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NEUROPEPTIDE Y-RELATED PEPTIDES AND THEIR RECEPTORS—ARE THE RECEPTORS POTENTIAL THERAPEUTIC DRUG TARGETS?

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INTRODUCTION

A decade has passed since the discovery of neuropeptide Y (also known as neuropeptide tyrosine or NPY) by Tatemoto & Mutt (1, 2). This 36-amino-acid peptide, arguably the most abundant and widely distributed of neuropeptides discovered to date, has stimulated several thousand scientific publications. NPY seems to fulfill the main neurotransmitter criteria, since it is stored in synaptic granulae (3), is released upon electrical nerve stimulation (4, 5) and acts at specific receptors (e.g. 6). Initially, much NPY-related work concerned its coexistence with classical transmitters (e.g., norepinephrine) and its possible role in cotransmission; these lines of investigation coincided with the general acceptance that neurons use multiple messenger molecules, at least one of which is commonly a peptide. NPY has been frequently used to study cotransmission, particularly in its relation to the "classical" neurotransmitter norepinephrine, in mediating sympathetic vasoconstriction (6–9). Although the role of NPY as a modulator of coreleased transmitter remains a focus for many investigators, it has been appreciated that NPY may also be an important messenger in its own right, perhaps particularly in the brain, where NPY

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potently induces, e.g. food intake (10–13) and anxiolysis (14, 15). Another important line of research described below has been to elucidate the evolution of NPY; this has resulted in the conclusion that NPY is one of the most highly conserved bioactive peptides known.

As with many other neurohormonal peptides, the absence of useful pharmacological antagonists has drastically limited our understanding of endogenous NPY mechanisms. Several proposed NPY antagonists of limited value for physiological studies (as a result of low selectivity and/or potency) have, however, been introduced and are discussed below. The only clear-cut evidence that an endogenous NPY mechanism (anxiolysis) could be selectively perturbed involved an antisense approach leading to reduced NPY receptor density (16).

On the other hand, peptidergic agonist analogs of NPY have been in use for several years. With the aid of these peptides various investigators have inferred that at least three different NPY receptor subtypes may exist (6, 17–19). The signal transduction pathways of these NPY receptor subtypes are likely to be similar but are far from well understood. Recent molecular cloning data have supported previous assumptions that NPY receptors belong to the G-protein-coupled superfamily of receptors.

SCOPE AND OBJECTIVES OF THE REVIEW

In this brief critical review, we aim to focus on certain functional aspects of NPY that may set this peptide apart from the many other neurohormonal peptides that have entered the scene over the past several decades.

To reiterate a point made above, the key dilemma in the field has been the absence of useful pharmacological NPY antagonists, making it difficult to evaluate which actions of NPY may indeed represent physiologically important phenomena. We will therefore describe in detail only selected actions of NPY that represent instances in which the peptide may be assumed to play a physiological role; it is perhaps in these cases that potential pharmaceutical agents affecting NPY receptor mechanisms will prove to be of value. Instances in which NPY appears to have effects similar to those of a range of other messenger molecules will thus be largely omitted (more complete lists of demonstrated NPY effects are given in other reviews, e.g. 6, 7, 19–21).

Major emphasis will be put on the study of NPY receptors, a field in which much progress has been made over the past few years, to the extent that the possibility of developing NPY-related pharmaceutical agents has been raised. First, however, follows a brief introduction of NPY and its related peptides, as well as their expression mechanisms and distributions in humans and other mammals.

THE NPY FAMILY OF PEPTIDES

NPY is a member of a peptide family that also includes peptide YY (PYY) (22), pancreatic polypeptide (PP) (23), and the nonmammalian (fish) pancreatic peptide Y (PY) (24). This family is sometimes referred to as the pancreatic polypeptide family, since PP was the first to be discovered; however, since NPY has been much more conserved during evolution and exhibits much greater biological activity than PP, this peptide family should more appropriately be called the NPY family (7, 25). Porcine NPY and PYY show 69% sequence identity (see Figure 1) and activate most NPY-responsive receptors with similar potencies, as pointed out below. In contrast, the pancreatic polypeptides differ considerably not only from NPY and PYY but also among various species (Figure 1).

Discovery

In retrospect, the story of NPY began in the late 1960s when Kimmel et al isolated insulin from the chicken pancreas and discovered (avian) PP as a by-product (23, 26). Subsequently, PP was isolated from many other species. Although it has little physiological significance, PP served as a forerunner of NPY and PYY. In 1980, using a technique to isolate peptides with C-terminal α-amide groups, Tatemoto & Mutt reported that porcine brain and gut contained large amounts of a peptide resembling PP (27). The PP-like peptide isolated from gut was named peptide YY (PYY) because of its N- and C-terminal tyrosines (Y being the abbreviation for tyrosine in the single-letter amino acid code). At first, PYY (22) was thought to be the PP-like peptide in both brain and gut, but subsequent work showed the (predominant) brain peptide to differ from PYY, and because of its occurrence in brain, it was named neuropeptide Y (NPY) (1, 2). Finally, the fourth, nonmammalian, 37-amino-acid nonamidated member of the peptide family, equally identical to NPY and PYY (64%), was found in fish (24); it is usually referred to as pancreatic peptide Y (PY).

Structure

NPY and its chemical relatives PYY and PP all feature a tertiary structure consisting of an N-terminal polyproline helix (residues 1–8) and an amphiphilic α-helix (residues 15–30), connected with a β-turn, creating a hairpin-like loop, which is sometimes referred to as the PP-fold (28). This domain has been identified from the crystal structure of avian PP, and nuclear magnetic resonance (NMR) studies support this three-dimensional configuration. The helices are kept together by hydrophobic interactions. The amidated C-terminal end (residues 30–36) projects away from the hairpin loop (29–31).

NPY Sequences

	1 10 20 30 36	Diff.
NPY human	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY	
NPY rat	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY	
NPY rabbit	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY	
NPY dog	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY	
NPY pig	YPSKPDNPGEDAPAEDLARYYSALRHYINLITRQRY	1
NPY cow	YPSKPDNPGEDAPAEDLARYYSALRHYINLITRQRY	1
NPY sheep	YPSKPDNPGDDAPAEDLARYYSALRHYINLITRQRY	2
NPY guinea-pig	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY	
NPY chicken	YPSKPDSPGEDAPAEDMARYYSALRHYINLITRQRY	1
NPY Rana	YPSKPDNPGEDAPAEDMAKYYSALRHYINLITRQRY	1
NPY goldfish	YPTKPDNPGEGAPAEELAKYYSALRHYINLITRQRY	5
NPY Torpedo	YPSKPDNPGEGAPAEDLAKYYSALRHYINLITRQRY	3
NPY dogfish	YPSKPDNPGEGAPAEDLAKYYSALRHYINLITRQRY	3
NPY Lampetra	FPNKPDSPGEDAPAEDLARYLSAVRHYINLITRQRY	6

PYY Sequences

	1 10 20 30 36	Diff.
PYY rat	YPAKPEAPGEDASPEELSRYYASLRHYLNLVTRQRY	
PYY pig	YPAKPEAPGEDASPEELSRYYASLRHYLNLVTRQRY	
PYY human	YPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY	2
PYY guinea-pig	YPSKPEAPGSDASPEELARYYASLRHYLNLVTRQRY	3
PYY frog	YPPKPENPGEDASPEEMTKYLTALRHYINLVTRQRY	9
PYY Raja	YPPKPENPGDDAAPEELAKYYSALRHYINLITRQRY	10
PYY dogfish	YPPKPENPGEDAPPEELAKYYSALRHYINLITRQRY	9
PYY Lampetra	FPPKPDNPGDNASPEQMARYKAAVRHYINLITRQRY	14
PYY Petromyzon	MPPKPDNPSPDASPEELSKYMLAVRNYINLITRQRY	14

PP Sequences

	1 10 20 30 36	Diff.
PP pig	APLEPVYPGDDATPEQMAQYAAELRRYINMLTRPRY	
PP dog	APLEPVYPGDDATPEQMAQYAAELRRYINMLTRPRY	
PP cat	APLEPVYPGDNATPEQMAQYAAELRRYINMLTRPRY	1
PP cow	APLEPEYPGDNATPEQMAQYAAELRRYINMLTRPRY	2
PP sheep	ASLEPEYPGDNATPEQMAQYAAELRRYINMLTRPRY	3
PP human	APLEPVYPGDNATPEQMAQYAADLRRYINMLTRPRY	2
PP rat	APLEPMYPGDYATHEQRAQYETQLRRYINTLTRPRY	8
PP mouse	APLEPMYPGDYATPEQMAQYETQLRRYINTLTRPRY	6
PP guinea-pig	APLEPVYPGDDATPQQMAQYAAEMRRYINMLTRPRY	2
PP chicken	GPSQPTYPGDDAPVEDLIRFYNDLQQYLNVVTRHRY	20
PP alligator	TPLQPKYPGDGAPVEDLIQFYNDLQQYLNVVTRPRF	19
PP bullfrog	APSEPHHPGDQATPDQLAQYYSDLYQYITFITRPRF	16

PY Sequences

	1 10 20 30 36	Diff.
PY gar	YPPKPENPGEDAPPEELAKYYSALRHYINLITRQRY	
PY salmon	YPPKPENPGEDAPPEELAKYYTALRHYINLITRQRY	1
PY bowfin	YPPKPENPGEDAPPEELARYYSALRHYINLITRQRY	1
PY eel	YPPKPENPGEDASPEEQAKYYTALRHYINLITRQRY	3
PY anglerfish	YPPKPETPGSNASPEDWASYQAAVRHYVNLITRQRY	11
PY sculpin	YPPQPESPGGNASPEDWAKYHAAVRHYVNLITRQRY	12

Figure 1 Alignments of all known NPY, PYY, PP, and PY amino acid sequences (25; D. Larhammar, personal communication). Dots mark differences (Diff.) from the top (master) sequence. The amide group has not been formally shown in every NPY sequence but is merely inferred from the presence of a glycine residue in the precursor and the presence of an amide group in all other NPY sequences.

