loops, transmembrane helices and intracellular loops. The nature of bonds between two receptors can vary from covalent (e.g. disulphide bonds) to non-covalent (for instance hydrophobic interactions between transmembrane helices or coiled coil structures) or a combination of both. Dimerization can occur in and affect different stages of a receptor's life, and can be seen as the natural way to regulate receptor activity or increase the functional repertoire of proteins (**Nikbin** *et al.*, **2003**).

GPCRs are integral membrane proteins, which face both the outside and the inside of the cell. The structural feature common to all GPCRs is the presence of seven hydrophobic transmembrane spanning α helical segments that are connected to each other by intracellular and extracellular loops. The amino terminus is on the extracellular side while the carboxy terminus is intracellular **(Fig. 9)**. This family of membrane receptors comprises the largest superfamily of proteins in humans. More than 1000 different GPCRs have been identified in different organisms. The endogenous ligands for this class of receptors are very diverse and include monoamines, peptides, glycoproteins, lipids, nucleotides and

ions as well as exogenous stimulants such as light, odors and taste (**Nikbin *et al.*, 2003**).

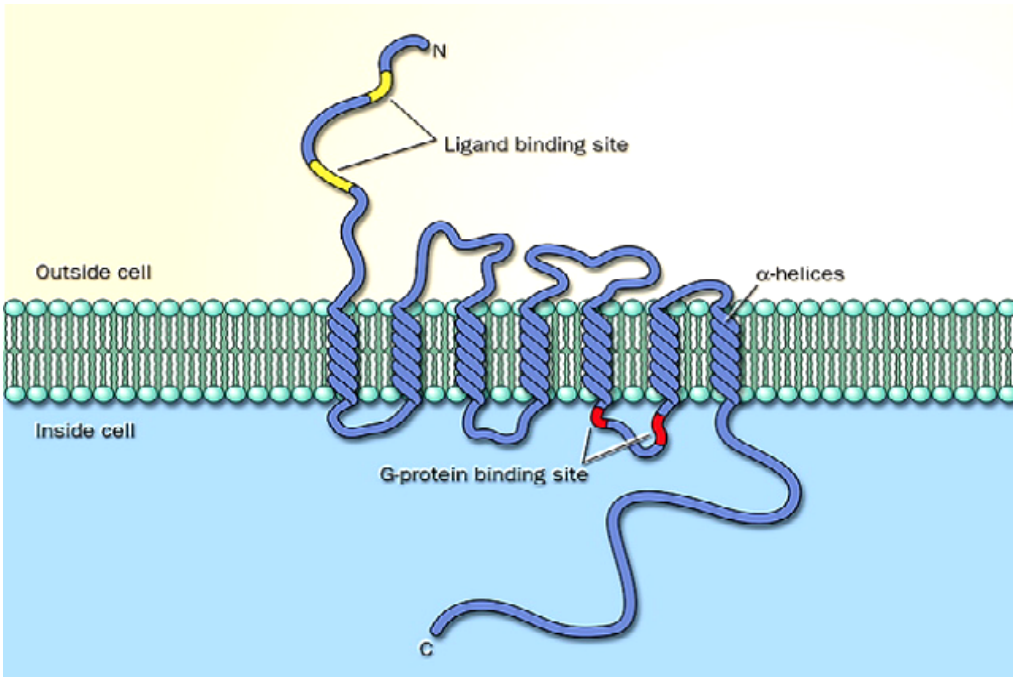


Fig. 9: Structure of GPCRs (**Nelson and Cox, 2000**).

GPCRs are so called because their action is mediated by trimeric G-proteins. Trimeric G-proteins are signaling molecules that bind to GDP and transduce signals from activated receptors to their intracellular target enzymes. They are associated with the plasma membrane and consist of alpha, beta and gamma subunits. They are inactive when the alpha subunit is bound to GDP, and become activated when a stimulated receptor induces the alpha subunit to exchange its GDP for GTP. The activated alpha subunit then dissociates from the beta and gamma subunits and activates target proteins such as phospholipase C and adenylate cyclase (**Nelson and Cox, 2000**).

Mode of action of GPCRs involves the activation of other membrane associated proteins after stimulation by an extracellular ligand. Hormone binding, for example, activates the receptor, which in turn bind to and activates the intracellular trimeric G-protein. This activated G-protein then activates target proteins associated with the plasma membrane, such as enzymes or ion channels which then brings about the physiological result specified by the hormone (**Nelson and Cox, 2000**).

The large number of Y receptors has made it difficult to delineate their individual contributions to the physiological processes. However, recent studies analysing NPY and Y receptors overexpressing and knockout models have

started to unravel some of the different functions of these Y receptors. Particularly, the use of conditional knockout models has made it possible to pinpoint a specific function to an individual Y receptor in a particular location. (**Lin *et al.*, 2004**).

Five distinct NPY receptors have been cloned, Y_1, Y_2, Y_4, Y_5 and y_6. They are widely expressed in brain and their distribution appears to be species specific. Among them, Y_1, Y_2, and Y_5 are abundantly expressed in the hypothalamic area controlling energy homeostasis, and only Y_2 receptor acts as an autoreceptor (**Mashiko *et al.*, 2003**). The anatomical and functional roles of these receptors are summarized in (**Table 3**).

Table 3: The anatomical and functional roles of NPY receptors (**Michel *et al.*, 1998**).

Y_1	**W**idely expressed in the brain and in peripheral blood vessels. It is involved in regulation of appetite, anxiety and blood pressure.
Y_2	**M**ost abundant in the hippocampus. It has a dampening effect on brain activity.
y_3	**N**ot cloned. Its pharmacological profile shows preference for NPY over PYY
Y_4	**S**elective for PP (pancreatic polypeptide) and is primarily expressed in the gastro-intestinal tract where it is presumably involved in regulation of pancreatic secretion and gut motility.
Y_5	**T**he "feeding" or "appetite" receptor expressed in the hypothalamus as well as several other brain regions.
y_6	**T**his receptor is peculiar in that it is non-functional in many species including humans and other primates. It seems to give rise to a functional receptor in mouse and rabbit, but its exact role is completely unknown.

Sequence comparisons show that receptors Y_1, Y_4, and y_6 are more closely related to each other than to the receptors Y_2 and Y_5 (**Fig. 10**). This is apparent not only from sequence identity but also from other features, such as cysteines, believed to form disulfide bonds and the size of the third cytoplasmic loop, which is large in Y_5. The receptors Y_2 and Y_5 are equally distantly related to one another as to the $Y_1/Y_4/y_6$ group. In fact the $Y_1/Y_4/y_6$ group, the Y_2, and the Y_5 are more distantly related to one another than any other GPCRs that bind the same endogenous ligand, despite the fact that Y_1, Y_2, and Y_5 each bind two distinct endogenous ligands, namely NPY and PYY. Two explanations are possible for the great differences between Y_1, Y_2, and Y_5. The first possibility is that they evolve rapidly and, hence, have accumulated all these differences in a short period of time, but this is unlikely because Y_1 is highly conserved between the frog *Xenopus laevis* and mammals. The second possibility is that the three genes arose very long ago and, hence, have had a long time to diverge from one another (**Michel *et al.*, 1998**).

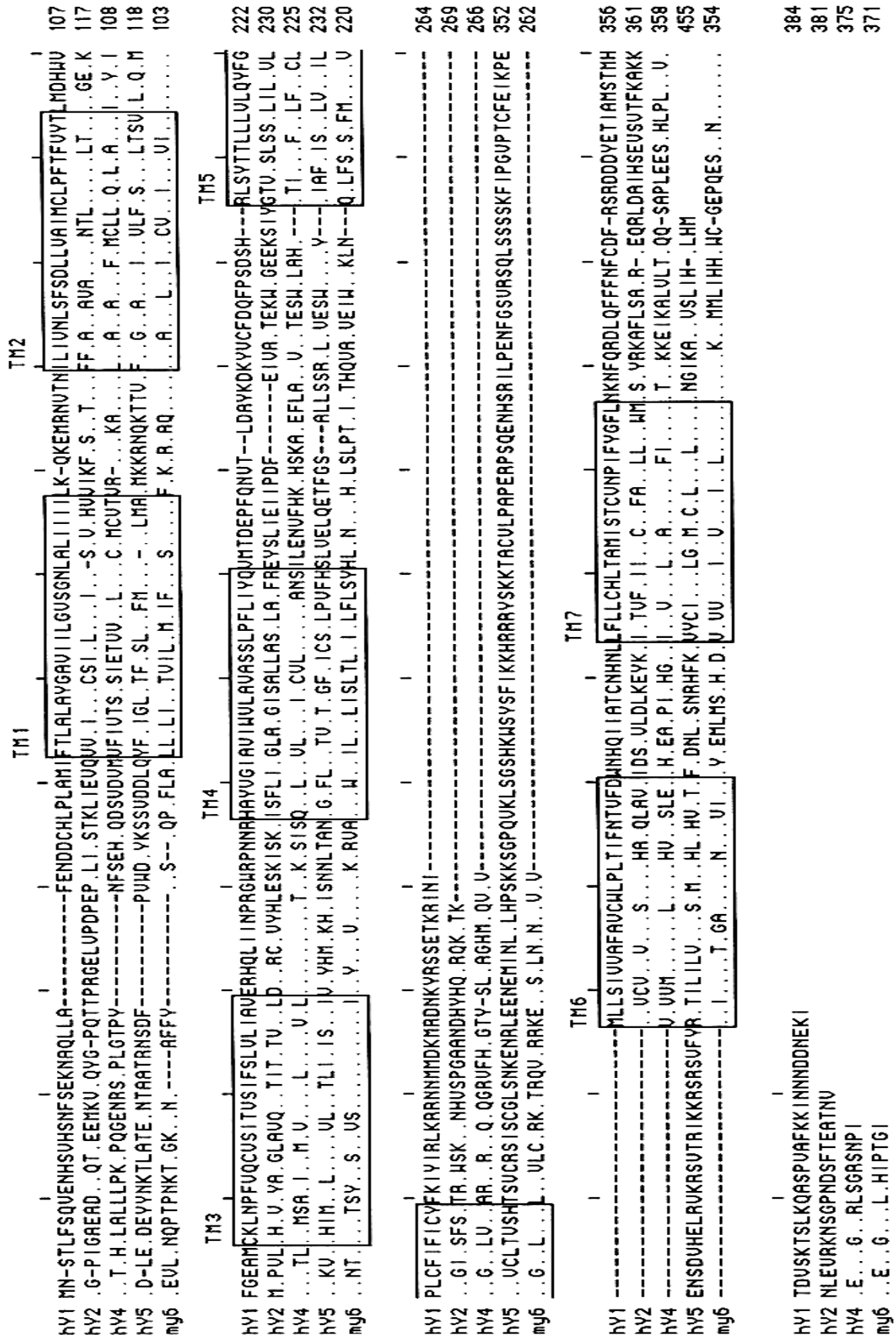


Fig. 10: Alignment of predicted human (h) Y_1, Y_2, Y_4, and Y_5 and mouse (m) y_6 receptor amino acid sequence. The putative transmembrane (TM) domains are indicated by boxes. Amino acid identities are indicated by dots (**Michel** *et al.*, **1998**).

