
Neuropeptide Y: History and Overview

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Abstract Neuropeptide Y (NPY) is a 36-amino acid peptide with structural similarities to peptide YY (PYY) and pancreatic polypeptide (PP). NPY, one of the most abundant neuropeptides known, is widely distributed throughout the central and peripheral nervous systems, while PYY and PP are predominantly distributed in the endocrine cells of the intestine and pancreas, respectively. Five NPY receptor subtypes denoted as Y_1 , Y_2 , Y_4 , Y_5 , and y_6 mediate the actions of NPY. NPY is involved in the regulation of diverse functions including food intake, blood pressure, circadian rhythms, stress, pain, hormone secretion, reproduction, and alcohol consumption. NPY has also been implicated in the pathophysiology of a number of diseases such as feeding disorders, seizures, hypertension, pain disorders, depression, and anxiety. This review will describe a brief history and an overview of the studies on NPY concerning the isolation, tissue distribution, receptor subtypes, receptor agonists and antagonists, physiological functions, and pharmacological activities.

Keywords Neuropeptide Y · Tissue distribution · Receptor subtype · Receptor antagonist · Review

1 Introduction

In this chapter, a brief history and an overview of the studies on neuropeptide Y (NPY) during the last 20 years are described. The main thrust of this chapter is to critically review the initial findings that have influenced later studies on NPY. Particular attention is focused on the studies concerning the receptors and physiological functions of NPY. The reader is referred to other excellent reviews in this book for more comprehensive discussion.

2 Isolation and Primary Structures of NPY

2.1 Discovery of NPY

In the last century, many neuropeptides and hormonal peptides were identified on the basis of specific biological responses mediated by them. Unlike most oth-

er known neuropeptides, however, NPY was first identified in brain extracts by its C-terminal tyrosine amide structure. In 1978, we developed a novel method for the detection of biologically active peptides based on the C-terminal amide structure that is a unique chemical feature of many peptide hormones and neuropeptides (Tatemoto and Mutt 1978). Since peptides with this structure are likely to be biologically active, the search for unknown peptide amides would result in the finding of novel peptides. We therefore carried out the isolation of previously unknown peptide amides from tissue extracts using a chemical method as the detection device.

In 1980, we isolated two novel peptide amides, which were designated peptide HI (PHI) and peptide YY (PYY) from porcine intestinal extracts (Tatemoto and Mutt 1980). Subsequently, we isolated a peptide with a C-terminal tyrosine amide from porcine brain extracts, which was named neuropeptide Y (Tatemoto et al. 1982). Using a similar approach, we isolated a series of other novel peptides such as galanin (Tatemoto et al. 1983), neuropeptide K (Tatemoto et al. 1985), and pancreastatin (Tatemoto et al. 1986).

2.2

Primary Structure of NPY

NPY is a linear polypeptide with 36 amino acid residues (Tatemoto 1982a). Since NPY contains many tyrosine (Y) residues in its structure, we named this peptide neuropeptide Y to distinguish it from PYY that possesses a very similar structure to NPY (Tatemoto 1982b). A comparison of the primary structures of NPY, PYY and pancreatic polypeptide (PP) reveals a high degree of sequence homology between NPY and PYY, with a lesser degree of homology between NPY and PP, as shown in Fig. 1. It was therefore proposed that NPY, PYY, and PP are members of a previously unrecognized peptide family (Tatemoto 1982a).

Later, human NPY was isolated from adrenal-medullary pheochromocytoma tissue. The primary structure of human NPY differs from that of the porcine peptide in only one position of the 36 residues (Corder et al. 1984). Subsequent studies identified the primary structures of NPY molecules from various animals, birds, frogs, and others (for a review see Larhammar et al. 1993). These