Distribution of the Peptides

PERIPHERY The observation that NPY is present in most sympathetic nerve fibers, particularly around blood vessels, i.e. perivascular fibers, has generated much interest (4–8, 32, 33). Dense plexuses of NPY-like immunoreactivity (NPY-LI) are found in vascular beds throughout the body. The possible functional role of NPY as a sympathetic cotransmitter is discussed in some detail below. It is sometimes overlooked that NPY is also present in nonadrenergic perivascular (34), enteric (35), cardiac nonsympathetic (36), and selected parasympathetic nerves (37).

PYY, on the other hand, occurs mainly in endocrine cells in the lower gastrointestinal tract (37, 38). However, small amounts of PYY immunoreactivity are also found in certain sympathetic fibers (39). PP is located predominantly in endocrine cells of the pancreatic islets (37, 40).

BRAIN Neurons containing NPY-like immunoreactivity are abundant in the central nervous system, and perhaps are most notably found in so-called limbic structures (41, 42). The picture that has emerged over the past decade, principally from immunohistochemistry studies, is that NPY is contained within very similar neurons throughout the cerebral cortex and nuclei of the forebrain but in a variety of neurons in the hypothalamus, brain stem, and spinal cord. Coexistence with somatostatin and NADPH-diaphorase is common in the cortex and striatum. Although NPY neurons in the cortex and striatum receive few inputs, they make numerous contacts with dendrites; among their targets are GABA-ergic neurons. In the cortex, but not in the striatum, NPY is also extensively colocalized with GABA (43).

Many studies (reviewed in 42) have pointed out the occurrence of NPY in a variety of brain stem monoaminergic neurons, i.e. arguing that coexistence involves the peptide and (*a*) norepinephrine (the A1 group of the ventrolateral medulla, the A2 group of the dorsal medulla and the locus coeruleus), (*b*) epinephrine (C1 and C2 groups, and solitary nucleus), or (*c*) serotonin (nucleus raphe pallidus). It is sometimes overlooked that NPY also occurs in nonmonoaminergic brain stem cranial nerve nuclei.

In contrast to NPY, there are only a few PYY-containing neurons in the brain; they are confined mainly to the brain stem and the cervical spinal cord (44, 45). Finally, PP does not seem to occur in the central nervous system (CNS) (46).

Evolutionary Conservation Implies Functional Importance?

At present, at least 40 sequences of peptides belonging to the NPY family are known (Figure 1). NPY sequences are known for several mammals (human, pig, cow, sheep, rat, rabbit, and guinea pig), chickens, goldfish, and

the ray *Torpedo marmorata* (Figure 1). Sequence comparison data indicate that NPY is one of the most highly conserved neuroendocrine peptides known. For example, human NPY is 92% identical to *Torpedo* NPY (25).

The "true reason" behind the remarkable conservation of NPY is a matter of pure speculation. Naturally, many investigators have been tempted to suggest that such a degree of conservation pressure would imply an important functional role(s) of NPY, perhaps one or several of those described below.

The evolutionary relationships of the PPs to NPY and PYY cannot be easily resolved with the available sequence information. It has been proposed that the PPs arose through duplication of the PYY gene (25) and that the PP lineage has undergone extensive sequence divergence since then. The latter hypothesis was based on the relationship between gut and pancreatic endocrine cells, as well as the observation that the PYY and PP genes have a much more compact exon-intron organization than the known NPY genes.

NPY Gene Expression Is Tightly Regulated

The cDNA that encodes human NPY was first obtained from a pheochromocytoma (47, 48), a tumor of the adrenal medulla that often contains high concentrations of NPY (see below). The corresponding mRNA was found to contain a single open reading frame that predicted a fairly simple precursor for NPY, comprising 97 amino acids. The predicted precursor contains a hydrophobic signal peptide of 28 amino acids, necessary for entry into the lumen of the endoplasmic reticulum and thus entry into the secretory compartment of the cell. The signal peptide is then eliminated, leaving a prohormone of 69 amino acids. Unlike many other peptides, there is no peptide flanking the N-terminus of NPY. However, in the prohormone, mature NPY (36 amino acids) is flanked at its C-terminus by 33 amino acids, three of which are the glycine-lysine-arginine motif, necessary for NPY amidation (a feature that is critical for all known actions of NPY with the exception of mast cell degranulation; 49, 50). The peptide formed by the remaining 30 amino acids of the precursor has been named CPON (C-flanking peptide of NPY). Although CPON has been found to be highly conserved (not to the extreme degree of NPY itself, but indeed at a level comparable to that of insulin; 25), no function has yet been assigned to this peptide. Predictably, CPON is found in every cell that produces NPY, and the two peptides are consequently coreleased (30, 51).

The NPY gene is expressed in cells derived from the neural crest and is tightly regulated by a number of factors. Thus, the NPY gene contains consensus sequences for a number of DNA-binding proteins that could act as regulatory factors. These include five potential G +C-rich SP-1-binding sites, two CCCCTC sites, a partial CAAT box, and one AP-1-binding site (52). The latter AP-1 site of the NPY gene shows no activity (53), an unexpected finding

in view of the potent actions of phorbol esters and nerve growth factor (52, 54) on NPY expression. Additional factors regulating NPY gene expression include activators of cyclic AMP (cAMP) and calcium- or phospholipid-dependent protein kinases (52, 54).

Thus, although NPY gene expression is tightly regulated by many factors, it is not known whether any specific factors are involved. In other words, potential pharmacological intervention of NPY gene expression may affect the expression of many other genes as well. A similar argument could be used against attempts to develop drugs interfering with NPY peptide-processing mechanisms; it is likely that such drugs would also affect the processing of many other bioactive peptides. Hence, if one wishes to achieve NPY specificity in a novel drug, the best chances are probably to seek a compound that exhibits affinity to NPY receptor(s).

NPY RECEPTORS

Very much like "classical" neurotransmitters (e.g. its frequently coexistent partner, norepinephrine), NPY appears to exert a wide range of effects on peripheral (blood vessels, heart, airways, gastrointestinal tract, kidney, pancreas, thyroid gland, platelets, mast cells, and sympathetic, parasympathetic, and sensory nerves) and central (pituitary hormone release, behavior, central autonomic control, and other neurotransmitter mechanisms) targets (reviewed in 6, 7, 20, 181). Some of these actions seem to be exerted by NPY per se, whereas others occur as a result of modulatory interactions with other agents, e.g. norepinephrine and glutamate (see below). In any event, it is likely that NPY (as well as its chemical relatives) acts upon membrane receptors that are dependent on guanine nucleotides, i.e. G-protein coupled receptors (6, 55, 56).

Radioreceptor ligand studies with iodinated or tritiated NPY have detected binding sites in the brain, vasculature, heart, kidneys, spleen, and uvea, whereas binding sites labeled by ^{125}I-PYY have been found in the brain and intestine (reviewed in 6). Moreover, autoradiographic studies of the brain have found regionally selective binding of ^{125}I-NPY and ^{125}I-PYY (57, 58), probably reflecting slightly differing affinities to the receptor subtypes described below.

NPY Receptors Are Heterogeneous

Y1 AND Y2 RECEPTORS Heterogeneity among NPY and PYY receptors, and the existence of Y1 and Y2 receptors, were first (59) proposed on the basis of studies on sympathetic neuroeffector junctions (see also 7, 59–61) and later corroborated for several other cell types and experimental systems (reviewed

in 6). With regard to NPY actions at sympathetic junctions (see below), we and others had, by the mid-1980s, demonstrated three types of NPY effects at sympathetic neuroeffector junctions: (*a*) a direct postjunctional response, e.g. vasoconstriction manifested in certain vascular beds; (*b*) a postjunctional potentiating effect on norepinephrine-evoked vasoconstriction; and (*c*) a prejunctional suppression of stimulated norepinephrine release (6, 59–61) (Figure 2). Incidentally, the last two phenomena are probably reciprocal; thus, norepinephrine may affect NPY mechanisms similarly (6, 62) (Figure 2).