To better characterize NPY receptor subtypes, several novel selective analogues of the NPY sequence have been developed in the last few years. These analogues show high selectivity towards single NPY receptors and facilitate insights into the localization and physiological role of each receptor subtype in health and disease. Besides the important role of NPY in the regulation of food intake and other physiological functions (**Koglin and Beck-Sickinger, 2004**). The synthesis of long-acting, potent NPY receptor selective agonists and antagonists could be used to treat a variety of disease (**Balasubramaniam, 2002**). In particular, Y_1 and Y_2 antagonists are developed for potential clinical use to treat feeding disturbances and anxiety (**Reubi et al., 2001**).

Behavioral effects of NPY and other peptides have been studied using antisense sequences that block translation of peptide or peptide receptor proteins, overexpression of peptides intact animals and generation of knockout mice lacking particular peptides or peptide receptors (**Siegel** *et al.*, **1998**). In the following few paragraphs, individual NPY receptors will be discussed in some detail.

Y_1

In 1990, the cloning of a cDNA from rats was described that was later found to encode a Y_1. Human Y_1 was determined as a 384 amino acid sequence and has seven putative transmembrane domains like other members of the GPCRs (**Larhammar** *et al.*, **1992**). Thereafter; species homologues from mice, humans and *Xenopus laevis* have been identified. Moreover, in humans and mice, the genomic organization of the Y_1 subtype gene has been determined and the gene has been located on human chromosome 4q (31.3-32). Very recently, the cloning of additional cDNAs has been reported from rats and humans that encode the proteins of 456 amino acids, according to one report and 445 amino acids, according to another report. The corresponding gene appears to reside on human chromosome 4q, i.e., the same location as the human Y_1 receptor gene but, apparently, in an opposite orientation. Messenger RNA for that receptor was detected by Northern blotting and in situ hybridization in several rat brain areas, including those believed to be important for the regulation of food intake, as well as in testis. Upon expression in 293 cells, the cloned subtype couples to inhibition of cAMP accumulation. This clone may represent a subtype previously referred to as "Y_1-like" or "food intake" receptor (**Michel** *et al.*, **1998**).

Y_1 mRNA has been detected in a variety of human, rat, and murine tissues including brain, heart, kidney, and gastrointestinal tract. Prototypical responses for the Y_1 subtype include vasoconstriction in most vascular beds, e.g. in the isolated perfused kidney, but vasoconstriction via other NPY receptor

subtypes has also been observed (**Michel** *et al.*, **1998**). The central NPY Y_1 receptor system appears to be involved in a variety of NPY induced pathways (**Pedrazzini, 2004**). It has been implicated in feeding, endocrinal activity and autonomic regulation (**Kishi** *et al.*, **2004**).

Y_2

A cDNA for Y_2 was first cloned from human SMS-KAN cells and subsequently from human brain cDNA libraries and the human neuroblastoma cell line KAN-TX. An order of potency of NPY ≈ PYY ≥ C-terminal fragment >> [Pro^{34}] substituted analog >PP is characteristic for the Y_2. Messenger RNA for the Y_2 has been detected in various parts of the CNS, although apparently low levels of Y_2 mRNA were found in human peripheral tissues. The prototypical response for Y_2 is the presynaptic inhibition of neurotransmitter release, e.g. of noradrenaline release in the prostatic half of the rat vas deferens, but postjunctional Y_2 receptors also exist in a variety of tissues. SMS-KAN cells are a human cell line containing an apparently homogeneous population of Y_2, and the rabbit kidney is also a rich source for this receptor type (**Michel** *et al.*, **1998**).

The predominantly presynaptically expressed Y_2 in the ARC has emerged as a prime candidate for mediating satiety as well as a candidate for regulating bone formation (**Herzog, 2002**). It is thought to act as an autoreceptor that can modulate the expression and secretion of NPY and other neurotransmitters (**Baldock** *et al.*, **2002**). NPY expression and Y_2 are unregulated with elevated activity, whereas expression of the proconvulsant Y_1 receptor decreases (**William** *et al.*, **2003**).

Activation of the Y_1 receptor by NPY results in vasoconstriction. NPY postsynaptic potentiation of the catecholamine effect on blood pressure also presynaptically inhibits the release of catecholamines through Y_2 receptor (**Cavadas** *et al.*, **2001**).

y_3

See **Table 3.**

Y_4

The gene for Y_4 was initially cloned from a human genomic library and the receptor derived from this clone was originally designated "PP_1". Human Y_4 mRNA is mainly expressed in the colon, small intestine, and prostate, although other peripheral tissues appear to lack it and various central nervous system (CNS) regions display low expression levels (**Michel** *et al.*, **1998**).

The Y_4 genomic clone encodes a predicted protein of 375 amino acids that is most homologous to Y_1 from human, rat, and mouse. Y_4 is involved in hypothalamic control of feeding (**Bard** *et al.*, **1995**).

Y_5

Y$_5$ activation suppresses the reproductive axis in both virgin and lactating rats and that Y_5 activation enhances food intake (**Toufexis** *et al.*, **2002**). It is the dominant receptor in antiepileptic actions of NPY (**William** *et al.*, **2003**). Both Y_2 and Y_5 receptors could mediate the NPY seizure suppressant effect (**Nanobashvili** *et al.*, **2004**).

y_6

Intronless gene encodes a 371 amino acid protein and, at the time, was designated Y_5. Homologues have also been cloned from rabbit, monkey, and human libraries and designated either "Y_{2B}" or "PP_2". In agreement with the authors of the respective publications, the use of these designations is no longer encouraged, and, instead, the designation "y_6" is recommended. Fluorescent *in situ* hybridization has localized the y_6 gene to the human chromosome 5 in the 5q31 region. Messenger RNA for this subtype is found in areas of murine brain by *in situ* hybridization but not in mouse tissues as assessed by Northern blotting. Similarly, mRNA for the y_6 was not detected in rabbit tissues by Northern blotting but readily seen using reverse-transcription polymerase chain reaction (RT-PCR) in rabbit brain areas including hypothalamus and hippocampus and in the small intestine and adrenals. In contrast, Northern blotting has detected mRNA for the y_6 in human tissues, including heart and skeletal muscle. The monkey and human sequences differ from those in mice and rabbits by a frame shift mutation located in the putative third intracellular loop of the receptor that result in a stop codon and a predicted truncated protein of only 290 amino acids. A physiological correlate of the cloned y_6 has not been described (**Michel** *et al.*, **1998**).

Chapter IV

NPY play different important physiological role in various organs.

Physiological Functions

The two main functions to which NPY is attributed are:

1. Regulation of NAergic transmission, pre and postsynaptically, especially in the vascular system of various organs, where it has a vasoconstrictor effect.
2. Control of food intake, since it appears to be a powerful stimulator of food and water intake (**Simonneaux and Ribelayga, 2003**).

Recent work suggests that NPY is implicated in a variety of physiological processes. It is a major regulator of food consumption and energy homeostasis (**Lin** *et al.*, **2004**) including feeding behavior, insulin secretion, hepatic glucose output, lipoprotein lipase activity and thermogenesis (**Larsen** *et al.*, **1999**). NPY also exhibits a large number of physiological activities in the central and peripheral nervous systems (**Silva** *et al.*, **2002**). It has anxiolytic and sedative properties within the central nervous system (**Nordmann** *et al.*, **1999**), and a vast range of effects in the immune system. Recent studies have shown that NPY is a potent growth and angiogenic factor, NPY-mediated vascular smooth muscle growth, leading to neointima formation (**Pons** *et al.*, **2004**). Dysregulation in NPY signaling causes alterations in bone formation and alcohol consumption (**Lin** *et al.*, **2004**).

NPY stimulates calcium (Ca^{2+}) channels in the vasculature. In some cell types, NPY induced Ca^{2+} mobilization from intracellular stores; although this appears to involve inositol phosphates in some cells, inositol phosphate-independent Ca^{2+} mobilization has been postulated in other cell types (**Michel** *et al.*, **1998**).

Several observations suggest that NPY is an important sympathetic co-transmitter involved in maintaining vascular tone, enhancing pressor responsiveness to norepinephrine, and sustaining blood pressure during hypovolemic hemorrhage. **Michalkiewicz** *et al.* (**2001**) reported that increasing NPY signaling through genetic manipulation results in **1.** Increased total vascular resistance, **2.** Increased pressor responsiveness to norepinephrine, and **3.** Reduced hemorrhagic hypotension (**Cavadas** *et al.*, **2001**, **Pons** *et al.*, **2004**, and **Pedrazzini, 2004**).

Endocrinal Activity

It has been reported that neuropeptides may play a role in the mechanism of hormone release. NPY inhibits aldosterone secretion from dispersed zona glomerulosa (ZG) cells, but this effect has probably to be considered non-specific and toxic in nature, since it is obtained only using micromolar concentrations of the peptide. In contrast, NPY appears to modulate the secretory response of dispersed rat ZG cells to their main agonists (adrenocorticotropic hormone; ACTH, angiotensin-II and potassium). However, there is indication that the main effect of NPY on the ZG in rats is indirect and involves the local release of catecholamines, which in turn, acting via beta-adrenoceptors, enhance the secretion of aldosterone. The prolonged treatment with NPY is also able to enhance the growth of the rat ZG. In contrast, the effects of NPY on glucocorticoid secretion from zona fasciculata-reticularis cells are negligible and doubtful (**Spinazzi** *et al.*, **2005**).

It was found that NPY potentiates the action of some vasoconstrictors, such as norepinephrine, angiotensin, and vasopressin (**Michalkiewicz** *et al.*, **2001**). NPY neurons are inhibited by both leptin and insulin, and thus stimulated in states of energy defect and fat loss (**Williams** *et al.*, **2004**). Indirect evidence is available that endogenous NPY system may play an important role in the modulation of adrenal functions under paraphysiological conditions (**Spinazzi** *et al.*, **2005**). Catecholamine synthesis and release are two separate but related processes that influence the level of catecholaminergic neurotransmission (**McCullough** *et al.*, **1998** and **Cavadas** *et al.*, **2001**). NPY is co-synthesized and co-released with catecholamines in the adrenal medulla (**Erdem** *et al.*, **2002**). It acts mainly via Y_1, Y_2 and Y_3, either to inhibit catecholamine secretion from bovine adrenal chromaffin cells, or to stimulate catecholamine secretion from adrenomedullary cells of humans and rats (**Spinazzi** *et al.*, **2005**).

It has been demonstrated that NPY inhibits catecholamine synthesis via the y_3 receptor subtype, in contrast to the Y_2 subtype that mediates inhibition of catecholamine release. Although inhibition of both synthesis and release by these receptor subtypes is via inhibition of Ca^{2+} entry, the two processes are associated with actions on distinct Ca^{2+} channel subtypes. Inhibition of L-type Ca^{2+} channels mediates inhibition of catecholamine synthesis, while inhibition of N-type Ca^{2+} channels inhibits catecholamine release (**McCullough** *et al.*, **1998** and **Cavadas** *et al.*, **2001**) **(Fig. 11**). On the other hand, NPY originating from the chromaffin cells of the adrenal medulla locally enhances the secretion of catecholamines, presumably by acting via the putative y_3 receptor (**Cavadas** *et al.*, **2001**). It was found that the stimulatory effect of NPY on adrenomedullary catecholamines biosynthetic pathway is blunted with age (**Erdem** *et al.*, **2002**).