		homology
NPY	<u>Y</u> <u>P</u> <u>S</u> <u>K</u> <u>P</u> <u>D</u> <u>N</u> <u>E</u> <u>G</u> <u>E</u> <u>D</u> <u>A</u> <u>P</u> <u>A</u> <u>E</u> <u>D</u> <u>L</u> <u>A</u> <u>R</u> <u>Y</u> <u>Y</u> <u>S</u> <u>A</u> <u>L</u> <u>R</u> <u>H</u> <u>Y</u> <u>I</u> <u>N</u> <u>L</u> <u>I</u> <u>T</u> <u>R</u> <u>Q</u> <u>R</u> <u>Y</u> *	100%
PYY	<u>Y</u> <u>P</u> <u>A</u> <u>K</u> <u>P</u> <u>E</u> <u>A</u> <u>E</u> <u>G</u> <u>E</u> <u>D</u> <u>A</u> <u>S</u> <u>P</u> <u>E</u> <u>E</u> <u>L</u> <u>S</u> <u>R</u> <u>Y</u> <u>Y</u> <u>A</u> <u>S</u> <u>L</u> <u>R</u> <u>H</u> <u>Y</u> <u>L</u> <u>N</u> <u>L</u> <u>V</u> <u>T</u> <u>R</u> <u>Q</u> <u>R</u> <u>Y</u> *	69%
PP	<u>A</u> <u>P</u> <u>L</u> <u>E</u> <u>P</u> <u>V</u> <u>Y</u> <u>E</u> <u>G</u> <u>D</u> <u>D</u> <u>A</u> <u>T</u> <u>P</u> <u>E</u> <u>Q</u> <u>M</u> <u>A</u> <u>Q</u> <u>Y</u> <u>A</u> <u>A</u> <u>E</u> <u>L</u> <u>R</u> <u>R</u> <u>Y</u> <u>I</u> <u>N</u> <u>M</u> <u>L</u> <u>T</u> <u>R</u> <u>P</u> <u>R</u> <u>Y</u> *	50%

Fig. 1 Comparison of the amino acid sequences of the porcine peptides, neuropeptide Y (NPY), peptide YY (PYY), and pancreatic polypeptide (PP). An *asterisk* indicates the amidated C-terminus. Identities are *underlined*

studies revealed that the structure of NPY has been strongly conserved throughout evolution.

2.3

NPY mRNA

Dixon and coworkers were the first to identify the sequence of a human cDNA encoding NPY from human pheochromocytoma cells. The 97-amino acid precursor has at least two processing sites, which would generate three peptides of 28 (signal peptide), 36 (NPY), and 30 (COOH-terminal peptide) amino acid residues (Minth et al. 1984). Subsequently, the structure of a human NPY gene identified from a human genomic DNA library was reported. The DNA sequences located within 530 bases of the start of transcription were found to be sufficient for transient expression in the two cell lines examined (Minth et al. 1986).

3

Cellular Localization of NPY

Since NPY was discovered by its chemical nature, no biological activity of the peptide was known when it was isolated. Therefore, we prepared a large quantity of natural NPY from more than 1,000 kg of porcine brains, and the natural NPY preparations thus obtained were sent to a number of laboratories in Europe and the USA to examine the biological activities of the peptide and to generate specific antisera against NPY for immunohistochemistry and radioimmunoassay studies. Between 1982 and 1985, the natural NPY preparations were used for many studies on the biological activity and localization of NPY, until synthetic NPY preparations became commercially available.

3.1

NPY in the Peripheral Nervous System

Lundberg et al. (1982) were the first to demonstrate NPY-like immunoreactivity in many peripheral neurons with a distribution mostly paralleling that of tyrosine hydroxylase (TH) and dopamine-beta-hydroxylase (DBH) containing neurons. Very high levels of NPY-like immunoreactivity were found in sympathetic ganglia and in tissues receiving a dense sympathetic innervation, such as the vas deferens, heart atrium, blood vessels, and spleen. NPY- and DBH-nerves had a roughly parallel occurrence in the heart, spleen, kidney, respiratory and urogenital tracts, around blood vessels, and within visceral smooth muscle. NPY thus seems to be a major peptide in the sympathetic nervous system (Lundberg et al. 1983). The presence of NPY-like immunoreactivity was also demonstrated in the neuronal elements of the gut and pancreas (Sundler et al. 1983). NPY has generally been found in the sympathetic neurons, costored and

coreleased with catecholamines, although NPY is also present in peripheral non-sympathetic neurons (for a review see MacDonald 1988).