Thus, in the mid-1980s, to compare the effects of NPY with those of several chemically related peptides and peptide fragments, we characterized some NPY-responsive smooth muscle preparations designed to represent each of the three above-described types of actions of NPY at sympathetic neuroeffector junctions (59). In all cases NPY and PYY were both active while PP displayed much weaker activity; additionally desamido-NPY (NPY free acid) was found

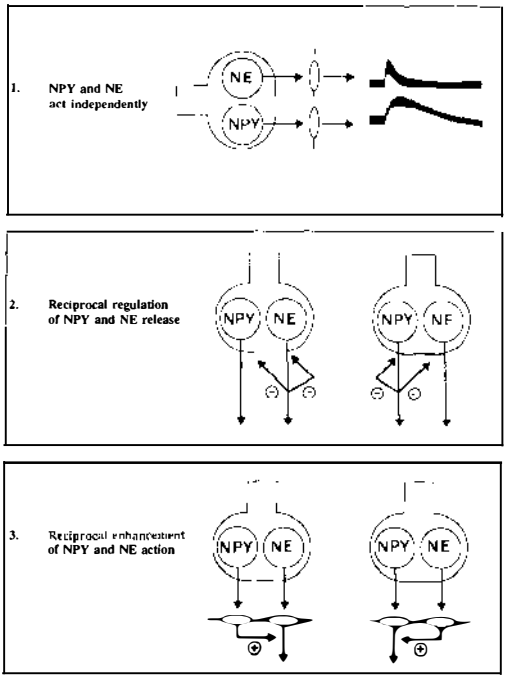

Figure 2 The three prinicipal types of synaptic/junctional actions and interactions of norepinephrine (NE) and neuropeptide Y (NPY). Models are based primarily on studies of a variety of assays of sympathetic neuroeffector junctions in the cardiovascular system. For simplicity, the presence and actions of additional cotransmitters, notably ATP, are not shown. Tracings in the upper right-hand corner show typical pressor responses to norepinephrine and NPY (note that the peptide response is slower in onset and more long-lasting, as is most often the case in other assays as well). See the text for a discussion and references.

to be virtually inactive, thus making it an ideal compound for use in any control experiment involving NPY. The main finding of this work was that long C-terminal amidated fragments of NPY and PYY, while being essentially inactive in the assays for postjunctional activity (per se effect as well as potentiation), retained their efficacy prejunctionally (inhibition of transmitter release). On the basis of the selective prejunctional effect of C-terminal NPY (or PYY) fragments, it was proposed that NPY/PYY receptor subtypes may exist (7, 59). The terms Y1 and Y2 were introduced to denote the receptor that required the whole NPY (or PYY) molecule for activation (Y1) and the receptor that was selectively stimulated by the long C-terminal NPY (or PYY) fragments (Y2) (7, 60). In these neuromuscular preparations, the postjunctional receptors were thus (predominantly) of the Y1 subtype. However, more recent experiments conducted by ourselves and others have indicated that although the Y1 receptor indeed appears to be the major vascular NPY (or PYY) receptor, the Y2 receptor can also occur postjunctionally on vascular smooth muscle (6). (There seems to be an analogy to α_2-adrenoceptors, which were originally thought to be exclusively prejunctional but were subsequently found to be located on vascular smooth muscle also.)

Y3 RECEPTORS Over the past few years it has become increasingly evident that some actions of NPY cannot be mimicked by PYY. This has become the major argument supporting the existence of an exclusively NPY-responsive Y3-type receptor. This receptor is likely to be present in, e.g., adrenal medulla, heart, and brain stem (17–19).

THE Y1 RECEPTOR

Presence on Vascular Smooth Muscle Cells

From initial bioactivity studies the Y1 receptor was suggested to be postjunctional at the vascular sympathetic neuroeffector junction (59). A large number of binding studies have demonstrated the presence of NPY/PYY receptors in vascular smooth muscle (6, 62–66). In attempts to identify the vascular NPY/PYY receptor subtype on vascular smooth muscle cells, it was found that the Y1 receptor agonist [Pro34]NPY and NPY were equally effective in displacing radiolabeled PYY in a monophasic manner, suggesting a homogeneous population of Y1 receptors (64, 66). These binding sites have recently been visualized by an autoradiographic approach at the electron-microscopic level. A small population of Y1-binding sites was also detected on the vascular endothelium (64). The recent cloning of the Y1 receptor cDNA has provided strong evidence (67) that fully supports the concept that vascular smooth muscle cells express the Y1 receptor (see below).

The NPY-evoked increase in arterial blood pressure appears to be fully mediated by Y1 receptors, since Y1 receptor agonists are as potent and efficacious as NPY/PYY (6, 66, 68, 69). In contrast, NPY 2-36 and shorter C-terminal fragments are markedly less potent or inactive (66). NPY is able to increase the vascular resistance in various vascular beds of many species (9, 70–72). By the use of the Y1 receptor agonist [Pro34]NPY or C-terminal NPY fragments in some bioactivity assays, the presence of Y1 receptors has been suggested in rat and guinea pig coronary vessels (66, 73), guinea pig inferior vena cava (66), and blood vessels in the rabbit maxillary sinus (74). Removal of the endothelium does not affect the NPY-evoked vasoconstriction, suggesting that the vascular rather than the endothelial Y1 receptor mediated the vasoconstriction (7, 75). NPY-evoked vasoconstriction in vivo (66, 76–78) and in many but not all isolated vessels in vitro (32, 79, 80) appears to rely in part on influx of extracellular Ca^{2+} into smooth muscle cells, since several L-channel-type Ca^{2+} channel antagonists were shown to attenuate NPY responses. A very different agent, Ins[1,2,6]P_3, was also found to reduce NPY-induced Ca^{2+} influx and associated vasoconstriction in vivo and in vitro (81, 82).

It is well known that NPY is also able to potentiate the vasoconstriction evoked by norepinephrine and other vasoactive agents both in vivo and in vitro (32, 76, 80, 83, 84). The Y1 receptor seems to mediate the enhancement of norepinephrine-evoked vasoconstriction, since NPY/PYY,
NPY/PYY fragments (e.g. PYY 13–36 and NPY 11–36), has this ability (59, 85). Whether this response is (in part) endothelium dependent is still controversial (86–88). Like the direct NPY-evoked contractile effect, the potentiation by NPY of norepinephrine-induced vasoconstriction has been suggested to be Ca^{2+} dependent (80, 89). It is important to point out that the potentiation is seen at low NPY concentrations, usually lower than those required to elicit direct vasoconstriction; for this and other reasons, it may be assumed that the same Y1 receptors are responsible for direct and potentiating effect of NPY in vasculature.

A third NPY mechanism promoting increased vascular tone may rely on the inhibition of vasodilation by the peptide (84, 90). In this case Y1 receptors, presumably the same population as referred to above, must also be activated (82).

Thus, the predominant vascular NPY/PYY receptor seems to be of the Y1 type. However, there is functional and biochemical evidence to suggest a mixture of vascular vasoconstriction-related Y1 and Y2 receptors in some vascular beds (66, 91). NPY is known to affect a variety of peripheral effectors outside the vascular bed (reviewed in 6). However, many of these responses have not been characterized in terms of receptor subtype classification, and it is not always clear whether the observed effects are secondary to vasoconstriction.

Y1 Receptors in Brain

Autoradiography studies using the Y1 receptor agonist [Pro34]NPY on frozen sections of rat brain have indicated that Y1 receptors are discrete (being much less abundant than Y2 receptors) and are localized primarily to distinct layers of the cerebral cortex, anterior olfactory nucleus, and a few thalamic and hypothalamic nuclei (92, 93). Furthermore, in situ hybridization with rat Y1 receptor cDNA (see below) has localized the expression of this receptor protein in the thalamus, cerebral cortex, dentate gyrus of the hippocampus and arcuate nucleus of the hypothalamus (94, 95; Wahlestedt et al, unpublished data).

The Y1 receptor in the CNS has been linked with a few different biological actions, for instance with NPY-induced stimulation of feeding behavior (e.g. 13). Central administration of NPY potently stimulates feeding behavior in rats, and this effect can be mimicked by the Y1 agonist [Leu31, Pro34]NPY, whereas the Y2 agonist NPY 13–36 is much less active. However, uncharacteristic of the Y1 receptor, NPY 2–36 is at least as potent as NPY itself (for references, see 13). Taken together with recent negative in situ hybridization data in hypothalamic regions thought to be related to feeding obtained by using Y1 receptor probes (94, 95), this seems to suggest the possibility that feeding is evoked by stimulation of a novel and unique NPY receptor (not Y1, Y2 or Y3, and pharmacologically most closely resembling the Y1 receptor). Stimulation of luteinizing hormone-releasing hormone is another central effect of NPY that has been suggested to be mediated by Y1 receptors (96). Finally, Heilig et al have argued that NPY-induced reduction of spontaneous locomotor activity (97, 98) and, more importantly, anxiolysis (14–16, 99, 100) may be mediated through central Y1 receptors (see below).