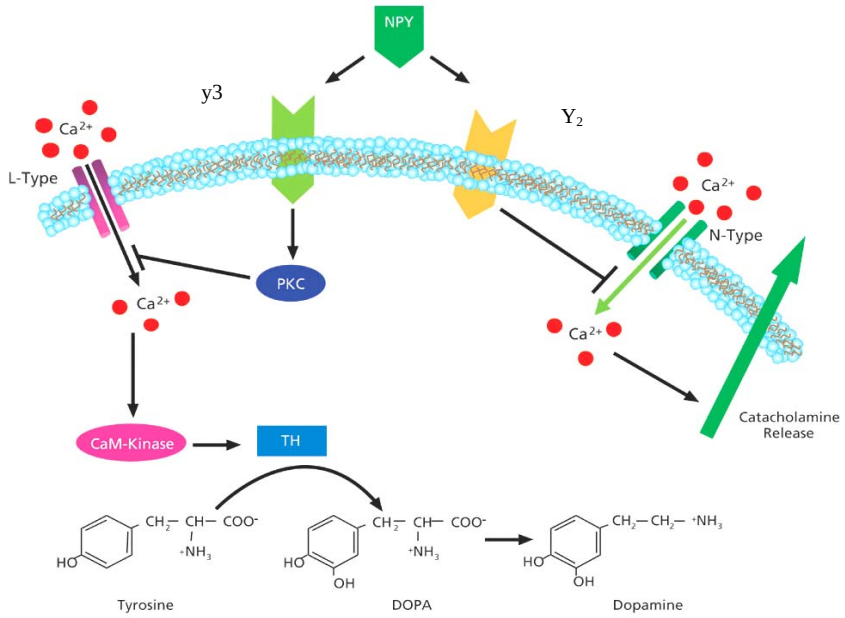


Fig. 11: NPY inhibition of catecholamine release (**McCullough** *et al.*, **1998** and **Cavadas** *et al.*, **2001**).

Food Behavior

It has been reported that neuropeptides may play a role in the control of appetite and in the mechanism of hormone release (**Fig. 12**). Neuropeptides such as beta-endorphin, NPY, galanin and leptin may affect hormones release; on the other hand the hormonal status may modulate neuropeptide activity (**Baranowska** *et al.*, **2003**). Also, it has been found that orexigenic neuropeptides, like NPY and agouti-related peptide (AgRP), and anorexigenic neuropeptides, like proopiomelanocortin (POMC) and alpha-melanocyte-stimulating hormone (α-MSH), are decisively involved in body weight regulation (**Franke** *et al.*, **2005**).

NPY is a powerful stimulant of food intake (**Ishiguchi** *et al.*, **2001**). In mammals, the regulation of appetite and food intake have been demonstrated to be an extremely complex process involving both orexigenic and anorexigenic neuronal systems that are morphologically and functionally connected in the hypothalamus. Among this network, NPY is the most potent orexigenic signal molecule (**Narnaware** *et al.*, **2000**). Available anatomical evidence suggests that NPY neurons in the ARC send projections to many hypothalamic areas, including the PVN and the medial preoptic area. The ARC-PVN NPY projection has been shown to represent a network which plays an important role as a conductor of the hypothalamic appetite regulating orchestra (**Li** *et al.*, **1998** and **Kalra& Kalra, 2004**).

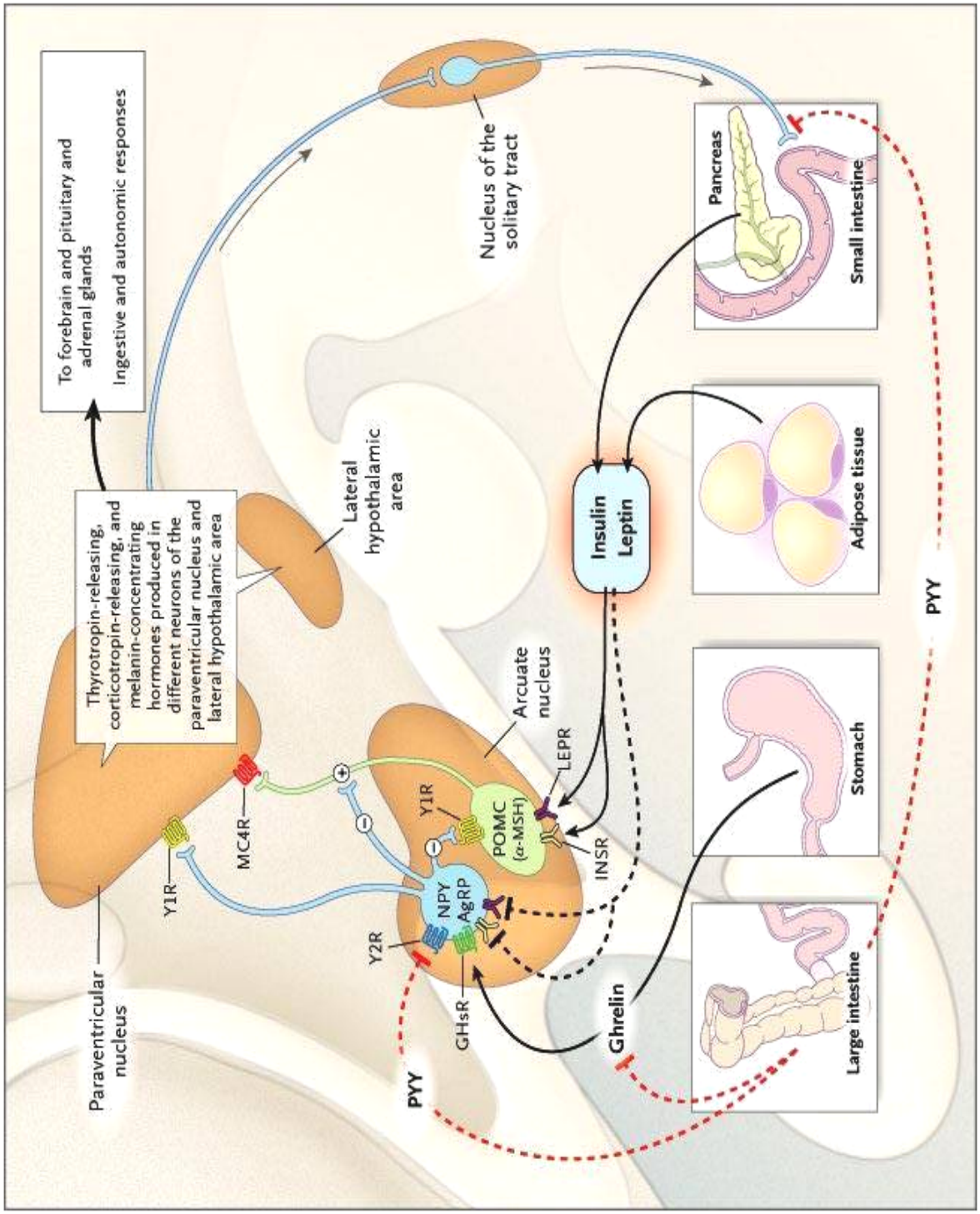


Fig. 12: Interactions between hormonal and neural pathways that regulate food intake and body-fat mass.

In this schematic diagram of the brain, *the dashed lines* indicate hormonal inhibitory effects, and *the solid lines* stimulatory effects. The paraventricular and arcuate nuclei each contain neurons that are capable of stimulating or inhibiting food intake. Y_1R and Y_2R denote the Y_1 and Y_2 subtypes of the NPY receptor, MC_4R melanocortin 4 receptor, PYY peptide $YY_{3–36}$, GHsR growth hormone secretagogue receptor, AgRP agouti-related protein, POMC proopiomelanocortin, ⍺-MSH ⍺-melanocyte–stimulating hormone, LEPR leptin receptor, and INSR insulin receptor (**Korner and Leibel, 2003**).

Central injection of NPY either in the cerebroventricular system or directly into various hypothalamic nuclei stimulates feeding behavior, food intake, and body weight gain (**Narnaware** *et al.*, **2000**). Stimulation of feeding behavior mediated by the peripheral system includes vasoconstriction, effects on the gastrointestinal motility and secretion, insulin release and secretion (**Reubi** *et al.*, **2001**).

The effects of NPY in stimulating food intake are mediated by NPY Y_1 and Y_5 (**Narnaware** *et al.*, **2000**). These two receptors induced hyperphagia after chronic central administration (**Raposinho** *et al.*, **2004**). Most Y_1 antagonists have been shown to inhibit food intake and treatment with Y_5 antisense oligoribonucleotides induces long lasting anorexia (**Larsen** *et al.*, **1999**). Another potentially related action of NPY is to delay gastric emptying, an action mediated by Y_2 receptors (**Cupples, 2003**). Both Y_1 and Y_2 are found in large quantities in the dorsal vagal complex (DVC) of the medulla. They are found pre- and postjunctionally in the nervous system. **Chen** *et al.* **(1997)** have shown that an Y_2 agonist applied to the DVC suppressed gastric motility in thyrotropin-releasing hormone (TRH) stimulated conditions. In contrast, a Y agonist had no effect on TRH stimulated gastric motility**,** although Y_1 absence impairs the perception of decreasing energy stores by the gonadotrope axis (**Gonzales** *et al.*, **2004**).

Food deprivation or diet restriction induces an increase in NPY concentration in hypothalamus and NPY mRNA gene expression in various brain nuclei, and refeeding normalizes both NPY concentrations and NPY mRNA levels (**Narnaware** *et al.*, **2000**).

Role of Leptin in Food Behavior

Leptin, a hormone produced in adipocytes, it is the *ob* gene product that circulates as a monomeric 16 kDa protein in rodent and human plasma (**Fig. 13**) and is also bound to leptin binding proteins that may form large high molecular weight complexes (**Kiess** *et al.*, **2000**). It acts as a feedback signal to the hypothalamus, playing a fundamental role in maintaining energy homeostasis. It inhibits eating, increases metabolism, and stimulates the reproductive axis. NPY serves as one of the downstream mediators of leptin's actions. These NPY neurons coexpress leptin receptor mRNA, and NPY gene expression is downregulated by leptin administration (**Bi** *et al.*, **2003**).

NPY is the most potent orexigenic agent known to man (**Juanenea** *et al.*, **2004**). In contrast, leptin provides an anorexigenic signal to centrally control the body needs in energy (**Pralong** *et al.*, **2002**) *i.e.* NPY stimulates and leptin inhibits food intake (**Ammar** *et al*, **2000**). NPY is released during sympathetic

stimulation and mediates the central effects of the adipostatic hormone leptin (**Zoccali** *et al.*, **2003**), which decreases food intake through actions in the hypothalamus (**Thorsell** *et al.*, **2002**). Under some conditions, leptin seems to inhibit NPY mRNA expression. It may be speculated that the leptin-NPY neuroendocrine system serves the purpose of directing attention to food acquisition when energy stores are depleted (low leptin, high NPY) and to other activities when energy levels are high (high leptin, low NPY (**Gerald and DiBona, 2002**).