3.2

NPY in the Central Nervous System

Bloom and his colleagues have shown that NPY-like immunoreactivity is widely and unevenly distributed in rat and human brains, and it is the most abundant neuropeptide known (Allen et al. 1983; Adrian et al. 1983). The highest concentrations of NPY were found in the paraventricular hypothalamic nucleus, hypothalamic arcuate nucleus, suprachiasmatic nucleus, median eminence, dorsomedial hypothalamic nucleus, and paraventricular thalamic nucleus (Chronwall et al. 1985). The extremely high concentrations and widespread distribution indicate important roles of NPY in many brain functions. Hokfelt and coworkers (1983) reported the coexistence of NPY-like immunoreactivity in catecholamine neurons in the medulla oblongata. During subsequent years, a number of *in vitro* studies have shown the coexistence of NPY not only with catecholamines but also with a variety of other neurotransmitters or neuropeptides (for a review see Everitt and Hokfelt 1989). It is suggested that the physiological roles of NPY in the central nervous system are very complex because of the interactions of NPY with other effector systems. More recently, localization of NPY mRNA by means of *in situ* hybridization was found to be comparable to that of NPY-immunoreactivity (Terenghi et al. 1987; Gehlert et al. 1987).

4

Studies on the Receptors and Physiological Functions of NPY (1982–1992)

During the first 10 years of NPY research, a number of important biological activities of NPY in both the central and peripheral nervous systems were discovered, and the presence of NPY receptor subtypes, Y_1 and Y_2 , was demonstrated using specific NPY receptor agonists.

4.1

Peripheral Actions of NPY

4.1.1

Cardiovascular Response

Lundberg and Tatemoto (1982) were the first to find a biological activity of NPY, demonstrating that NPY induced potent vasoconstriction, more potent than noradrenaline, which was resistant to alpha-adrenoceptor blockade. It was also shown that NPY caused strong contractions of cerebral arteries (Edvinsson et al. 1983), and that NPY produced an inhibition of colonic motility and a vasoconstriction of long duration (Hellstrom et al. 1985). NPY induced renal vasoconstriction and inhibited renin release by inhibiting adenylate cyclase in the

vascular smooth muscle and renin-producing cells (Hackenthal et al. 1987). NPY produced potent pressor responses (Allen et al. 1984; Petty et al. 1984), while NPY (18–36), a C-terminal NPY fragment, exhibited substantial hypotensive action (Boublik et al. 1989).

Interestingly, NPY was found to prevent the blood pressure fall induced by endotoxin in conscious rats with adrenal medullectomy (Evequoz et al. 1988), and plasma levels of NPY were found to markedly increase in patients with septic shock (Watson et al. 1988). These data suggest that NPY plays a role in maintaining blood pressure during endotoxic shock.

4.1.2

Hormone Secretion

Allan et al. (1982) found that NPY inhibited the contraction of electrically stimulated mouse vas deferens, suggesting inhibitory actions of NPY on noradrenaline release at a pre-synaptic level. Subsequently, NPY was shown to depress the secretion of ^3H -noradrenaline and the contractile response evoked by field stimulation in the vas deferens (Lundberg and Stjarne 1984).

4.2

Central Actions of NPY

4.2.1

Cardiovascular Response

Fuxe et al. (1983) first demonstrated an effect of central administration of NPY. They found that NPY induced hypotension and bradypnea in the rat, suggesting the involvement of NPY in the cardiovascular and respiratory controls of the central nervous system.

4.2.2

Circadian Rhythms

Albers and Ferris (1984) found that microinjection of NPY into the suprachiasmatic region of the hypothalamus (SCN) phase-shifted the circadian rhythm of hamsters housed in constant light. It is suggested that NPY functions as a chemical messenger that is important for the light–dark cycle entrainment of circadian rhythms.

4.2.3

Food Intake and Energy Expenditure

In 1984, NPY was found, for the first time, to stimulate feeding behavior in rats (Clark et al. 1984; Levine and Morley 1984; Stanley and Leibowitz 1984). Since then, a number of studies have shown that NPY is the most potent orexigenic

peptide identified to date. Later, cerebrospinal fluid NPY concentrations were found to be significantly elevated in anorectic and bulimic patients. These levels normalized in long-term weight-restored anorectic patients who had a return of normal menstrual cycles (Kaye et al. 1990). This suggests that NPY plays a role in eating disorders. It was also reported that NPY decreased rectal temperature after intracerebroventricular administration (Morioka et al. 1986). These observations suggest that NPY is involved in the regulation of food intake and energy expenditure.