Y1 Peptide Ligand Signal Epitopes

NPY and its related peptides, in contrast to most other small peptide messengers, retain a distinct tertiary structure in solution (101). There has been a considerable interest in structure-function studies of NPY-related peptides in attempts to develop receptor-specific agonists and antagonists. These experiments have been extensively pursued by using different NPY fragments and analogs in both receptor assays and different biological preparations. In general, PYY is equipotent and equally effective with NPY in all Y1 (and Y2) receptor assays studied (6).

N-TERMINAL END OF NPY Characteristic of the Y1 receptor is that a truncation of the first N-terminal residue Tyr1 (NPY 2–36) results in a marked loss of biological activity or affinity (19, 66, 67, 73). Further N-terminally truncated NPY fragments are less potent or even inactive. For instance, NPY 13–36 is about 100–400 times less potent than NPY at this receptor (19). The demand for an intact N-terminus is distinctly illustrated by the fact that a single

substitution of the Y1 receptor agonist [Pro34]NPY from Lys4 to Glu4 ([Glu4, Pro34]NPY) results in a loss of affinity to the Y1 receptor (28). Furthermore, systematic D-substitutions in any of the first five amino acid residues in the N-terminal end of NPY result in a marked loss of potency at the Y1 receptor (102). Thus, the Y1 receptor requires an intact N-terminus of NPY to become fully activated.

DEMAND ON THE C-TERMINUS OF NPY This is slightly less stringent, since substitutions from Ile to Leu at position 31 and from Gly to Pro at position 34 results in analogs ([Pro34]NPY and [Leu31, Pro34]NPY), which retain full activity on Y1 receptors while being virtually inactive at Y2 receptors (cf 19). It has been suggested that deamidated NPY-related peptides no longer form a stable tertiary structure (101), perhaps explaining their virtual lack of affinity and activity in any NPY test system with the exception of the mast cell degranulation assay (see below).

HAIRPIN LOOP OF NPY The hairpin loop of NPY (the PP fold) has been suggested to present the N- and C-terminal close together for recognition at the Y1 receptor (28). Substitution of the hairpin loop with small bridging constructs has been performed to delineate the part of the ligand that activates the receptor (103, 104). Several centrally truncated NPY analogs with intact N- and C-termini are quite potent at the Y1 receptor, suggesting that the center part of the NPY molecule is not involved in Y1 receptor recognition (19, 105). The importance of the close steric arrangement of the N- and C-termini is illustrated by the fact that substitution from Tyr to Pro in position 20 in [Pro20]NPY breaks the hairpin-like loop, resulting in a loss of affinity for the Y1 receptor (cf 19).

Molecular Biology of the Y1 Receptor

Although Y1 receptors were expressed heterologously in *Xenopus* oocytes a few years ago (6), this expression system did not allow cloning of the receptor. Instead, its initial cloning from a rat forebrain cDNA library relied on its homology with already cloned members of the G-protein-coupled superfamily of receptors (94). However, Eva et al were not able to identify their clone, named FC5, and it remained an "orphan" receptor clone when published. The distribution of FC5, as assessed by in situ hybridization in rat brain, was rather distinct (94), and the pattern showed certain similarities with the autoradiographic distribition of the Y1 receptor (see e.g. 93). Therefore, we (67) hypothesized that the "orphan" FC5 clone (95) might correspond to a rat Y1 receptor. The corresponding cDNA was isolated by the polymerase chain reaction (PCR). The resulting PCR product was then used to screen a human fetal brain cDNA library, and following identification of a positive clone, hY1-5, (67) and its sequencing and insertion into an expression vector, COS1

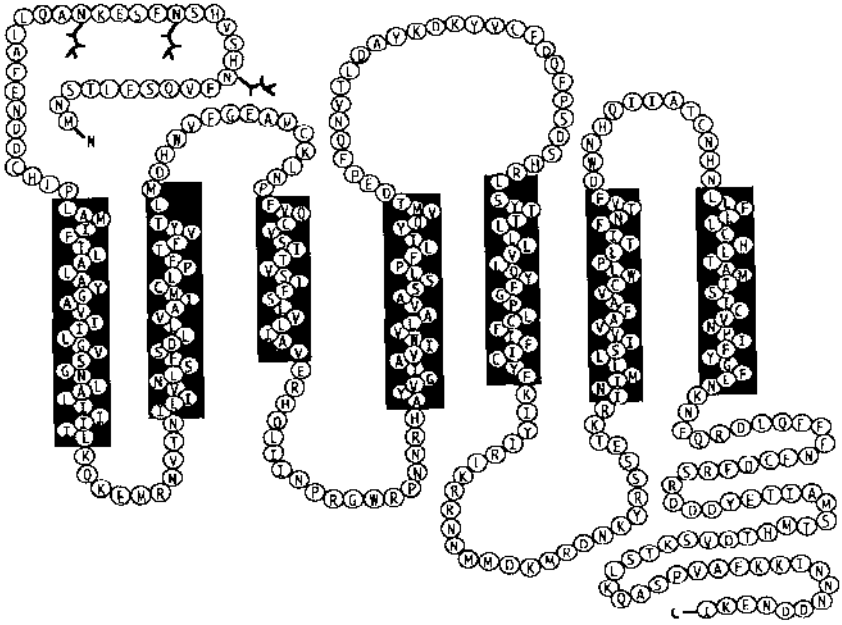

Figure 3 Amino acid sequence and putative membrane topography of the human NPY and PYY Y1 receptor (67). Shaded areas represent the seven hydrophobic membrane-spanning domains, the N-terminus presumably being extracellular. Possible N-linked glycosylation is indicated.

cells were transfected. The latter cells bound ^{125}I-PYY with high affinity and a rank order of potency of competing ligands appropriate for a Y1 receptor, i.e. PYY ≥ NPY ≥ [Leu31, Pro34]NPY >> NPY2–36 > C2NPY > pancreatic polypeptide > NPY 13–36 > NPY 18–36. Also, since appropriate second-messenger responses to NPY and PYY, i.e. reduction of cAMP accumulation and elevation of intracellular Ca^{2+}, were found, it was concluded that hY1-5 indeed encodes a human Y1 receptor (67). Independently, a human Y1 receptor was cloned by Herzog et al (106). The amino acid sequence of this putative human Y1 receptor is shown in Figure 3.

THE Y2 RECEPTOR

Y2 Receptors Are Present on Sympathetic Neurons and Other Peripheral Cells

Y2 receptors in the periphery are generally considered to be localized at prejunctional sites at the sympathetic neuroeffector junction, suppressing the release of transmitters (59, 85). Y2 receptors may also be located on other nerve fibers, like parasympathetic (107) and sensory (108) C-fibers.

The rat vas deferens is a widely used preparation for the study of Y2 receptors. In attempts to characterize this receptor, it has been shown that NPY and PYY and a large number of C-terminal fragments of NPY/PYY (2–36 to 22–36) quite effectively suppress the electrically paced (sympathetically mediated) twitches in a concentration-dependent manner (59, 109, 110). Similarly, NPY and PYY, as well as several C-terminal NPY fragments, have the ability to attenuate stimulated ion secretion in the rat small intestine, suggesting the presence of Y2 receptors (111, 112). Furthermore, C-terminal NPY fragment (NPY 11–36 to NPY 16–36) have been shown to be as effective as the parent molecule in suppressing the release of norepinephrine in the rat mesenteric artery, which is consistent with the presence of Y2 receptors on sympathetic fibers (59, 85).

Both NPY and NPY 13–36 are able to suppress sensory C fiber-mediated contractions of the guinea pig uterine cervix (107), which suggests a direct action via Y2 receptors on these nerve fibers. Moreover, there is functional and biochemical evidence to suggest the presence of vasoconstriction-related Y2 receptors in the pig spleen (114), guinea pig caval vein (66), and rat mesenteric artery (74). Y2 receptors have recently been suggested to inhibit mucociliary activity in the rabbit maxillary sinus (74). The Y2 receptor is also abundant in the kidneys, having been localized to the proximal tubules by an autoradiographic approach at the electron microscopic level (116). Finally, Y2 receptors have also been suggested to occur on rat platelets (117).

Y2 Receptors Are Widely Distributed in the Brain

Autoradiographic data obtained from the rat brain by using radiolabeled PYY, displaced by Y1 or Y2 selective ligands, have suggested a differential localization of Y1 and Y2 receptors (92, 93). It appears that the Y2 receptor is the quantitatively predominant receptor type in the rat brain (56, 93, 118).