As previously mentioned, NPY synthesis is particularly high in neurons of the ARC, with many of these neurons also expressing leptin receptors. Both Y_2 and leptin receptors are found on NPY expressing neurons in the ARC. Deficiency of either the leptin receptor or the Y_2 receptor leads to increased NPY expression (**Baldock** *et al.*, **2002**).

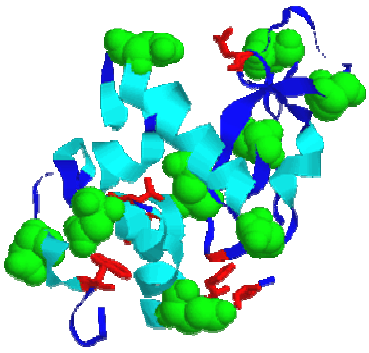


Fig. 13: 3D structure of leptin
http://www.dq.fct.unl.pta

Role of Insulin in Food Behavior

Insulin is known to be an important hormonal factor (**Fig. 14**) influencing lipogenesis by increasing uptake of glucose and activating lipogenic and glycolytic enzymes. Chronic activation of the Y_5 receptor led to an increase in the 2-h fasting plasma insulin level even in the pair-fed group. Previous studies have shown that acute central injection of NPY produces a transient increase in the plasma insulin level, stimulates hyperphagia indicating that insulin release might also be involved in NPY-mediated obesity (**Mashiko** *et al.*, **2003**).

Selective activation of central NPY Y_1 receptor subtype induced hyperinsulinemia independent of food ingestion, whereas the NPY Y_5 receptor-induced hyperinsulinemia was dependent on food ingestion (**Gao** *et al.*, **2004**). It is worth noting that the deletion of the Y_1 receptor in mice leads to hyperinsulinemia, and it has been proposed that NPY acts through Y_1 to inhibit insulin release (**Ishiguchi** *et al.*, **2001**).

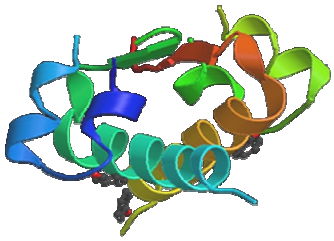


Fig. 14: 3D structure of insulin

www.bio.davidson.edu

Puberty in Females

Puberty results from withdrawal of the "gonadostat" mechanisms and from increased gonadotropin sensitivity to gonadotropin-releasing hormone (GnRH). It has been hypothesized that GnRH release may be modulated by a non-steroid-mediated mechanism. The onset of puberty derives from the complex interplay among neuropeptides, neurotransmitters, and neurosteroids that occurs in the awakening of hypothalamic-pituitary-ovarian axis (**Genazzani** *et al.*, **2000**). The adult reproductive axis is driven by an intermittent discharge of GnRH generated by a network of hypothalamic neurons known as the GnRH pulse generator. NPY is a fundamental component of the neurobiological brake restraining the onset of puberty (**El Majdoubi** *et al.*, **2000**). In particular, an increase in NPY expression participates in the inhibition of the reproductive activity under poor nutritional conditions (**Gonzales** *et al.*, **2004**).

Increased NPY activity has an inhibitory effect on the gonadotropin axis and represents a direct mechanism for inhibiting sexual maturation and reproductive function in conditions of food restriction and/or energy expenditure. By modulating the hypothalamo-pituitary-gonadal axis both directly and indirectly, leptin may thus serve as the signal from fat to the brain about the adequacy of fat stores for pubertal development and reproduction. Normal leptin secretion is necessary for normal reproductive function to proceed and leptin may be a signal "metabolic cue" allowing for the point of initiation of and progression toward puberty (**Kiess** *et al.*, **2000**) through the activation of the GnRH neuronal system at the end of the prepubertal period. On the other side there is a possibility of altered leptin pulsatility during prepubertal period that can consequently influence hypothalamus and GnRH neuronal system (**Macut and Micic, 1998**).

More than two decades of clinical studies have provided us with the opportunity to develop and implement criteria that distinguish three phases during pre-menarche, paralleling rising levels of estrogens, namely

"pre-estrogenization", "onset of estrogenization" and "full estrogenization". The relationships between somatic features and levels of leptin, NPY, β-endorphin, gonadotropin and estradiol in pubertal girls before menarche were studied by **Blogowska** *et al.* (**2003**). They found that the onset of estrogenization in girls was marked by weight loss, followed by an increase in total fat and lean body mass, and pubertal acceleration of growth. At the time of full estrogenization, lower NPY and β-endorphin levels were observed, accompanied by an increase in gonadotropin secretion.

Constitutional delay of puberty (CDP), in healthy girls is defined as failure to develop secondary sexual features past the age of 13 years. The inhibitory action of NPY on the gonadotropic and somatotropic systems in experimental animals and stimulation by NPY of the hypothalamic-pituitary-adrenal axis has been reported normal course of puberty (NP). NPY was higher in girls with CDP than in girls with NP. The results corroborate the involvement of NPY in sexual maturation and its role in delayed puberty (**Koglin and Beck-Sickinger, 2004**).

Lactation

Lactation in the rat is accompanied by changes in hormone secretion and behavior that participate in adaptation to the lactating condition. The hypothalamus appears to be the site where the majority of these changes are regulated by incoming neural signals induced by the suckling stimulus. It has been shown by several laboratories that the levels of NPY are significantly elevated in a number of hypothalamic areas in chronic lactating animals; this is further confirmed by the increase in NPY mRNA levels in the hypothalamus. The diverse functions of NPY make the peptide a candidate for modulating many of the alterations in hypothalamic function during lactation (**Li** *et al*, **1998**).

During lactation, there is significant energy demand due to milk production; this demand is met primarily by increased food intake. The central role of NPY is inducing the feeding response makes it a possible candidate in mediating the hyperphagia during lactation. In fact, elevated NPY concentrations in PVN, dorsomdial hypothalamic nucleus (DMH), and ARC as well as elevated NPY mRNA levels in the ARC and DMH have been reported in the chronic lactating rat. **Li** *et al.* (**1998**) demonstrated that NPY neurons are activated by the acute suckling stimulus. Taken together, the sustained activation of NPY neurons by the suckling stimulus throughout the course of lactation is likely to play an important role in mediating changes in hypothalamic regulation of hormone accompanied by an increase in food intake to meet the energy demands resulting from milk production.

Alcohol Consumption

In recent years, evidence has emerged suggesting that NPY is involved with neurobiological responses to ethanol and other drugs of abuse. An overview of physiological, pharmacological, and genetic research shows that **1.** Administration of ethanol, as well as ethanol withdrawal, alters central NPY expression, **2.** NPY modulates ethanol consumption under certain conditions, and **3.** NPY signaling modulates the sedative effects of several drugs, including ethanol, sodium pentobarbital, and ketamine. NPY may influence ethanol consumption by regulating basal levels of anxiety, by modulating the sedative effects of ethanol, and/or by modulating ethanol's rewarding properties (**Thiele** *et al.*, **2004**).

Bone Formation

Studies revealed that distinct populations of hypothalamic neurons are involved in the control of bone mass and demonstrated that monosodium glutamate (MSG) sensitive neurons control bone formation in a leptin-independent manner (**Elefteriou** *et al.*, **2003**). Leptin can inhibit bone synthesis by action within the hypothalamus independently of increasing body fat or body weight. Central infusion of NPY for 28 days in wild-type mice had the same inhibitory effect on bone function as leptin had, suggesting that the increased hypothalamic NPY expression of leptin-deficient mice does not mediate the associated increase in bone density (**Baldock** *et al.*, **2002**). Although NPY deficiency does not affect bone mass (**Elefteriou** *et al.*, **2003**), intracerebroventricular (ICV) administration of NPY or leptin also causes bone loss (**Baldock** *et al.*, **2002**).

The potential for simultaneous activation of the different types of hypothalamic Y receptors by central NPY infusion makes it difficult to distinguish the true role of these receptors in bone physiology (**Baldock** *et al.*, **2002**). The predominantly presynaptically expressed Y_2 receptor in the ARC of the hypothalamus has emerged as a prime candidate for mediating satiety as well as a candidate for regulating bone formation (**Herzog, 2002**). **Baldock** *et al.* (**2002**) found that the hypothalamic Y_2 receptors are crucial for bone formation; they are involved in a tonic inhibition of this process, since selective deletion of hypothalamic Y_2 receptors in mature conditional Y_2 knockout mice results in an identical increase in trabecular bone volume within 5 weeks. This hypothalamus-specific Y_2 receptor deletion stimulates the osteoblastic activity and increases the rate of bone mineralization and formation, with no effect on osteoblast or osteoclast surface measurements. The lack of any changes in plasma total calcium, leptinemia, or hypothalamo-pituitary-corticotropic, -thyrotropic, -somatotropic, or -gonadotropic output suggests that Y_2 receptors

do not modulate bone formation by humoral mechanisms, and that alteration of autonomic function through hypothalamic Y_2 receptors may play a key role in a major central regulatory circuit of bone formation. The absence of detectable levels of Y receptor mRNAs in bone tissue is further evidence that this effect of Y_2 deficiency occurs by a central mechanism.

Both Y_2 and leptin receptors are found on NPY expressing neurons in the ARC. Deficiency of either the leptin receptor or the Y_2 receptor leads to increased NPY expression in the ARC. It is not clear whether the high hypothalamic NPY expression common to mice deficient in leptin action or Y_2 receptors is causally related to the high bone density, because 28-day intracerebroventricular NPY infusion actually decreased bone density (though it probably also resulted in hyperleptinemia). Y_2 receptor signaling specifically is important in regulating bone mass, while hypothalamus specific Y_2 receptor deletion did not induce any obvious endocrine imbalances that would have impacts on bone homeostasis. In conclusion, Y_2 receptor deficiency does not influence bone formation via modulation of humoral factors (**Baldock** *et al.*, **2002**).

NPY Homologue, NPF

In invertebrates, neuropeptide F (NPF) peptides share structural similarity with the vertebrate NPY and are considered to be its homologues. From a completely sequenced *Drosophila melanogaster* genome, short NPF (sNPF) and Drosophila NPF (dNPF) were found. Both neuropeptides are 36 amino acids long with a carboxyl terminus Arg-X-Arg-Phe-amide, and Tyr residue at positions 10 and 17 from the carboxyl terminus (X is Val in case of dNPF, Leu in case of sNPF and Gln in case of NPY with the replacement of Phe by Tyr) (**Fig. 15**) (**Lee *et al.*, 2004**).