4.2.4

Hormone Secretion and Reproduction

Kalra and Crowley (1984) found that central administration of NPY suppressed luteinizing hormone (LH) release in ovariectomized rats, while it stimulated LH release in ovariectomized rats pretreated with estrogen and progesterone. Kalra and coworkers found that NPY not only stimulated food intake but also inhibited sexual behavior in rats (Clark et al. 1985). NPY inhibited excitatory synaptic transmission in the hippocampus by acting directly at the terminal to reduce a calcium influx (Colmers et al. 1988). These observations suggest that NPY is involved in the central control of hormone and neurotransmitter release.

4.2.5

Stress, Depression, Anxiety, and Pain

Fuxe et al. (1983) demonstrated that central administration of NPY induced EEG synchronization. It is therefore suggested that NPY produces behavioral signs of sedation. Subsequently, Heilig and Murison (1987) found that intracerebroventricular administration of NPY protected against stress-induced gastric erosion in the rat. Stress-induced erosion was reduced by approximately 50% by NPY, suggesting the anti-stress action of NPY as a manifestation of its sedative properties. In addition, it was reported that the administration of NPY into the third ventricle of the brain enhanced memory retention. It is suggested that NPY modulates memory processes (Flood et al. 1987).

Interestingly, NPY-like immunoreactivity was found to be significantly lower in cerebrospinal fluid from patients with a major depressive disorder compared with healthy controls (Widerlov et al. 1988). It was also found that antidepressant drugs increased the concentrations of NPY-like immunoreactivity in the brain (Heilig et al. 1988). These observations support the hypothesis that NPY is involved in the pathophysiology of depressive illness.

Furthermore, Heilig et al. (1989) found that centrally administered NPY produced anxiolytic-like effects that were mediated through interactions with noradrenergic systems in animal anxiety models. In a hot plate test, spinally administered NPY produced a dose-dependent elevation in the nociceptive threshold in rats, suggesting the involvement of NPY in the mechanism of pain control (Hua et al. 1991).

4.2.6

Seizures

Marksteiner and Sperk (1988) observed significantly increased levels of NPY-like immunoreactivity in the frontal cortex of rats that had undergone strong limbic seizures induced by kainic acid. The increase could be prevented by early injection of an anticonvulsant (Marksteiner et al. 1990). These observations suggest that NPY is involved in the control of seizures.

4.3

NPY Receptor Binding and Intracellular Signaling

The specific binding of the iodinated NPY to membranes from the cerebral cortex was demonstrated. The binding of iodinated NPY was characterized by a K_d value of 0.38 nM (Unden et al. 1984). A study on autoradiographic localization of NPY receptors indicated that the receptors were discretely distributed in the rat brain with high densities found in areas such as the olfactory bulb, superficial layers of the cortex, ventral hippocampus, and area postrema (Martel et al. 1986).

NPY was found to be a potent inhibitor of cyclic AMP accumulation in feline cerebral blood vessels (Fredholm et al. 1985). NPY was shown to inhibit adenylate cyclase through a pertussis toxin-sensitive G protein (Kassis et al. 1987). In addition to inhibiting adenylate cyclase, NPY was found to elevate intracellular calcium (Motulsky and Michel 1988). It was also shown that guanine nucleotide-binding protein G_o mediated the inhibitory effects of NPY on dorsal root ganglion calcium channels (Ewald et al. 1988).

4.4

NPY Receptor Agonists and Antagonists

Centrally truncated synthetic NPY agonists were synthesized and shown to be biologically active (Beck et al. 1989; Krstenansky et al. 1989). Fuhlendorff et al. (1990) reported that [Leu³¹, Pro³⁴] NPY was a specific Y1 receptor agonist that could be useful in delineating the physiological importance of Y1 receptors. About the same time, the first NPY receptor antagonists, Ac-[3-(2,6-dichlorobenzyl)Tyr²⁷, D-Thr³²] NPY(27-36) and Ac-[3-(2,6-dichlorobenzyl) Tyr^{27,36}, D-Thr³²] NPY(27-36), designated PYX-1 and PYX-2, respectively, were synthesized based on the C-terminal structure of the NPY molecule (Tatemoto 1990). PYX-2 was found to block the stimulatory action of NPY on carbohydrate ingestion (Leibowitz et al. 1992).