A dense population of Y2 receptors occurs in the hippocampus (92, 93). Previous ligand-binding data from rat and pig hippocampus homogenates suggest that this tissue may contain a homogeneous population of Y2-binding sites (119–121). Importantly, electrophysiological studies by Colmers et al have clearly demonstrated the presence of the Y2 receptor excitatory inputs to rat hippocampal CA1 neurons (122). Thus, NPY, PYY, and several C-terminal NPY fragments were able to suppress the release of glutamate onto rat hippocampal CA1 neurons via a prejunctional mechanism (122, 123). There was a gradual loss of potency with a progressive N-terminal truncation of the molecule, which is characteristic of Y2 receptors (122). Similarly, in a behavioral study, NPY 20–36 (just like NPY itself) was shown to enhance memory retention, probably reflecting a Y2 receptor-mediated action in the rostral part of the hippocampus (124). The presence of Y2 receptors and the associated inhibition of Ca^{2+} influx have also been clearly demonstrated in rat dorsal root ganglion neurons (125).

Y2 Peptide Ligand Signal Epitopes

N-TERMINAL END OF NPY This is less important for activation of the Y2 receptor than of the Y1 receptor. Hence, NPY 2–36 is, in contrast to the Y1 receptor, about equipotent with NPY or PYY, and N-terminally truncated NPY fragments from NPY 2–36 to 22–36 are rather potent. Table 1 shows the relative potencies of various NPY-related analogs at Y2 receptors. For instance, NPY 13–36 is about 5–10 times less potent than the parent molecule. PYY and its C-terminal fragments appear to be slightly more potent than the corresponding NPY peptide (59, 60, 109). The breaking point of biological activity seems to be between position 25 and 26. Thus, the 12 C-terminal amino acid residues of NPY/PYY are the minimum length required to activate this receptor (109, 110).

C-TERMINAL END OF NPY The Y2 receptor is very stringent in its demand for this part of the NPY/PYY molecules, since an intact C-terminal end of NPY/PYY is required for activation of the Y2 receptor. This is best illustrated by the fact that a substitution from Gly to Pro in position 34 in [Pro34]NPY results in a loss of affinity for the Y2 receptor. It is well established that N-terminal fragments of NPY are inactive (6, 31, 59, 60, 126, 127). From studies in which amino acid residues have been substituted systematically, it has been suggested that His26 is important for the recognition of the Y2 receptor (128). Not only is the amino acid sequence of the C-terminal of NPY essential, but also the C-terminal amide group, since desamido-NPY and the C-terminally extended form NPY-Gly-Lys-Arg fail to activate the Y2 receptor (59).

Table 1 Proposed agonist rank order of potency of NPY and related peptides at the receptors for NPY and related peptides in prototypical tissues and cultured cells (see text for species and other information):

Receptor	Peptides	Tissue
Y1	PYY ≥ NPY ≥ [Pro34]NPY >> NPY 13–36	Cloned and expressed Y1 receptor, blood vessels, smooth muscle cells, cerebral cortex, cortical neurons, SK-N-MC neuroblastoma cells
Y2	PYY > NPY > NPY 13–36 >> [Pro34]NPY	Nerve endings, renal tubules, hippocampus, SK-N-BE(2) neuroblastoma cells
Y3	NPY ≥ [Pro34]NPY ≥ NPY 13–36 >> PYY	Brain stem (NTS), cardiac membranes, adrenal medullary chromaffin cells

HAIRPIN LOOP The hairpin loop thus serves to bring the N- and C-termini close together (28). Several NPY analogs with the center part of the molecule substituted with links between the N- and C-termini were shown to be quite potent, suggesting that the hairpin loop of the molecule is not involved in the recognition at the Y2 receptor per se (19). The importance of an intact C-terminus but not a hairpin loop is also illustrated by the observation that Pro in position 20 in [Pro20]NPY, which breaks the hairpin loop, is a full Y2 receptor agonist (104). Exchange of the hairpin loop of NPY with that in PP in PP (1–30)/NPY (31–36) results in a hybrid peptide that retains high affinity for the Y2 receptor (104). Conversely, NPY (1–30)/PP (31–36) does not recognize this receptor. The proposed rank order of potency of NPY-related peptides on Y2 receptors is shown in Table 1.

Biochemistry and Molecular Biology of Y2 Receptors

The Y2 receptor has not yet been cloned, but several groups have pursued its biochemical isolation. Thus, the structure of Y2 receptors from several tissues and species have been probed by using affinity-labeling techniques (120, 129, 130). In rat and pig hippocampal membranes and in basolateral vesicles from the rabbit kidney, an M_r 50,000 molecule was the major labeled species, in contrast with the predicted M_r of 70,000 for the Y1 receptor (129).

Several groups have solubilized Y2 receptors in a functional state (i.e. gentle solubilization resulting in a state in which the receptor is still able to bind ligand) from the cerebral cortex, kidneys, and spleen (129–133). Since very few peptide hormone receptors retain their original ligand affinity and specificity in the purified state, the kidney Y2 receptor may be an interesting model to probe for functional activity when reconstituted with purified G-proteins in phospholipid vesicles.

THE Y3 RECEPTOR

In an increasing number of binding and bioactivity studies on various tissues and cultured cells, the pharmacological order of potency of NPY and related peptides differs markedly from those of Y1 and Y2 receptors, pointing to the existence of a Y3 receptor. The main characteristic of these Y3 receptors is that they recognize NPY, whereas PYY is several orders of magnitude less potent. There is much evidence to suggest the existence of such specific NPY receptors in both the brain and periphery. These NPY receptors have thus been referred to as Y3 receptors (17, 18).

Y3 Receptors in the Brain: Presence in the NTS

By injecting NPY and related peptides into the nucleus of the tractus solitarius (NTS), receptors mediating the cardiovascular effects thus evoked were recently characterized (134, 135). Unilateral injections into the NTS of NPY,

the Y1 receptor agonist [Pro34]NPY, or the Y2 receptor agonist NPY 13–36 evoked comparable dose-dependent decreases of arterial blood pressure and heart rate in anesthetized rats. However, the Y1/Y2 receptor agonist PYY was inactive, as were PP and desamido-NPY (134, 135). Since Y1 and Y2 receptor agonists were equally active, one explanation would be that both Y1 and Y2 receptors mediate the same cardiovascular effects in the NTS. However, injection into the NTS of PYY, which is about equipotent with NPY on peripheral Y1/Y2 receptors (e.g. 6, 28), had no effect. Also, Tseng et al (136) found PYY to be virtually inactive in the NTS. Moreover, quantitative autoradiography has shown that NPY, but not PYY or PP, displaced radiolabeled NPY in the rat brain stem (137). Functional and biochemical studies have suggested interactions between NPY and α_2-adrenoceptors in the NTS (138–140) but not between PYY and α_2-adrenoceptors (139). Together these data indicate the existence of a specific NPY receptor, i.e. the Y3 subtype referred to above.

The cardiovascular effects of NPY in the NTS have been linked to G-proteins, which are sensitive to pertussis toxin (140). Furthermore, in vitro NPY seems to suppress the stimulated formation of adenylate cyclase in the NTS (139). Thus, the specific NPY receptor, Y3, in the NTS seems to be coupled to G-protein(s).

As noted above, NPY-containing neurons are abundant in the CNS (41, 42), while PYY-immunoreactive neurons seem to be present only in the brain stem, (44, 45). Therefore, it may be of functional relevance that NPY acts upon a specific NPY receptor in the NTS, thereby preventing local PYY from interfering with NPY signaling.

It is possible that Y3 receptors are also present in the hippocampus. Thus, in CA3 neurons of the rat hippocampus NPY, [Leu31, Pro34]NPY, and NPY 13–36, but not PYY or PP, were found to potentiate the excitatory response to the glutamate receptor agonist N-methyl-D-aspartate (NMDA) (142). In contrast, a recent slice-patch study found that NPY did not alter NMDA conductances in CA3 pyramidal neurons (143).

Y3 Receptors in the Periphery

NPY-specific binding sites (Y3) have recently been suggested to occur also in the periphery. In rat cardiac membranes and bovine adrenal chromaffin cells, NPY, [Pro34]NPY, and NPY 13–36 potently displaced radiolabeled NPY, whereas PYY and PP were several orders of magnitude less potent (18, 121, 144). It is thought that Y1 as well as Y2 receptors equally well recognize [Tyr36]^{125}I-NPY and [Tyr1]^{125}I-NPY (145). In contrast, in cardiac membranes [Tyr1]^{125}I-NPY has a higher specific binding than [Ty1]^{125}I-NPY, further supporting the existence of binding sites that are distinct from Y1 and Y2 receptors (144). Moreover, in sharp contrast to NPY, radiolabeled PYY did not bind to chromaffin cells (18). In another study, NPY but not PYY was

shown to inhibit nicotine-stimulated release of catecholamines from the adrenal medulla (146). Like the NPY receptors in the NTS, the NPY receptors in the adrenal medulla (18) and heart (144) seem to be coupled to G-proteins. Conceivably, these properties are those of a common specific NPY receptor subtype, Y3, which is similar in the brain, heart, and adrenal medulla. The proposed rank order of potency of NPY-related peptides on the tentative Y3 receptor is shown in Table 1.