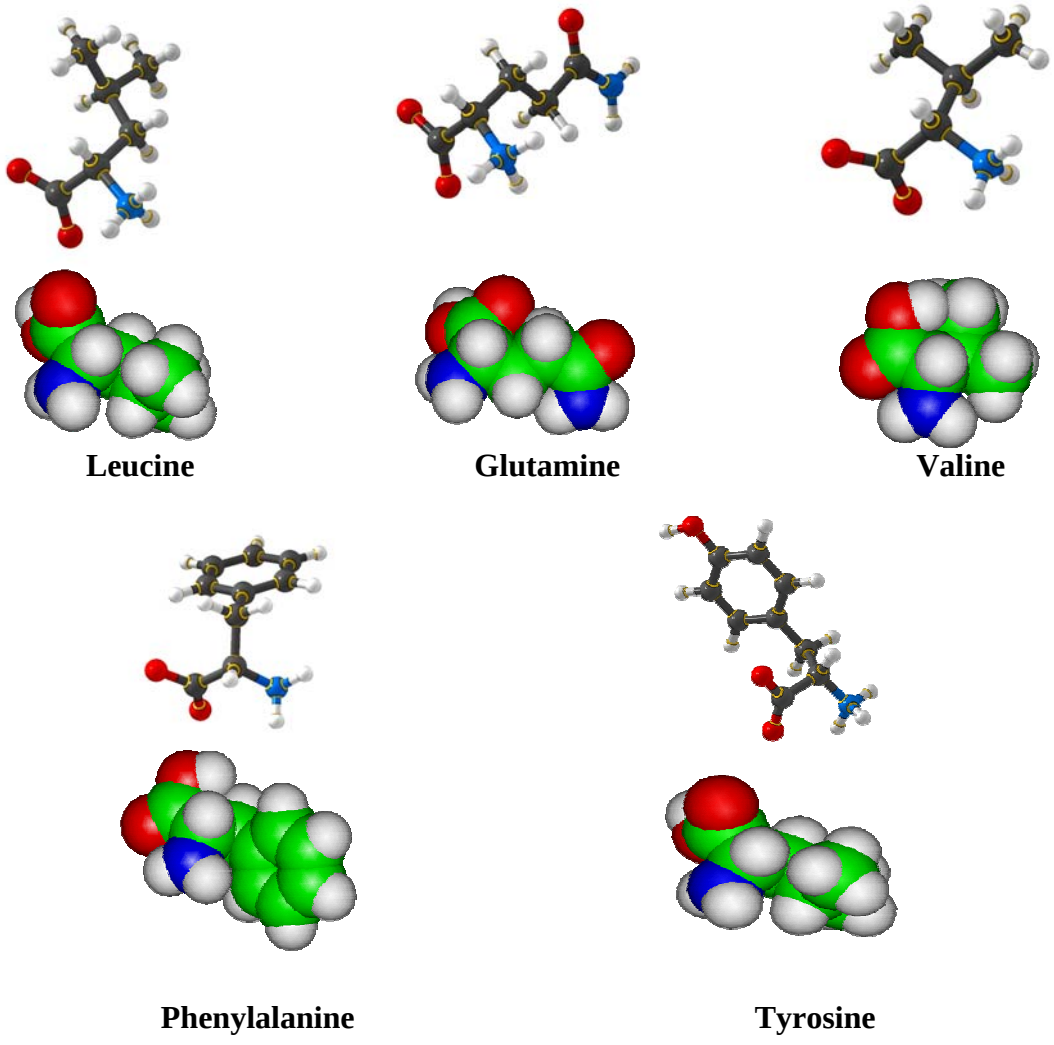


Fig. 15: Chemical structure of some amino acids
(Note: Carbon is in green or grey, Oxygen is in red, Hydrogen is in white and Nitrogen is in blue.)

dNPF

dNPF was expressed in the brain and midgut of *Drosophila* larvae and adults. The DmNPFR, the receptor for dNPF, was cloned and showed the homology with the vertebrate NPY receptor family; it is expressed in larval CNS and midgut (**Lee** *et al.*, **2004**).

It was found that dNPF neural network in the larval CNS is changed by the gustatory stimulation of sugar, indicating that dNPF is an integral part of the chemosensory system that regulates eating behavior (**Lee** *et al.*, **2004**). Also, it was found that dNPF overexpression prolonged feeding, and suppressed hypermobility and cooperative burrowing behaviors (**Wu** *et al.*, **2003**).

sNPF

sNPF is expressed in the nervous system of late stage embryos and larvae. In the adult brain, the sNPF peptide was localized in the medulla and the mushroom body, and it was not detected in the gut (**Lee** *et al.*, **2004**).

NPFR76F, the receptor for sNPF, was also cloned and showed the similarity with NPY Y_2. It is expressed in the CNS of embryos and larvae (**Lee** *et al.*, **2004**).

From the feeding assay, the gain-of-function sNPF mutants expressed in nervous systems promoted food intake, whereas the loss-of-function mutants suppressed food intake. Also, sNPF overexpression in nervous systems seemed to increase appetite and produced bigger and heavier flies. These findings indicate that the sNPF is expressed in the nervous systems to control food intake and regulate body size in *Drosophila melanogaster*. Like other neuropeptides, the sNPF peptide may be involved in regulating various physiological processes other than regulating food intake. Because the sNPF peptide and transcript were expressed during all developmental stages; it is involved in learning and memory (**Lee** *et al.*, **2004**). In locusts, it accelerates ovarian maturation, and in mosquitoes, it inhibits host-seeking behavior (**Mertens** *et al.*, **2002**). These unknown multi-functions of the sNPF peptide in various biological processes are the subjects of future studies (**Lee** *et al.*, **2004**).

Structural similarity of NPF with porcine NPY indicates that its mode of action is similar. These structural data can serve as a starting point in the design of new antiparasitic drugs (**Miskolzie and Kotovych, 2002**).

Chapter V

NPY is correlated to different diseases diagnostically and therapeutically. Several NPY analogues are being developed for potential clinical therapeutic strategy.

Clinical Relevance of NPY

Evidence suggests that NPY is involved in the pathophysiology of several disorders, such as depression, anxiety, epilepsy, seizures, sleep, learning, memory, circadian rhythm, drug addiction, pain, cardiovascular diseases, rhinitis, endothelial cell dysfunctions (**Silva** *et al.*, **2002** and **Sajdyk** *et al.*, **2004**) diabetes, hypertension, pheochromocytoma **(Spinazzi** *et al.*, **2005)**, congestive heart failure, gastrointestinal regulation, intestinal disorders (**Balasubramaniam, 2002**) intestinal electrolyte secretion, urinary sodium excretion (**Rose** *et al.*, **1995**) and regulation of excitability in extreme physiological circumstances (**William** *et al.*, **2003** and **Michalkiewicz** *et al.*, **2001**). It also has a potential role in affective disorders, bone formation and cravings (**Nara-ashizawa** *et al.*, **2001**). Some of the disorders in which NPY is implicated will be discussed in this chapter.

Obesity

The control of feeding and metabolism in mammals is an exquisitely controlled physiological process (**Hohmann** *et al.*, **2004**). Feeding behavior and food intake are important determinants of balance between energy intake and energy expenditure in the regulation of body weight (**Gerald and DiBona, 2002**).

Obesity, defined as increased body fat content, is a growing problem in Western societies, and it is estimated that more than 30% of the population of the United States is obese, as defined by a body mass index exceeding 27 kg/m^2 (**Larsen** *et al.*, **1999**). Chronic administration of NPY produces continuous hyperphagia and remarkable body weight gain. In addition to its potent effect on feeding, NPY also has some anabolic effect on carbohydrate metabolism, brown fat thermogenesis, and white adipose tissue lipid storage. Furthermore, central NPY may induce peripheral endocrine and metabolic alterations via efferent routes, as well as hyperphagia. These finding indicate that NPY-mediated obesity is caused by both an increase in energy intake and a decrease in energy expenditure (**Mashiko** *et al.*, **2003**). The association of increased body mass index with hypertension, ischemic heart disease, hyperlipidemia, and most certainly non-insulin-dependent diabetes makes pharmacological agents counteracting severe obesity high in demand, and it has been speculated that NPY antagonists could subserve such a role (**Larsen** *et al.*, **1999**). Repeated administration of NPY leads to obesity, perhaps by interaction with other regulatory molecules, such as leptin and insulin (**Hohmann** *et al.*, **2004**).



It is well known that circulating leptin levels constitute a reliable reflection of body adipose tissue mass, with increased concentrations during weight gain and decreased levels during weight loss, but the constantly elevated plasma levels render obese subjects less sensitive to leptin and make leptin a poor candidate as a pharmacological tool to reduce body weight, i.e. the utility of leptin-mimics for treatment of obesity might be limited (**Thorsell** *et al.*, **2002**).

Antagonizing the robust stimulation of food intake by NPY represents a new potential therapeutic approach for the treatment of obesity (**Blum** *et al.*, **2004**). Counteracting the central actions of NPY on feeding and energy metabolism will not only reduce food intake, but will also lead to lowered fat deposition and increased resting metabolic rate, ultimately lowering the tendency toward hyperinsulinemia, insulin resistance, glucose intolerance, dyslipidemia, and hypertension that otherwise prevails in obese patients (**Larsen** *et al.*, **1999**).

NPY-mediated obesity might be regulated by multiple subtypes of NPY receptors, as in the case of feeding regulation**.** Several lines of evidence show that both Y_1 and Y_5 receptor subtypes are involved in NPY-mediated obesity (**Mashiko** *et al.*, **2003**). The overall phenotype of Y_1 mimics metabolic syndrome, which is characterized by obesity, a prediabetic state, and a susceptibility to develop hypertension (**Pedrazzini, 2004**). Y_5 mediated obesity may involve metabolic changes, such as decreased lipolysis and thermogenesis, as well as hyperphagia. Y_5 activation produces obesity due to increases in both energy intake and energy storage. Therefore, the Y_5 receptor can play a key role in regulating energy homeostasis, and may also play a key role in NPY-mediated obesity (**Mashiko** *et al.*, **2003**).

Diabetes Mellitus

Diabetes Mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized, producing hyperglycemia (**Nathan, 1993** and **Sacks, 1999**). The hyperglycemia is the consequence of a relative or absolute deficiency of insulin resulting in disturbances of carbohydrate, protein and lipid metabolism (**Abbas** *et al.*, **1994** and **Sacks, 1996**).

Diabetic vascular complications are a combination of specific abnormalities and an acceleration of the vasculopathies also found in the non-diabetic population. At the cellular level, the principal factors that initiate and promote the progression of diabetic vascular disease are hyperglycaemia, insulin

resistance, dyslipidaemia, and various cytokines and vasoactive hormones (**Fenner and King, 1997**). Because of the potent ability of NPY to stimulate food intake, it is suspected to play a role in obesity and diabetes (**Gerald** *et al.*, **1996**). NPY is a potent vasoconstrictive peptide and has a mitogenic effect on blood vessels. It is an angiogenic factor at concentration below those required for vasoconstriction. It seems that the primary function of the peptide is regulation of endothelial function (**Zukowska-Grojec, 1997**).

As previously mentioned, NPY is a main brain neurotransmitter which expressed in neurons of the hypothalamic ARC that project mainly to PVN an important site of NPY release. The ARC-PVN projection appears to be active in insulin-deficient diabetic rats, and could contribute to the hyperphagia and polydipsia, the early and possibly the primary abnormalities of the diabetic syndrome (**Williams** *et al.*, **1998**).