4.5

NPY Receptor Subtypes

Wahlestedt et al. (1986) first suggested the presence of two receptor subtypes for NPY and its related peptides. They studied the effects of NPY, PYY, and the

C-terminal fragments of NPY or PYY on different smooth muscle preparations *in vitro*, and found that PYY(13–36) reproduced the NPY- and PYY-induced suppression of noradrenaline release. Thus, the C-terminal portion seems to be sufficient for exerting pre-junctional effects of NPY and PYY, while the whole sequence seems to be required for post-junctional effects. Later, Schwartz and coworkers showed that two subtypes of NPY/PYY-binding sites occurred in different cells, supporting the hypothesis of NPY receptor subtypes (Sheikh et al. 1989).

Since 1989, a number of the studies on NPY receptor subtypes have been published. Using selective receptor agonists, it was shown that Y_1 and Y_2 receptors were independently expressed in the brain and the majority of NPY receptors in the brain were of the Y_2 type (Aicher et al. 1991). It was found that the hypothalamic Y_1 receptors mediated the stimulatory effect of NPY on carbohydrate intake and meal size, while the Y_2 receptors had the opposite effect of suppressing carbohydrate intake (Leibowitz and Alexander 1991). Presynaptic inhibition by NPY observed in rat hippocampal slice was shown to be mediated by a Y_2 receptor (Colmers et al. 1991). Involvement of Y_1 receptor subtype in the regulation of LH secretion was demonstrated by using NPY, NPY(2–36), [Leu³¹, Pro³⁴] NPY, NPY(13–36) and other NPY fragments (Kalra et al. 1992).

5

Studies on the Receptors and Physiological Functions of NPY (1992–2002)

The main topics for the last 10 years have been the cloning of NPY receptor subtypes, Y_1 , Y_2 , Y_4 , Y_5 , and y_6 , and subsequent studies on the biological functions of these receptor subtypes. Thus, in addition to studies on NPY-transgenic and deficient animals, a number of animals lacking specific NPY receptor subtypes were generated and the physiological functions of these animals were studied. Moreover, specific receptor agonists and antagonists for each NPY receptor subtype were developed and their physiological and pharmacological properties were evaluated. The synthesis of selective and potent receptor agonists and antagonists has provided useful tools to study the physiological functions of NPY receptor subtypes and to develop novel pharmacological treatments.

5.1

Cloning of NPY Receptor Subtypes

Recent advances in molecular biology have resulted in the identification of five NPY receptor subtypes, Y_1 , Y_2 , Y_4 , Y_5 , and y_6 receptors (for a review see Michel et al. 1998). These receptor subtypes were found to share only modest sequence homologies (30–50%). Moreover, each of the receptor subtypes seems to be characterized by a distinct tissue localization and unique pharmacological profile. The next section describes a brief history of the cloning of NPY receptor subtypes.

5.1.1

Y₁ Receptor

In 1992, the primary structures of rat and human Y₁ receptors were identified (Krause et al. 1992; Herzog et al. 1992; Larhammar et al. 1992). The Y₁ receptor, the first NPY receptor to be cloned, was found to be a 384-amino acid protein belonging to a G protein-coupled receptor family. The functionality of the expressed NPY receptor was demonstrated by inhibition of adenylate cyclase and mobilization of intracellular calcium, both being characteristic of an NPY receptor. The distribution of Y₁ receptor expression correlated with that of NPY-immunoreactive nerves and the apparent actions of NPY in the intestine, kidney, and heart (Wharton et al. 1993).

5.1.2

Y₂ Receptor

The cloned Y₂ receptor consists of 381 amino acids, and has only 31% identity to the structure of the Y₁ receptor (Rose et al. 1995; Gerald et al. 1995; Gehlert et al. 1996; Rimland et al. 1996). The Y₂ receptor expressing cells have high affinity binding sites for NPY, PYY, and NPY(13–36), whereas [Leu³¹, Pro³⁴] NPY binds with lower affinity. The Y₂ receptor is localized on a number of NPY-containing neurons in the brain, suggesting that this receptor has a characteristic of an autoreceptor (Caberlotto et al. 2000).