Y3 Peptide Ligand Signal Epitopes

The main characteristic of the NPY-specific Y3 receptor is therefore that, in contrast to Y1 and Y2 receptors, it does not recognize PYY. The N-terminus of NPY appears to have some importance for activation of this receptor, since NPY 13–36 has 20–40 times lower affinity than the intact molecule. The shape of the hairpin loop of NPY could be very important for receptor recognition. As shown in Figure 1, the main differences between NPY and PYY are found in the 13–23 segment, where PYY differs from NPY in 7 of 11 positions. As at the Y1 receptor, a change to Leu at position 31 and to Pro at position 34 of NPY has virtually no effect on the binding affinity of the Y3 receptor. Moreover, like Y1 and Y2 receptors, Y3 receptors require an amidated C-terminal in order to become activated.

Has the Y3 Receptor Been Cloned?

Like the Y1 receptor, a proposed bovine Y3 receptor clone (147) was isolated on the basis of its nucleotide homology with other members of the G-protein-coupled superfamily of receptors. Thus, Rimland et al (147) were able to isolate a fragment of the receptor by PCR with a bovine locus coeruleus cDNA library as the template; this was followed by the identification and sequencing of a full-length clone, called LCR1. After some time as an orphan receptor, LCR1 was found to confer upon transfected cells high-affinity ^{125}I-NPY binding sites (147). More recently, we (141) isolated a corresponding human clone and were unable to establish that this clone confers upon transfected cells NPY-binding sites or NPY-associated second-messenger responses. This was not altogether surprising since the Y1 and the proposed Y3 receptors seem to be only distantly related; for instance, the proposed Y3 receptor appears to be more closely related to the interleukin-8 receptor than to the Y1 receptor (141). It is therefore unclear whether the Y3 receptor really is cloned, and the literature awaits confirmatory results with the LCR1 clone and/or corresponding clones isolated from other species.

ARE THERE OTHER RECEPTOR SUBTYPES?

We believe that the putative subclassification of NPY/PYY receptors into Y1, Y2, and Y3 subtypes will be further corroborated in the next few years,

particularly as the receptor-cloning work progresses. Nevertheless, five lines of evidence have suggested even further heterogeneity among the NPY/PYY/PP receptors, indicating the existence of additional subtypes. First, and perhaps most importantly, it may be that food intake is mediated by a hypothalamic receptor that is similar but not identical to the Y1 receptor (see the sections on the Y1 receptor and on food intake). Second, it was recently proposed that NPY and PYY may bind to brain sigma- and phencyclidine-binding sites (148); however, this finding was not reproduced by others (149), and therefore this issue is unresolved. Third, PP appears to have its own receptors distinct from Y1, Y2, and Y3 (see e.g. 28) For instance, PP has been shown to bind with high affinity to binding sites on a pheochromocytoma cell line (PC 12); NPY or PYY bound poorly to these PP-selective binding sites (28, 150). Moreover, in the rat brain, selective PP-binding sites have been found in various areas that are permeable at the blood-brain barrier, such as the area postrema and adjacent nuclei (151). Also, chicken brain may indeed have specific avian PP-binding sites (152). Fourth, we and others have observed that short C-terminal fragments, in sharp contrast to NPY itself, induce hypotension upon systemic administration (6, 7, 49, 50, 102, 153, 154); as discussed in the following section, it has been argued that this mast cell degranulation occurs independently of specific NPY receptors. Fifth, a *Drosophila* NPY receptor was recently cloned (155); it is not known whether mammalian homologs exist (see below).

Non-Receptor-Mediated Mast Cell Degranulation

The effects of NPY in the peripheral cardiovascular system have been concerned mainly with vasoconstriction and increase in arterial pressure. However, several investigators have, in addition, observed vasodepressor responses to NPY and its C-terminal fragments in vivo but not in vitro. Thus, systemic injection of C-terminal NPY fragments, such as NPY 13–36 (7), NPY 17–36, and NPY 18–36 (153, 154, 156, 157), elicits a marked hypotension in rats. However, the mechanism behind the depressor response was not revealed initially. During characterization of this response, it was shown that high doses of NPY 18–36 amd NPY 22–36, as well as of NPY itself, evoke a brief and transient pressor response, followed by long-lasting depressor response in both conscious (50) and pithed (49) rats. The depressor response, but not the pressor response, to NPY or C-terminal NPY fragments is prevented by pretreatment with histamine H1 antagonists or the histamine liberator compound 48/80, indicating that NPY and C-terminal NPY fragments are capable of releasing histamine from mast cells (49, 50).

The effect profile of the NPY-related peptides on rat peritoneal mast cells suggests that histamine is released by a mechanism that is distinct from those

associated with Y1, Y2, and Y3 receptors. Conceivably, positively charged amino acid residues at the C-termini of NPY and PYY activate G-proteins in the mast cell membrane by a nonreceptor mechanism, as has been suggested for tachykinins and certain other peptides (for a review, see 158). In support of a nonreceptor mechanism is the very rapid kinetics (<10 s) of the NPY-evoked histamine release (50, 159). Whether the NPY-evoked release of mast cell histamine is physiologically relevant is unclear. Interestingly, mast cells are particularly numerous around blood vessels and nerves (160, 161). Possibly, therefore, NPY-evoked release of mast cell histamine contributes to sympathetic control of the microcirculation in certain vascular beds.

With respect to the development of NPY-related drugs for systemic use, it may prove to be advantageous to avoid compounds with too many positively charged amino acids. In this way, mast cell degranulation is not likely to occur.

A Drosophila NPY Receptor Has Been Cloned

Very recently, Li et al. (155) isolated a cDNA clone, PR4, from *Drosophila melanogaster* by a PCR-based homology approach. Upon microinjection of in vitro-transcribed mRNA from this cDNA clone, *Xenopus laevis* oocytes responded to NPY and related peptides in a manner typical of phosphatidyl inositide (PI)-coupled agonists, i.e. by an electrophysiological response reflecting Ca^{2+} mobilization and consequent activation of Ca^{2+}-dependent Cl^- channels. Such coupling makes it less likely that this *Drosophila* receptor corresponds to any of the mammalian NPY/PYY receptor subtypes discussed above, since they couple differently. Also, the rank order of potency, PYY > C2-NPY > NPY > [Pro34]NPY, differs from rank orders for previously discussed NPY/PYY receptors. Finally, attempts to isolate mammalian clones corresponding to PR4 have been unsuccessful (M. Forte, personal communication).

NPY RECEPTOR SIGNAL TRANSDUCTION

Inhibition of cAMP Accumulation

It is well established that most, if not all, NPY/PYY receptors couple to inhibition of adenylate cyclase and hence decreased levels of cAMP (6, 67, 162–166). Thus, inhibition of adenylate cyclase and/or cAMP accumulation has been demonstrated in peripheral tissues such as arteries (163–166), vas deferens (167), spleen (114), and pancreas (166). The same phenomenon has been observed in brain regions, e.g. cortex (7,168), hippocampus (166, 169), striatum (168), and medulla oblongata (141). Adenylate cyclase of neu-

roblastoma cells (SK-N-MC) also responds similarly to NPY (see e.g. 6, 170). In at least three instances when Y1 receptors were studied, i.e. rat arterial cells (164), SK-N-MC (6, 170), and human erythroleukemia cells (171), the phenomenon was blocked by pretreatment of the cells with pertussis toxin, indicating a G-protein linkage between the receptor and adenylate cyclase. Other effects associated with NPY and possibly Y1 receptors have also been shown to be sensitive to pertussis toxin (for references, see 6). However, other studies of possibly Y2-containing tissues, i.e. vas deferens (7), hippocampus (172), and sympathetic nerve terminals of the heart (173), have found that pertussis toxin does not affect the actions of NPY.

Elevated Intracellular Ca^{2+} Levels

In many cell types, e.g. vascular smooth muscle cells (81, 174), dorsal root ganglion cells (175), erythroleukemia cells (171), SK-N-MC cells (18, 176), and bovine adrenal chromaffin cells (18), NPY raises the intracellular Ca^{2+} concentrations. In addition, NPY has weak to moderate effects on PI turnover in the brain (7, 60, 177), dorsal root ganglion cells (175, 178, 179), vas deferens (167), and vasculature (62). At present, it cannot be excluded that the effect of NPY on PI turnover is secondary to a more powerful effect on intracellular Ca^{2+}, which in turn may stimulate phospholipase C, resulting in an apparent PI response (62, 166). Calcium ion influx into cultured cells has been demonstrated following stimulation of Y1 and Y3 receptors (6, 18, 67, 81).