Chua *et al.* (**1991**) identified NPY as a gene whose expression in hypothalamus together with enkephalin gene may be two important determinants of the expression of the diabetes mutation. The effect of uncontrolled diabetes to decrease proopiomelanocortin (POMC) hypothalamic gene expression (reduced food intake) while increasing hypothalamic agouti-related protein (AgRP) gene expression (that increase food intake), suggests that reduced hypothalamic melanocortin signaling, along with increased NPY and decreased corticotrophin releasing hormone signaling, could contribute to diabetic hyperphagia (**Havel** *et al.*, **2000**).

Serum NPY concentration was significantly elevated in diabetic patients, which may be due to NPY overflow via sympathetic activation and/ or clearance secondary to sympathetic activity and to hemodynamic changes. The increase in circulating NPY level also could be explained by over activity of NPY neurons due to reduction of insulin hormone inhibitory effect (**Frankish** *et al.*, **1995**) or hyperglycaemia that stimulates hypothalamic NPY release (**Hounsom** *et al.*, **2001** and **Ishiguchi** *et al.*, **2001**). **Malabu** *et al.* (**1994**) stated that the effect of diabetes to increase hypothalamic NPY expression was normalized by insulin treatment.

Anorexia Nervosa

Anorexia and Bulimia Nervosa are disorders of unknown etiology that invariably begin during adolescence and near in time to puberty in young women. These disorders are associated with aberrant eating behaviors, body image distortions, impulse and mood disturbances, as well as characteristic temperament and personality traits. It is well known that malnutrition produces

changes in neuroendocrine function. More recently, disturbances in neuronal systems have been found to play a role in the modulation of feeding, mood and impulse control. These neuronal systems include neuropeptides [corticotropin releasing hormone (CRH), opioids, NPY and PYY, vasopressin and oxytocin, cholecystokinin (CCK), and leptin] and monoamines [serotonin, dopamine, and norepinephrine]. Disturbances of most of these neuronal systems have been found when people are ill with an eating disorder, but it was not certain whether they were a cause or consequence of symptoms (**Barbarich** *et al.*, **2003**).

Impaired appetite and weight loss are common in patients with depression and are prevalent in modern societies. **Kishi and Elmquist** (**2005**), concentrate on the established brain machinery of food intake and body weight, especially on the melanocortin and NPY systems. These systems play a critical role in receiving and processing critical peripheral metabolic cues such as leptin and ghrelin. It is also notable that both systems modulate emotion and motivated behavior as well. The neuroendocrine disturbances in obesity and in anorexia nervosa are opposite. The feedback mechanism between leptin and NPY is disturbed in both in obesity and in anorexia nervosa (**Baranowska** *et al.*, **2003**). Under normal conditions, food intake is controlled in the hypothalamus by: **1.** transducing metabolic/sensorial inputs arising from the periphery into neuronal response; **2.** integrating the information originating from different tissues; and **3.** triggering the appropriate feeding responses. Thus, the anorexia associated with a number of chronic diseases, including cancer, may result from an abnormal input of information to the hypothalamus, or in its defective transduction and integration, or in the induction of exaggerated and inappropriate feeding responses. Currently available data suggest that the pathogenesis of secondary anorexia is multifactorial, and involves most of the neuronal signaling pathways modulating energy intake, including hormones (e.g. leptin), neuropeptides (e.g. NPY), cytokines (e.g. Interleukins [IL-1, IL-6], Tumor necrosis factor [TNF]) and neurotransmitters (e.g. serotonin and dopamine) (**Fanelli and Laviano, 2002**).

Chronic Renal Failure

NPY does not participate in the regulation of kidney function and arterial pressure in normal conscious or anesthetized rats. When sympathetic nervous system activity is increased, as in heart failure and intense renal sympathetic nerve stimulation, respectively, a small contribution of NPY to maintenance of arterial pressure and to sympathetic renal vasoconstrictor responses may be identified (**DiBona and Sawin, 2001**).

Glomerular filtration rate is altered only little if at all by NPY, indicating greater affect on the vas efferens than the vas afferens. NPY also has a

vasoconstrictor action on rennin secretion and stimulatory action on Na^+ K^+ ATPase in the renal tubular cells. Despite the profound reduction of renal blood flow, systemic NPY infusion can cause diuresis and natriuresis indicating that renovascular, but not tubular, NPY receptors may be tonically activated by endogenous NPY (**Bischoff and Michel, 1998**). A changed vasomotor reactivity of renal arteries may lead to defect autoregulation of renal hemodynamic with damage of diabetic kidneys. The urinary excretion rate of albumin in the diabetics rats related to the largest noradrenaline induced contraction of renal but not intrarenal arteries. NPY decreased the contractile response of noradrenaline. The high blood glucose slightly increased this effect of NPY (**Torffvit** *et al.*, **1997**).

It has been revealed that the plasma concentration of NPY and leptin is increased in patients with end stage renal disease (ESRD) (**Zoccali** *et al.*, **2003**), while loss of renal function may diminish the excretion of NPY, inducing anorexia in chronic renal failure (**Li** *et al.*, **2003**).

Cancer

Besides the important role of NPY in the regulation of food intake and other physiological functions, recently NPY receptors have been identified to play an important role in several types of cancer (**Koglin and Beck-Sickinger, 2004**) and in tumor biology, based on the high density of NPY receptors in breast, ovarian and prostate cancers (**Korner** *et al.*, **2004a, b** and **Magni& Motta, 2001**). Peptide receptors can be used successfully for *in vivo* targeting of human cancers. The molecular basis for targeting rests on the *in vitro* observation is that peptide receptors can be expressed in large quantities in certain tumors. The clinical impact is at the diagnostic level: *in vivo* receptor scintigraphy uses radiolabeled peptides for the localization of tumors and their metastases (**Korner** *et al.*, **2004**). It is also at the therapeutic level: peptide receptor radiotherapy of tumors emerges as a serious treatment option. Peptides linked to cytotoxic agents are also considered for therapeutic applications. The use of nonradiolabeled, noncytotoxic peptide analogus for long-term antiproliferative treatment of tumors appears promising for only a few tumor types, whereas the symptomatic treatment of neuroendocrine tumors by somatostatin analogs is clearly successful (**Reubi, 2003**).

The high frequency of NPY receptors in steroid hormone-producing ovarian sex cord-stromal tumors, together with the known influence of NPY on steroid hormone and catecholamine secretion in the rodent adrenal gland, led to the investigation of NPY receptor expression in the human adrenal gland and related tumors. NPY receptors are described in human ovarian tissue, and may

play a role in the pathogenesis and also in the pathophysiology of ovarian malignancies (**Korner** *et al.*, **2004a, b**).

Neuroendocrine molecules are now believed to play a significant role in the progression of human prostate cancer (CaP). **Magni and Motta** (**2001**) evaluated the presence and the function of the receptors for NPY in human CaP cell line, and their activation results in cell proliferation, suggesting that NPY-related mechanisms might be relevant in certain stages of CaP, such as the progression of the disease.

Pharmacological characterization of the expressed NPY receptor subtypes in the various tumors revealed the presence of Y_1, Y_2, or both. In addition, Y_1 receptors were observed in intra- and peritumoral blood vessels as well (**Korner** *et al.*, **2004a, b**). The study of **Reubi** *et al.* (**2001**) provides the first evidence that NPY may play a role in human cancer. It is remarkable that a great majority, *i.e.*, 85% of the patients with breast cancers, often have a high expression of NPY receptors in their cancers. In all cases, the NPY receptor subtype Y_1 is expressed, whereas Y_2 is only expressed in 24% of the cases, and, when it is expressed, it never represents the predominant subtype of the tumor and is never found alone. They also give here the first evidence that Y_1 receptors may be of functional relevance in tumor cell proliferation**.**

The observation that neoplastic human breast tissue can express a different NPY receptor subtype than non-neoplastic breast strongly suggests an alteration of the NPY receptor subtype expression during the process of neoplastic transformation of the breast. The normal condition appears to be a predominant Y_2 expression by the non-neoplastic breast. Neoplastic transformation of breast tissue may induce a switch of expression from receptor subtype Y_2 to Y_1. This switch in receptor expression possibly may be related to the increasing dedifferentiation of the tissue (**Reubi** *et al.*, **2001**).

Long-term treatment with Y_1 selective analogues may be used to inhibit tumor proliferation. One may think of targeting breast tumors and metastases with radiolabeled Y_1 analogues for diagnostic *in vivo* scintigraphic tumor detection or for receptor-mediated radiotherapeutic treatment of these tumors, in analogy to *in vivo* targeting of other peptide receptors. NPY should therefore be added to the list of small regulatory peptides relevant to cancer, and to breast cancer in particular. It may be attractive to combine Y_1 radioligands with radiolabeled vasoactive intestinal peptide (VIP), gastrin-releasing peptide, and somatostatin analogues, as all four peptide families can have their receptors overexpressed in breast cancers. Such a cocktail of four peptide radioligands, given concomitantly, may substantially increase the diagnostic sensitivity of the

scintigraphic procedure as well as the radiotherapeutic efficacy (**Reubi** *et al.*, **2001**).

Cardiovascular Diseases (CVD)

Congestive heart failure and other cardiac diseases are characterized by increased activity of the sympathetic nervous system, whereas at the same time parasympathetic activity is often suppressed. Such imbalance may be a result of or at least enhanced by presynaptic inhibitory effects of sympathetic neurotransmitters on acetylcholine release (**Schwertfeger** *et al.*, **2004**). NPY plays a signaling role in cardiovascular regulation, particularly regarding its functional cooperation with norepinephrine. It could have stimulated vasoconstriction directly via its own vascular receptors or indirectly by enhancing vascular responsiveness to other endogenous vasoconstrictors including norepinephrine, vasopressin, and angiotensin II. It could also be attributed to the action of the excess NPY in the central nervous system (**Michalkiewicz** *et al.*, **2001**).

In humans, higher circulating levels of NPY were reported in situations of increased blood pressure, including exercise or some forms of hypertension. Vasopressin is known to contribute to maintaining arterial blood pressure after hemorrhage, and NPY has been demonstrated to have anatomical connections and stimulatory functional interactions with this neurohormone (**Michalkiewicz** *et al.*, **2001**). Sympathetic nerve activity, including that in the kidney, is increased in heart failure with increased plasma concentrations of norepinephrine and the vasoconstrictor co-transmitter NPY (**DiBona and Sawin, 2001**). The plasma level of NPY is greatly elevated in patients with congestive heart failure (**Ullman** *et al.*, **2002**).

Sympathetic neurotransmitter NPY inhibits parasympathetic neurotransmission in the human heart through activation of presynaptic Y_2 receptors; since NPY plasma levels are high in chronic heart failure patients (**Schwertfeger** *et al.*, **2004**). Moreover, Activation of the Y_1 receptor by NPY results in vasoconstriction and postsynaptic potentiation of the catecholamine effect on blood pressure (**Cavadas** *et al.*, **2001**).

The presence of dipeptidyl peptidase IV influences the cardiovascular responses of NPY by acting as a converting enzyme, shifting NPY activities away from Y_1. Thus, agonists and antagonists aimed at the NPY system represent a new avenue for drug treatment, which may help alleviate several cardiovascular disorders in which vascular remodeling plays a major role, such

as atherosclerosis, restenosis following balloon angioplasty, hypertension and peripheral vascular disease (**Pons** *et al.*, **2004**).