5.1.3

Putative Y₃ Receptor

The Y₃ receptor is distinguished from the other NPY receptors by its high affinity for NPY but relatively low affinity for PYY. However, evidence for the existence of such a subtype is not clear as the clone initially reported as a Y₃ receptor (Rimland et al. 1991) failed to confer NPY binding sites (Herzog et al. 1993; Jazin et al. 1993). Therefore, the evidence is not sufficient to grant the presence of a Y₃ receptor (Michel et al. 1998).

5.1.4

Y₄ Receptor

A unique feature of the Y₄ receptor is a high affinity for PP. Therefore, the Y₄ receptor is probably a PP receptor. The cloned human Y₄ receptor has 43% sequence homology with the human Y₁ receptor (Lundell et al. 1995; Bard et al. 1995; Yan et al. 1996). Both NPY and PYY have low affinities for this receptor. Y₄ receptor is present in the intestine, prostate, and pancreas (Lundell et al. 1995). The Y₄ receptor mRNA is sparsely expressed in the brain, except in the brainstem (Parker and Herzog 1999).

5.1.5

Y₅ Receptor

The cloning of a novel NPY receptor designated Y₅ receptor was reported (Gerald et al. 1996; Hu et al. 1996). The complementary DNA encoded a 456-amino-acid protein with less than 35% overall identity to the other known NPY receptors. [D-Trp³²] NPY had a high affinity for the Y₅ receptor, while it had low affinities for the other known NPY receptors. The Y₅ receptor, originally cloned as the 'feeding' receptor in the hypothalamus, was also found in the peripheral nervous system such as the testis, spleen, and pancreas (Statnick et al. 1998).

5.1.6

y₆ Receptor

The cloning of a novel NPY receptor proposed to be a Y₅ receptor was reported (Weinberg et al. 1996). However, other researchers reported the same clone as a PP receptor or Y_{2b} receptor (Gregor et al. 1996; Matsumoto et al. 1996). To avoid confusion, it was renamed the y₆ receptor. The y₆ receptor gene is present in chicken, rabbit, cow, dog, mouse, and human, but it is completely absent in rat (Burkhoff et al. 1998). Sequence data revealed the y₆ gene to be the orthologue of the mouse Y₅ gene. Rabbits encode functional y₆ receptor, but the y₆ receptors in primates are functionally inactive due to a frameshift mutation occurring during early primate evolution (Matsumoto et al. 1996).

5.2

Selective NPY Receptor Agonists and Antagonists

Based on the C-terminal structure of the NPY molecule, the first nonpeptide Y₁ receptor antagonist BIBP 3226 was designed and synthesized (Rudolf et al. 1994), demonstrating that such a nonpeptide compound could be a useful tool for studying physiological functions and exploring therapeutic relevance.

Furthermore, synthesis of both peptide and nonpeptide Y₁ receptor antagonists such as [D-Tyr^{27,36}, D-Thr³²] NPY(27–36), SR120819A, 1229U91, BIBO3304, LY-357897, J-115814, and CP-617,906 have been reported. More recently, T4-[NPY(33–36)]4 and BIIE0246 have been described as selective Y₂ receptor antagonists. After the cloning of the Y₅ receptor, a number of Y₅ receptor antagonists including CGP71683A and L-152,804, and Y₅ receptor agonists such as [D-Trp³⁴] NPY and [Ala³¹, Aib³²] NPY were synthesized (for reviews see Balasubramaniam 1997; Pheng and Regoli 2000; Parker et al. 2002). The Y₁ receptor antagonist 1229U91 has been shown to exhibit an agonist activity for the Y₄ receptor (Parker et al. 1998). However, no selective antagonist for the Y₄ receptor has yet been reported.

5.3

NPY Receptor Subtypes and Their Physiological Functions

The cloning of NPY receptor subtypes has made it possible to generate specific receptor subtype-deficient animals. The generation of such animals has provided unique models to examine the physiological functions of NPY. The next section focuses on the physiological functions of NPY and its receptor subtypes revealed by the use of receptor agonists and antagonists and genetically modified animals.