Coupling May Not Distinguish Receptor Subtypes

The study of second-messenger systems may not be helpful in providing a basis for receptor subtype classification, since the Y1, Y2, and Y3 receptors seem capable of activating the same intracellular pathways in many systems, resulting in reduced cAMP accumulation and elevated intracellular Ca^{2+} concentrations (6, 17, 18). It is still not known whether one and the same G-protein (probably G_i or G_o) mediates the coupling to cAMP and Ca^{2+}. However, through the recent cloning and heterologous expression of the human Y1 receptor, it has become evident that stimulation of this receptor subtype a given cell (CHO or COS-1) can result in changes in both cAMP and Ca^{2+} levels; however, in 293 cells no Ca^{2+} coupling was observed (67, 106, 162). Thus, there is a distinct possibility that NPY/PYY receptors can couple to multiple G-proteins, although this has not been definitively shown. To date there is, however, no evidence that convincingly demonstrates that Y1, Y2, and Y3 receptors would differ in their coupling to G-proteins and second messengers.

THERAPEUTIC POTENTIAL OF NPY-RELATED DRUGS: WHICH DISORDERS AND SYSTEMS MIGHT BE TARGETED?

Like many other biological messenger molecules, NPY has been suggested to be linked with human disease. These include hypertension; eating disorders; affective disorders (depression, anxiety, and cocaine withdrawal); congestive heart failure; cardiac and cerebral vasospasm; pheochromocytoma and ganglioneuroblastoma; and Huntington's, Alzheimer's, and Parkinson's diseases (see also 7, 181). In the present context we have chosen to focus on three disorders, i.e. hypertension, eating disorders, and depression and anxiety, in which there is evidence that NPY may indeed contribute to specific symptoms. In most of the other instances listed in Table 2, it could well be argued that the changes in NPY levels that were observed might merely represent epiphenomena, i.e. that NPY expression may be altered along with the expression of (many) other peptides and proteins.

Hypertension: NPY Is a Potent Vasoconstrictor

BACKGROUND It is now well established that NPY is a cotransmitter with norepinephrine (and ATP) in the postganglionic sympathetic nerves. Several years ago it was found that NPY (or PYY) given exogenously affects both pre- and postjunctional ("synaptic") mechanisms, presumably by activating receptors on nerve terminals (usually associated with inhibition of neurotransmitter release) and on effector cells (such as vascular smooth muscle cells), respectively. Many studies indeed have been concerned with the actions of NPY at sympathetic neuroeffector junctions. Obviously, these studies were stimulated by observations of the prevalence of the peptide in sympathetic, notably perivascular, nerves and its coexistence with norepinephrine in these nerves (e.g. 33). Briefly, we and others have demonstrated three effects of NPY at sympathetic neuroeffector junctions: (*a*) a direct postjunctional response, e.g. vasoconstriction manifested in certain vascular beds; (*b*) a postjunctional potentiating effect on norepinephrine-evoked vasoconstriction; and (*c*) a prejunctional suppression of stimulated norepinephrine release (e.g. 6, 7) (Figure 2). The two latter phenomena are probably reciprocal; thus norepinephrine may affect NPY mechanisms similarly (6, 7, 62). Recently, we have argued that the vascular cooperation of NPY and norepinephrine in vivo as well as in vitro is accounted for in large part by threshold synergism phenomena rather than receptor-receptor interactions and that, when present, α-adrenoceptor reserve prevents the lowering of the norepinephrine response threshold by NPY (62). Hence, it is likely that the NPY receptors responsible for potentiating norepinephrine-evoked vasoconstriction could in principle be

identical to those mediating direct effects. It is conceivable that prejunctional norepinephrine and NPY release is "cross-regulated" by feedback stimulation of "autoreceptors" for both messengers and that they activate similar, if not identical, mechanisms in the terminals leading to inhibition of stimulated norepinephrine as well as NPY release. The three actions of NPY at sympathetic neuroeffector junctions are illustrated schematically in Figure 2. The top right panel of Figure 2 also illustrates that responses to NPY generally are slower in onset and more long lasting than are responses to norepinephrine; this is exemplified by recordings of pressor responses to norepinephrine and to NPY in conscious rats. Obviously, however, blood pressure measurements following systematically applied NPY will reflect actions of the peptide on pre- as well as postjunctional receptors in numerous vascular beds. The complexity of the in vivo setting was further emphasized by recent findings that high doses of NPY as well as certain C-terminal NPY fragments can reduce blood pressure by way of mast cell degranulation and histamine release (49, 50).

Further, it has been suggested that NPY may be a "missing link" between the sympathetic nervous system and the renin-angiotensin system, because the peptide inhibits renin secretion and prevents renal hypertension (182).

For simplicity, we have not dealt with the fact that ATP is also colocalized with norepinephrine and NPY in sympathetic nerves. As expected, NPY also modulates purinergic transmission and enhances ATP-evoked vasoconstriction (183, 184). The agent Ins[1,2,6]P_3, which is discussed below, was found to attenuate NPY- and ATP-evoked but not norepinephrine-evoked vasoconstriction in guinea pig basilar artery, perhaps indicating that the two former agents share a common signaling mechanism (81).

Thus, NPY appears to be a mediator involved in both peripheral and central (see below) cardiovascular control. Its coexistence and cooperation with norepinephrine in sympathetic nerves is well established; however, the relative contribution of NPY to sympathetic tone has been difficult to evaluate, largely as a result of the lack of NPY antagonists. Nevertheless, we have provided evidence that the relative sympathetic vasoconstrictor contribution of NPY increases during situations of high sympathetic nerve activity (7, 62). During resting conditions, little NPY is released (185–187) and sympathetic nerve activity is reflected largely by adrenoceptor activation. Increased sympathetic nerve activity is accompanied by both adrenergic desensitization and increased NPY release. This may result in a dual effect of NPY. First, NPY may restore the impaired responsiveness to norepinephrine, and second, NPY per se may become a more efficacious vasoconstrictor agent (62). It has been hypothesized (188) that NPY and norepinephrine (in vascular models, in most cases acting at α_1-adrenoceptors)

both affect calcium ion homeostasis but by different mechanisms. Thus, unlike norepinephrine, NPY may not mobilize calcium ions by an Ins[1,4,5]P_3-dependent action.

CIRCULATING NPY CONCENTRATIONS CORRELATE WITH SYMPATHETIC NERVE ACTIVITY There is ample evidence that the measurement of NPY concentrations in the circulation of humans and several animal species provides an index for sympathetic nerve activity, because circulating NPY derives mainly from sympathetic nerves. Thus, upon sympathetic stimulation, NPY overflow into the local venous effluent from organs of experimental animals or human heart is increased, as are systemic plasma levels in humans (cf 9). A dissociation between rises in plasma NPY concentration and evoked pressor responses has been noted (189); this probably reflects a delay in the diffusion of "synaptic" NPY into the general circulation.

In Figure 4, we have made a preliminary attempt to summarize data from many different laboratories (these data are reviewed in detail in 188) to illustrate the point that circulating NPY levels correlate to increased sympathetic nerve activity. Although the absolute concentrations of the peptide shown in Figure 4 may be of limited interest because of variability among different immunoassays used by different investigators, it can be seen that "stressful" conditions and situations in humans are associated with marked increments in NPY levels. A special case (not relating to high sympathetic nerve activity) in Figure 4 is pheochromocytoma, a tumor of adrenal medullary origin, which often but not always produces high levels of NPY (cf 188). Finally, Figure 4 shows that rats have markedly higher circulating NPY concentrations than the other species studied. It is likely that much of the circulating NPY in rats derives from platelets (rats appear unique in that their megacaryocytes express the NPY gene; 190). Consequently, it is not surprising that the highest circulating NPY levels found in rats occurred after platelet aggregation (117, 190).

NPY IN HYPERTENSIVE PATIENTS Elevated sympathetic nerve activity is likely to be implicated in the pathophysiology of mild to severe hypertension (see e.g. 191). Because vasoconstriction has been found to persist after α-adrenoceptor blockade in hypertensive patients (192), it is conceivable that sympathetic cotransmitters, i.e. NPY and ATP, are involved (see also 81). In this context it is of considerable interest that circulating NPY concentrations have been reported to be elevated in hypertensive patients but not reversed after treatment with conventional antihypertensive drugs (193).