High serum total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels constitute the main risk factor for atherosclerotic vascular diseases. Both genetic and environmental factors are involved in the regulation of serum cholesterol levels (**Uusitupa** *et al.*, **1998**). NPY enhance the development of atherosclerosis. This effect may be mediated by the prepro-NPY genotype on LDL-C level with increased lipidemia (**Karvonen** *et al.*, **1998** and **Uusitupa** *et al.*, **1998**). This is because of the angiogenic properties of NPY that could be implicated in the development of atherosclerosis (**Pons** *et al.*, **2003**)**,** as well as NPY is considered to be an important modulator of the autonomic nervous system (**Niskanen** *et al.*, **2000**).

Anxiety and Depression

Extensive animal studies suggest NPY to be involved in coping with a wide range of stressors through actions within the brain (**Heilig** *et al.*, **2004** and **Heilig, 2004**). Impaired central NPY signaling could be involved in the pathophysiology of anxiety and depression (**Heilig** *et al.*, **2004**). The regulation of emotionality including fear and anxiety, modulate autonomic control of cardiovascular function. NPY mediates central inhibition of sympathetic outflow, potentially coupled with attenuation of parasympathetic tone, *i.e.*, mechanisms that may be associated with the reported anxiolytic action (**Tovote** *et al.*, **2004**). Behavioral antistress actions of NPY are noteworthy in that their magnitude surpasses that of other endogenous compounds; they are produced across a wide range of animal models, normally thought to reflect different aspects of emotionality. This suggests that NPY acts with a high potency on a common core mechanism of emotionality and behavioral stress responses (**Heilig, 2004**).

Anxiolytic and sedative effects of NPY are thought to involve inhibition of CRH. Enhanced secretion of CRH plays a critical role in the pathophysiology of major depression, characterized by sleep disturbances, anxiety and loss of appetite (**Antonijevic** *et al.*, **2000**). It has been proposed that NPY counteracts the effects of corticotropin-releasing factor (CRF) to maintain no net change in emotional state, e.g., emotional homeostasis (**Sajdyk** *et al.*, **2004**). Endogenous NPY has an important role in reducing anxiety as well as regulation of excitability (**Sajdyk** *et al.*, **2004** and **William** *et al.*, **2003**). It serves as a physiological stabilizer of neural activity in circuits involved in the regulation of arousal and anxiety **(Sajdyk** *et al.*, **2004)**. Exogenous NPY reduces experimental anxiety in a wide range of animal models (**Thorsell** *et al.***, 2000**).

Many neuropeptides and neuropeptides receptor agonists and antagonists have been shown to have anxiogenic or anxiolytic effects when administered centrally to animals. Opiates have long been used for that purpose (**Siegel** *et al.*, **1998**). Data support a role of Y_1 receptors in the behavioral and neuroendocrine responses to stress (**Eva** *et al.*, **2004**). Anxiolytic-like effects of ICV administered NPY in rats are mediated via both Y_1 and Y_5 receptors, whereas sedation is mediated via Y_5 receptors (**Sorensen** *et al.*, **2004**). Antistress actions of NPY are mimicked by Y_1 agonists, and blocked by Y_1 antagonists, although Y_5 receptors may substitute for Y_1 actions in some cases. Blockade of Y_2 receptors produces antistress effects indistinguishable from those produced by Y_1 agonism, presumably through potentiation of presynaptic release of endogenous NPY. Together, available data point to the potential of the NPY system as a target for novel pharmacological treatments of stress-related disorders, including anxiety and depression. Development of Y_2 antagonists presently appears to offer the most promising strategy for developing these clinical treatments (**Heilig, 2004**).

Y_1 is essential for NPY-induced enhancement of sedation and place this activity of NPY in the posterior hypothalamic area, a region of the brain previously implicated in the regulation of the wake-sleep cycle (**Naveilhan** *et al.*, **2001**). NPY promotes sleep period time and also reduced sleep latency and time awake. It inhibits the hypothalamo-pituitary-adrenocortical (HPA) axis in humans, pointing to a possible role of NPY agonists for the development of novel treatment strategies for affective disorders (**Antonijevic** *et al.*, **2000**).

Neuropsychiatric Illness

NPY is implicated in the control of many physiological processes. It is also suggested the involvement of NPY in several neuropsychiatric illnesses (**Krysiak** *et al.*, **2001**). For the last few years, there has been an increase in the number of studies on the role of NPY in the benefits of psychopharmacotherapy (**Obuchowicz** *et al.*, **2004**). More researches can provide humanity with the vital information necessary for designing new drug therapy for these diseases.

Epilepsy and Seizure Susceptibility

Seizures result from the imbalance of excitation over inhibition. The bulk of excitation is mediated by the neurotransmitter glutamate, whereas inhibition results mainly from the actions of γ-aminobutyric acid (GABA). In the neocortex and hippocampus, the intrinsic sources of GABA are the interneurons, which lately have come under intense scrutiny. It has become clear that a large number of distinct types of interneurons can be differentiated in part by the array

of neuropeptides they coexpress. The neuropeptide complement of interneurons plays important roles in the way that interneurons regulate excitability (**William** *et al*., **2003**).

Evidence from studies in rodents and humans support an antiseizure action of NPY in focal, acquired epilepsy. However, the effects of NPY in generalized genetic epilepsy remain unexplored. NPY modulates pathological oscillatory thalamocortical activity and may represent a new therapeutic approach for the treatment of generalized epilepsies (**Stroud** *et al*., **2005**). NPY may play a more prominent role in determining seizure thresholds and severity of seizures than in events leading to epileptogenesis (**Shannon and Yang, 2004**). An increase in the normal expression of NPY results in a decreased susceptibility to seizures while the absence or dysregulation of NPY signaling increases seizure susceptibility (**William** *et al*., **2003**).

It appears that elevated activity in hippocampal circuitry, such as that accompanying epileptiform discharges, results in increased NPY expression in interneurons and dentate granule cells, but that prolonged overstimulation in some epilepsy models and in humans ablates NPY (and other) interneurons. Thus several laboratories determined that NPY peptide and mRNA expression was increased in epilepsy models *in vivo*. At about the same time, it was reported that NPY/GABA interneurons in the hippocampus were selectively ablated in rat epilepsy models *in vivo* and in epilepsy patients, although it was recently questioned whether NPY cells are indeed selectively vulnerable in human epilepsy. Work by several laboratories suggested that NPY might be involved in the hippocampal response to epileptogenesis (**William** *et al*., **2003**).

NPY can have potent actions in models of epilepsy, but the ultimate test of its relevance to epilepsy is in the human (**William** *et al*., **2003**). Recurrent epileptic seizures in the rat enhance the expression of NPY and its mRNA in various brain areas including the hippocampus, cerebral cortex and the amygdale (**Vezzani and Sperk, 2004**). Furthermore, the ectopic expression of NPY in dentate granule cells, after even mild elevations in activity, can be considered adaptive, as the mossy fibers have a very sensitive presynaptic response to NPY. NPY also acts in epileptic human brain tissue, arguably the ultimate pharmacologic target in this field of research (**William** *et al*., **2003**).

Both Y_2 and Y_5 receptors could mediate the seizure suppressant effect of NPY (**Nanobashvili** *et al*., **2004**). Expression of Y_2 receptors is also increased whereas Y_1 receptors are reduced. Similar changes in Y_1 and Y_2 receptors were observed in the hippocampus of patients with temporal lobe epilepsy (TLE) (**Vezzani and Sperk, 2004**). Y_5 receptors mediate inhibitory effects of NPY in

kindling and display anticonvulsant rather then antiepileptogenic effects upon agonist stimulation (**Benmaamar *et al.*, 2005**).

Stimulation of presynaptic Y_2 receptors inhibits glutamate release, and exerts an anticonvulsant action in experimental models. Y_1 receptors mediate a weak excitatory component of NPY action. These findings suggest that changes in the NPY system induced by seizures represent an endogenous adaptive mechanism aimed at counteracting hyperexcitability underlying epileptic activity (**Vezzani and Sperk, 2004**). Exogenously applied NPY receptor ligands can inhibit epileptic seizures in various rodent *in vitro* and *in vivo* models. Agonists at Y_2 and/or Y_5 receptors and antagonists at Y_1 receptors appear to inhibit seizures, depending on the seizure model studied. Although progress has been made, further studies are needed using transgenic animals as well as novel selective agonists and antagonists to firmly identify the NPY receptors mediating antiepileptic effects. This may lead to the development of future antiepileptic drug treatments targeting the NPY (**Woldbye and Kokaia, 2004**).

Parkinson's disease

Parkinson's disease (PD) is a hypokinetic characterized by tremor, rigidity, akinesia (difficulty in initiating movements) and bradykinesia (slowness of movements). Acquired PD is associated with head trauma, brain cancers and possibly environmental toxins (**Longstaff, 2000**).

High levels of NPY are found in basal ganglia; although alterations occur in the level of various neuropeptides in basal ganglia in PD it is not known whether NPY is affected. In PD, the number of NPY mRNA-expressing cells was increased, the increase is more pronounced in the nucleus accumbens and in the ventral part of caudate nucleus (**Cannizzaro *et al.*, 2003**).

Alzheimer

Alzheimer's disease (AD) is the most common dementia of the old and its incidence increases with age. AD is insidious, progressive and causes defects in memory, cognition, attention and motivation. Most AD sufferers also have symptoms of PD (**Longstaff, 2000**).

NPY level is altered in AD; a strong tendency to reduction was noted. There was a correlation with the duration of disease demonstrating a significant reduction in NPY levels in subjects with dementia of Alzheimer type (DAT) (**Edvinsson *et al.*, 1993**).

Schizophrenia

Schizophrenia is a chronic mental disorder characterized by loosing of associatetions, autism (morbid self-absorption with extreme withdrawal and failure to relate to other persons) ambivalence (simultaneous existence of conflicting emotional attitudes toward a goal, object or person) and inappropriate effect, as well as by hallucinations and delusions (a false believe inconsistent with an individual's own knowledge and experience); variously classified, according to predominant symptoms or time at onset, as adolescent type, catatonic, childhood type or paranoid. NPY level is decreased in several postmortem studies of schizophrenia, in the cortex (**Siegel** *et al.*, **1998**).

Chapter VI

This chapter includes some of the most recent studies on NPY. Last update was in April, 2005.

The Most Recent Studies on NPY

NPY and its receptors can be considered as promising field for scientific research. In this chapter some of the most recent studies were reviewed to point out the importance of this neuropeptide.

NPY Y_1 and Y_2/Y_5 receptor interaction in NPY-induced modulation of macrophage functions related to inflammation (**Dimitrijevic** *et al.*, **2005**).

NPY and Y_1 receptor are involved in intestinal inflammation and suggest that inhibition of NPY Y_1 receptor signaling may provide a novel therapeutic approach in the treatment of colonic inflammation (**Hassani** *et al.*, **2005**).

NPY and leptin plasma levels are increased in patients with obstructive sleep apnea syndrome (OSAS). Yet, whereas the former appear independent of obesity, the latter are mostly associated with obesity (**Barcelo** *et al.*, **2005**).