5.3.1

Cardiovascular Response

BIBP3226 antagonized vasoconstriction induced by NPY. This suggests that endogenous NPY acting on the Y_1 receptor is likely to account for the long-lasting component of sympathetic vasoconstriction in response to high-frequency stimulation (Malmstrom and Lundberg 1995). It was reported that the incubation of the subcutaneous arteries with Y_1 receptor antisense oligodeoxynucleotides attenuated NPY-induced vasoconstriction (Sun et al. 1996). Furthermore, Y_1 receptor-deficient mice showed a complete absence of blood pressure responses to NPY, suggesting the importance of Y_1 receptors in the NPY-mediated cardiovascular response (Pedrazzini et al. 1998).

However, it was also reported that the depressor effect of intrathecal NPY injection was primarily mediated by a Y_2 receptor (Chen and Westfall 1993). Furthermore, a Y_2 receptor agonist evoked vasoconstriction in the spleen, while a Y_2 receptor antagonist BIIE0246 antagonized the response. These suggest that the Y_2 receptor is also involved in NPY/PYY-evoked vasoconstriction (Malmstrom 2001).

5.3.2

Circadian Rhythms

NPY has been implicated in the phase shifting of circadian rhythms. Microinjection of a Y_2 receptor agonist produced phase advances that were significantly greater than those produced by the injection of a Y_1 receptor agonist. This suggests that NPY phase shifts circadian rhythms via the Y_2 receptor (Huhman et al. 1996; Golombek 1996). There is, however, some evidence that the Y_1/Y_5 receptors, in addition to the Y_2 receptor, may also be involved in the mechanism of NPY action by altering the levels of circadian clock-related genes (Fukuhara et al. 2001).

5.3.3

Food Intake and Energy Expenditure

NPY has been implicated to be a central stimulator of feeding behavior by interacting with a number of other hormones and neuroregulators that play roles in

the regulation of body weight. A novel obese gene product, leptin, was found to regulate food intake by inhibiting the synthesis and release of NPY in the central nervous system (Stephens et al. 1995). It was reported that the mild obesity found in Y_1 receptor-deficient mice was caused by impaired insulin secretion and low energy expenditure (Kushi et al. 1998). Furthermore, NPY-induced food intake was remarkably reduced in Y_1 -deficient mice (Kanatani et al. 2000). These results suggest the importance of Y_1 receptors in the regulation of food intake and body weight through the central control of energy expenditure.

It was found that the Y_5 receptor was also involved in NPY-induced food intake (Gerald et al. 1996). The Y_5 receptor-deficient mice responded significantly less to NPY-induced food intake than wild-type mice (Marsh et al. 1998). On the other hand, the results obtained using Y_2 receptor-deficient mice indicated an inhibitory role for the Y_2 receptor in the central regulation of body weight and food intake (Naveilhan et al. 1999). Hypothalamus-specific Y_2 receptor-deleted mice showed a significant decrease in body weight and a significant increase in food intake, suggesting an important role of hypothalamic Y_2 receptors in body weight regulation (Sainsbury et al. 2002). In addition, it was reported that peripheral injection of PYY(3–36) in rats inhibited food intake and reduced weight gain. PYY(3–36) also inhibited food intake in mice, but not in Y_2 receptor-deficient mice. This suggests that the anorectic effect requires the Y_2 receptor (Batterham et al. 2002).

5.3.4

Hormone Secretion and Reproduction

NPY has been known to be a putative neuroregulator of the reproductive axis in the central nervous system. A selective Y_5 agonist inhibited LH secretion, while the inhibitory action was fully prevented by Y_5 receptor antagonists (Raposinho et al. 1999). It was also shown that Y_5 receptor activation suppressed the reproductive axis in both virgin and lactating rats (Toufexis et al. 2002). These results suggest that the actions of NPY on the reproductive axis are predominantly mediated by the Y_5 receptor. On the other hand, using Y_1 receptor-deficient mice, crucial roles for the Y_1 receptor in controlling food intake, the onset of puberty, and the maintenance of reproductive functions were demonstrated (Pralong et al. 2002).

5.3.5

Anxiety, Pain, Stress, and Depression

It has been shown that NPY exhibits anxiolytic, antinociceptive, anti-stress, and anti-depressive actions. Involvement of the Y_1 receptor in the anxiolytic-like action of NPY was demonstrated (Wahlestedt et al. 1993; Heilig et al. 1993). NPY may produce not only an anxiolytic effect via the Y_1 receptor, but also an anxiogenic effect via the Y_2 receptor (Nakajima et al. 1998). It was reported that NPY transgenic mice displayed anxiolytic behaviors (Inui et al. 1998). Moreover,

transgenic rats with hippocampal NPY overexpression were insensitive to restraint stress, had no fear suppression behavior, and displayed impaired spatial learning (Thorsell et al. 2000). It was also reported that Y_1 receptor-deficient mice developed hyperalgesia to acute pain, and showed a complete absence of the pharmacological analgesic effects of NPY (Naveilhan et al. 2001). These data suggest that NPY and its receptors are involved in the mechanisms of anxiety, stress, learning, and nociception.

Using an animal model of depression, alterations in the NPY levels and Y_1 receptor mRNA were observed after treatment with an anti-depressant drug (Caberlotto et al. 1998). When compared with healthy controls, the levels of NPY appeared to be low in patients who had recently attempted suicide. Patients who had repeatedly attempted suicide were found to have the lowest NPY levels (Westrin et al. 1999). These data suggest the possible involvement of NPY and Y_1 receptors in depression.

5.3.6

Seizures

NPY has been implicated to function as an endogenous anticonvulsant. It was reported that NPY-deficient mice were susceptible to seizures induced by a GABA antagonist (Erickson et al. 1996). Kainic acid-induced limbic seizures in NPY-deficient mice progressed uncontrollably and ultimately produced death in 93% of the mice, whereas intracerebroventricular NPY infusion could prevent such death (Baraban et al. 1997). Furthermore, the transgenic rats with NPY overexpression showed a significant reduction in the number and duration of kainic acid-induced seizures (Vezzani et al. 2002).

It was found that NPY, acting predominantly via Y_2 receptors, could dramatically inhibit epileptiform activity in vitro models of epilepsy (Klapstein and Colmers 1997). NPY was also found to potently inhibit seizures induced by kainic acid via Y_5 receptor (Woldbye et al. 1997). Moreover, mice lacking the Y_5 receptor were more sensitive to kainic acid-induced seizures (Marsh et al. 1999). In human epilepsy it is suggested that abundant sprouting of NPY fibers, concomitant upregulation of Y_2 receptors, and downregulation of Y_1 receptors in the hippocampus of patients with Ammon's horn sclerosis is involved in the anticonvulsant mechanism by the NPY system (Furtinger et al. 2001).

5.3.7

Ethanol Consumption

Thiele et al. (1998) first reported that NPY-deficient mice showed increased ethanol consumption, while transgenic mice with NPY overexpression had a lower preference for ethanol. These data suggest that alcohol consumption and resistance are inversely related to the NPY levels in the brain. Recently, it was reported that knockout mice lacking the Y_1 receptor showed increased ethanol consumption. It is suggested that the Y_1 receptor regulates voluntary ethanol con-

sumption and some of the intoxicating effects caused by administration of ethanol (Thiele et al. 2002).

It was shown that blockade of central Y₂ receptors by a Y₂ receptor antagonist, BIIE0246, reduced ethanol self-administration in rats. It is therefore suggested that the Y₂ receptor is a candidate target for developing novel pharmacological treatments for alcoholism (Thorsell et al. 2002).

6 Conclusions and Future Studies

NPY has been shown to be involved in the regulation of diverse physiological functions and has been implicated in a variety of disorders such as anxiety, depression, obesity, epilepsy, and alcohol dependence. Thus, the NPY system has emerged as a potential drug target for a number of disorders.

During the last decade, the cloning of NPY receptor subtypes has made it possible to clarify the functional importance of the subtypes and to discover novel compounds with selective affinity to individual receptor subtypes. Indeed, a number of impressive advances have been made in the development of nonpeptide antagonists to NPY receptor subtypes. However, further studies are needed to clarify the potential of these compounds as useful drugs. In contrast, synthesis of nonpeptide NPY receptor agonists has not yet been successful, thereby hampering the development of drugs for the treatment of disorders such as anxiety, depression, pain disorders, and epilepsy. In addition, such an agonist may be of clinical importance for modulating the circadian-clock responses to light.

Advances in the development of orally-active nonpeptide NPY receptor agonists and antagonists that are capable of crossing the blood-brain barrier will facilitate our understanding of the physiological roles of NPY and will undoubtedly underscore the importance of NPY in the fields of pharmacology and clinical medicine.

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