Low concentrations of Lithium (Li^+) reduce the rate of internalization of NPY Y_1 and Y_5 receptors and of pancreatic polypeptide Y_4 in preference to sodium and potassium ions. This Li^+-induced decrease in Y_1 receptor internalization could be alleviated by Y_1 receptor agonists. Lithium treatment induces a concentration-related decrease of intermediate and higher endosomal densities that contain the internalized Y_1 ligand-receptor complex. This indicates an inhibition of endosome processing and maturation. Internalization of human transferrin shows Li^+ sensitivity similar to that of the Y_1 receptor, and a similar Li^+-induced decrease in endosomal processing. Lithium treatment thus decreases activity of the endosome system shared in the recycling endocytosis of the Y_1 and transferrin receptors (**Parker** *et al.*, **2005**).

As previously mentioned NPY has been implicated in the control of food intake and energy balance based on many observations in animals. **Ding** *et al.* (**2005**) have studied single nucleotide polymorphisms (SNPs) within the regulatory and coding sequences of the human NPY gene. One variant (1128 T>C), which causes an amino acid change from leucine to proline at codon 7 in the signal peptide of NPY, was associated with increased body mass index (BMI) in two separate Swedish populations of normal and overweight individuals. In vitro transcription and translation studies indicated the unlikelihood that this signal peptide variation affects the site of cleavage and targeting or uptake of NPY into the ER. However, the mutant, and to a lesser extent the wild-type, signal peptide by themselves markedly potentiated

NPY-induced food intake, as well as hypothalamic NPY receptor signaling. Their findings in humans strongly indicate that the NPY signaling system is implicated in body weight regulation and suggest a new and unexpected functional role of a signal peptide.

Central NPY injection has been reported to cause hyperphagia and in some cases also hypometabolism or hypothermia. Chronic central administration induced a moderate rise of short duration in body weight, without consistent metabolic/thermal changes (**Szekely** *et al.*, **2005**).

Amphetamine (AMPH) is a well-known anorectic agent. The mechanism underlying the anorectic response of AMPH has been attributed to its inhibitory effect on hypothalamic NPY. AMPH treatment might reduce the expression of NPY at both transcriptional and posttranslational levels (**Hsieh** *et al.*, **2005**).

Although nicotinic receptors have been demonstrated in hypothalamic appetite-regulating areas and nicotine administration alters food intake and body weight in both animals and humans, the mechanisms underlying the effects of smoking on appetite circuits remain unclear. Conflicting effects of nicotine on the major orexigenic peptide, NPY, have been observed in the brain, but the effects of smoking are unknown. Short-term cigarette smoke exposure led to reduced body weight, food intake, and fat mass. The reduction in plasma leptin concentration may have been too modest to increase NPY production; alternatively, change in NPY or its function might have been offset by nicotine or other elements in cigarette smoke (**Chen** *et al.*, **2005**).

Malnutrition is defined as abnormalities caused by an inadequate diet, but this term is often used inappropriately to describe the syndrome of loss of body weight with muscle mass being replaced by fatty tissue and declining serum proteins present in adults and children with chronic kidney disease (CKD). This syndrome is more accurately described as cachexia, and manifests as growth failure in children with CKD. Cachexia is common and is an important risk factor for poor quality of life and increased mortality and morbidity in both adults and children with CKD. Anorexia, acidosis and inflammation are important causes of cachexia. There is experimental evidence that hypothalamic neuropeptide signaling system may have an important role in the pathogenesis of cachexia in CKD. Understanding the molecular mechanism of cachexia in CKD may lead to novel therapeutic strategies (**Mak** *et al.*, **2005**).

NPY is a sympathetic neurotransmitter recently found to be potently angiogenic and growth promoting for endothelial, vascular smooth muscle and neuronal cells. NPY and its cognate receptors, Y_1, Y_2 and Y_5, are expressed

in neural crest-derived tumors. NPY and its receptors may become targets for novel approaches in the treatment of these diseases, directed against both tumor cell proliferation and angiogenesis (**Kitlinska** *et al.,* **2005**).

Numerous peptide receptors are overexpressed in human cancer, permitting *in vivo* tumor targeting. Among such receptors, those for the neurotransmitter NPY are overexpressed in various tumors. Since NPY can play a role in the kidney, NPY receptor expression and/or endogenous production of peptides of the NPY family (NPY, PYY, PP) were evaluated in 40 renal cell carcinomas (RCCs) and 18 nephroblastomas. Fifty-six percent of RCCs expressed the Y_1 receptor subtype in moderate density, and 80% of nephroblastomas expressed Y_1 and Y_2 subtypes in moderate to high density. Y_1 was also highly expressed in intratumoral blood vessels. In selected cases, NPY was observed in nerve fibers in close association with intratumoral blood vessels and in the vicinity of tumor cells, while no PYY or PP was detected immunohistochemically in these sites. NPY receptors on renal tumor cells and tumor blood vessels may therefore be the molecular targets of endogenous NPY released by intratumoral nerve fibers. With regard to clinical applications, NPY receptors may act as *in vivo* targets for receptor-directed therapy of RCCs and nephroblastomas for which alternative therapeutic approaches are still required (**Korner** *et al.,* **2005**).

NPY is a potent inhibitory neurotransmitter expressed in the central neurons that control blood pressure. Nitric oxide (NO) also serves as an inhibitory neurotransmitter, and its deficit causes sympathetic overactivity, which then contributes to hypertension. Thus, acting at the level of the central nervous system distinctively via Y_1 receptor-mediated mechanism, endogenous neuropeptide Y exerted a potent antihypertensive function, and its enhanced signaling ameliorated NO deficiency hypertension (**Michalkiewicz** *et al.,* **2005**).

NPY is the most abundant peptide in the mammalian heart, but its cardiac actions are not fully understood. **Heredia** *et al.* (**2005**) find that NPY has a positive inotropic effect in isolated rat cardiac myocytes, which involves increase in Ca^{2+} release after activation of Y_1 NPY receptor and subsequent stimulation of phospholypase C (PLC).

NPY plays a role in angiogenesis, cardiovascular regulation and hormone secretion. The leucine7 to proline7 (Leu7Pro) polymorphism of prepro NPY is associated with vascular diseases and has an impact on hormone levels in healthy subjects. It is related to decreased level of basal sympathetic activity, decreased insulin secretion, and increased responsiveness to noreepinepherine (**Jaakkola** *et al.,* **2005**).

The seriousness of scorpion envenomation results essentially from left cardiac function with pulmonary oedema and/or a state of shock. Adrenergic myocarditis, toxic myocarditis and myocardial ischemia are the 3 mechanisms that explain the cardiac dysfunction. Myocardial ischemia is not only due to the release of catecholamines but also the effect of the cytokines and/or NPY on the coronary vessels. The cardiac damage can be due or enhanced by the depressive effect of the cytokines on the myocardial cells. The frequently observed hyperglycaemia only enhances the state of the already damaged myocardium (**Bahloul** *et al.*, **2005**).

Glossary

Autonomic : Not subject to voluntary control.

Axon : The long outgrowth of the body of a nerve cell which conducts impulses from the body toward the next neuron.

Cachexia : A state of malnutrition, emaciation (a wasted lean habit of body) and debility (weakness).

Chromaffin cell : Cell that is staining strongly with chrome salts.

Circadian : Denoting a period of about 24 hours.

Crest : A projection, or projecting structure or ridge, especially one surmounting a bone or its border.

Diuresis : Excretion of urine; often used to indicate increased function of the kidney.

Efferent : Conducting or progressing away a center or specific site of reference.

Epilepsy : A disorder characterized by convulsive seizures or disturbances of consciousness, or both, and associated with disturbance of electrical activity of the brain.

Generic : 1- Pertaining to a genus. 2- distinctive of a kind.

Homeostasis : A tendency to uniformity or stability in the normal body states of the organism, adj. homeostatic.

Homologous : Arising from common primordial and similar in basic plan and development, but having a different function.

Hypothermia : Greatly decreased temperature.

Hypovolemic : Hypovolemia; abnormally decreased volume of circulating fluid (plasma) in the body.

Internalization : A mental mechanism whereby certain external attributes, attitudes or standards are unconsciously taken within oneself.

Inotropic : Affecting the force of cardiac contractions.

Mitogenic : An agent that induces mitosis.

Myocytes : Cells of muscular tissue.

Natiuresis : The excretion of sodium in the urine.

Neointima : Neo; word element, new. Intima; the innermost coat of a blood vessel.

Orexigenic : Increasing the appetite.

Osteoblast : An immature bone-producing cell.

Osteoclast : A large, multinuclear cell frequently associated with resorption of bone.

Paraventricular : Para: beside or beyond. Ventricular: a small cavity or chamber, as in the brain or heart.

Pheochromocytoma : A tumor of chromaffin tissue of the adrenal medulla or sympathetic paraganglia.

Presynaptic : Before synaptic place, pertaining to synapse (the functional junction between two neurons, a region of protoplasmic continuity) or to synapsis (the pairing off and union of homologous from male and female pronuclei at start of mitosis.

Phylogenetic : Phylogeny is the complete developmental history of a race or group of organism.

Psychopharmaco-therapy : Treatment for mental disordes.

Restenosis : Recurrent stenosis, especially of a cardiac valve after surgical correction of the primary condition.

Rhinitis : Inflammation of nasal mucous membrane.

Scintigraphic : A gaphic record of the particles registered by scintiscanner after administration of radioactive isotope.

Seizure : A sudden attack, as of disease, psychomotor seizure is an episode of mental confusion with apparently purposeful but coarse and coordinated muscular movements.

Stenosis : Narrowing or contraction of a body passage or opening.

Thermogenesis : The production of heat in organisms.

Glossary

Acini : The smallest lobules of a compound gland.

Afferent : Conducting toward a center or specific site of reference.

Agonist : An agent which resists the action of another.

Amygdala : A lobule of the cerebellum.

Analogous : 1- Serving a similar function, but not arising from common rudiments and not similar in basic plan and development.
2- Having similar properties.

Angiogenic : 1- Pertaining to angiogenesis; development of blood vessels in the embryo.
2- Of vascular origin.

Angioplasty : Plastic repair of blood vessels or lymphatic channels.

Anorectic : An agent that diminishes the appetite for food.

Anorexigenic : 1- Producing anorexia (lack or loss of appetite for food).
2- An agent that diminishes or controls the appetite.

Antagonist : An agent, which exerts an action opposite to that of another.

Anxiety : An exaggerated feeling of apprehension, uncertainty and fear.

Apnea : Temporary cessation of breathing.

Arcuate : Bent like a bow.

Atherosclerosis : A condition characterized by degeneration and hardening of the walls of arteries and sometimes valves of the heart.

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Notes

List of Errata

Page no.	Line no.	Word(s)	Correction(s)
9	9	97 amino **acid**	97 amino **acids**
9	20	The amino acid sequence **which written** in black	The amino acid sequence **written** in black
10	Fig.6 title	Note: **Carbon is in green**, Oxygen is in red, Hydrogen is in white, **Nitrogen is in blue or grey** and Sulfur is in yellow.	Note: **Carbon is in green or grey**, Oxygen is in red, Hydrogen is in white, **Nitrogen is in blue** and Sulfur is in yellow.