The only proposed NPY antagonist that has been used in a controlled clinical trial in hypertensive patients is Ins[1,2,6]P_3. Preliminary data seem to indicate

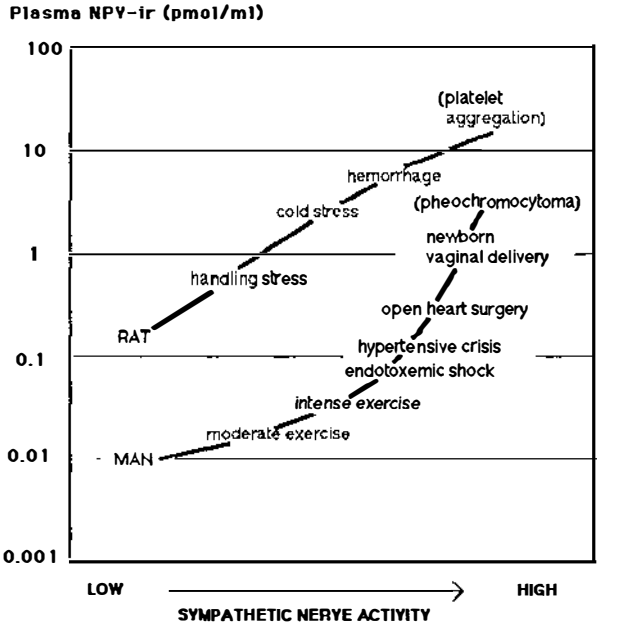

Figure 4 Circulating NPY concentrations increase in many instances associated with high sympathetic nerve activity. The absolute values in resting and stimulated NPY concentrations may vary significantly between different laboratories using various immunoassays. The sole purpose of this figure is to illustrate the gradually elevated levels of NPY that may occur in various experimental and clinical situations. Extreme concentrations of circulating NPY may occur independent of sympathetic nerve activity as a result of pheochromocytoma (human) or platelet aggregation (rat). Also, rat plasma contains higher levels of NPY since platelets in rats contain NPY, in contrast to other species that have been studied. NPY-ir (-immunoreactivity) is given in picomoles per milliliter of plasma.

that Ins[1,2,6]P_3 has the ability to lower blood pressure, particularly in hypertensive patients whose blood pressure was elevated by physically challenging bicycle exercise (82). This type of challenge is known to release NPY (Figure 4) (194), and therefore it is conceivable that the endogenously released NPY was inhibited in these patients.

Since NPY is preferentially released during situations of high sympathetic nerve activity, it is possible that NPY blockers will prove to be particularly effective in suppressing "stress-induced" elevations of blood pressure. In this way, potential NPY-blocking pharmaceutical agents may have different therapeutic profiles (selectively suppressing sympathetic pressor peaks) from those of conventional antihypertensives.

Hypertension: Central Aspects

The NTS, the site of termination in the brain stem of primary afferent fibers of arterial baroreceptors, is richly innervated by neurons containing NPY (195). Moreover, injection of NPY into the NTS is known to evoke a long-lasting hypotension and bradycardia (134–136, 196, 197).

Glutamate may be the presumed endogenous transmitter of primary baroreceptor afferents (198). Local pretreatment of NTS with NPY, [Pro34]NPY, or NPY 13–36 prevented (for hours) the fall in arterial blood pressure and heart rate evoked by subsequent injections of glutamate into the same site in the NTS. Pretreatment of the NTS with desamido-NPY, PYY, or PP did not affect the cardiovascular responses evoked by glutamate in the NTS, suggesting this inhibition to be Y3 receptor mediated (cf above). Although the mechanism behind the NPY-evoked refractoriness to glutamate is still unclear, it appears that NPY can suppress glutamatergic baroreceptor inputs and thereby affect blood pressure regulation at a brain stem level(s) (135, 197). As reviewed by MacAuley et al. (91), NPY also affects blood pressure when injected into the hypothalamus and other central sites.

NPY in Overeating and Obesity

A multitude of evidence suggests that NPY participates in the regulation of eating behavior and specifically that the peptide is an extremely potent orixigenic agent. The first indication was the finding that NPY, given intracerebroventricularly (icv) elicits eating in satiated rats (10). Subsequent work by Stanley & Leibowitz (11,199) indicated that NPY injections into the area of the hypothalamic paraventricular nucleus of the rat were still more effective. Thus, low doses of hypothalamic NPY elicit eating of a single normal meal, while high doses elicit consumption of an enormous initial meal followed by a series of somewhat smaller meals; repeated injections cause marked and sustained overeating, leading to dramatic obesity (cf 13). When compared with the smaller transient effects of other neurotransmitters that stimulate feeding, NPY appears unique in the sense that it might be capable of mediating even the most pronounced weight gains observed in experimental settings or in nature.

NPY has been found to be an appetite-stimulating agent in a variety of species and at different stages of development. Moreover, hypothalamic NPY levels are altered by a number of conditions that have been linked to disturbances in consummatory behaviors in animals and humans (reviewed in 13).

Elevated concentrations of NPY in the CSF have been found in both underweight amenorrheic anorectics and the same amenorrheic patients restudied within 6 weeks after weight restoration. An inverse relationship

between CSF NPY levels and caloric intake in healthy female volunteers was also found (200, 201). Thus, it could be argued that the increased concentrations of NPY in the CSF of these subjects may be a secondary, compensatory response to decreased food intake.

NPY in Affective Disorders: Depression, Anxiety, and "Psychostimulant Withdrawal Syndrome"

In several psychiatric and neurological disorders, alterations of NPY levels have been reported (reviewed in 181; see also Table 2). Although it should be mentioned that NPY-containing neurons show a potential to survive even advanced cases of neurodegenerative disorders (e.g. Huntington's disease, Alzheimer's disease, and Parkinson's disease), it is difficult to believe that NPY plays any unique role in these disorders and consequently that potential NPY-ergic drugs may significantly benefit such patients.

In contrast, affective disorder symptomatology, notably anxiety, may involve NPY-mediated causal component(s). At the very least, NPY could serve as a marker for depression and the clinically similar "psychostimulant withdrawal syndrome." The arguments are as follows.

As with some other neuropeptides, e.g. corticotropin-releasing factor, it has been tempting to draw parallels between central effects of NPY and symptoms and phenomena of major depression. Among these well-known observations in depressive patients, one may note alterations in pituitary hormone release, appetite, and circadian rhythms, i.e. instances in which NPY has indeed been found to have very potent effects (cf 181). Stimulated by such parallelisms, we and others have measured NPY concentrations in the CSF of patients with major depression (202–204) and in brain tissue of suicide victims believed to have suffered from the syndrome (205; Wahlestedt et al, unpublished data). The picture that has emerged is that in depressed patients, NPY synthesis in the brain is reduced, perhaps predominantly in the cerebral cortex, and that reduced amounts of the peptide are present in the CSF. The NPY content in CSF is high compared with that of other neuropeptides and does not show a so-called gradient, indicating its homogeneous distribution and, in all likelihood, its relative stability in this body fluid. Interestingly, Widerlöv et al. (14) pointed out a specific negative correlation between NPY concentrations in CSF and anxiety symptoms in depressed patients (both psychological and somatic anxiety); in other words, low levels of NPY were observed in patients showing severe anxiety (one of the most common symptoms in depressives).

The rat has been used as an experimental animal in attempts to address indirectly the hypothesis that NPY may be involved in the pathophysiology of depressive illness and may relate to anxiety symptoms in particular. Briefly, a rat model of depression, olfactory bulbectomy, has been associated with reduced cortical NPY concentrations (14, 203), whereas repeated electrocon-

vulsive shocks (14, 206, 207) as well as antidepressant drugs (either adrenergic or serotonergic uptake inhibitors; 208) instead increased cortical NPY levels. A common brain region in these rat studies was the cerebral cortex, with most data deriving from measurements of the frontal part. Interestingly, not only antidepressants, but also lithium, which is used frequently in the treatment of affective disorders, appears to enhance NPY (but not proenkephalin) gene expression (209).

Three different rat models developed to study anxietylike behavior, the elevated X-maze (Montgomery), the punished drinking test (Vogel), and the punished responding test (Geller-Seifter), have been used to show that NPY is an anticonflict/anxiolytic agent, with potency and efficacy matching those of, e.g., the benzodiazepines (cf 14, 15, 99, 100).

Recent evidence indicates that this anxiolytic action of NPY involves stimulation of Y1 receptors, perhaps in the amygdala. Thus, injections of minute amounts (50 pmol) of either NPY or the Y1 agonist [Leu31, Pro34]NPY into the central nucleus of the amygdala reproduced fully the effect of icv injections on conflict behavior. In the latter microinjection experiments, no effect of NPY on food intake was observed, further indicating that anxiolysis can occur without affecting consummatory behavior (100).

Another very recent line of evidence supporting the view that central Y1 receptors mediate NPY-induced anxiolysis relies on the use of antisense oligodeoxynucleotides (AS D-oligos) to the rat Y1 receptor (16). Thus, down-regulation of Y1 receptors (but not Y2 receptors) was achieved in vivo by repeated icv injections of an AS D-oligo corresponding to the N-terminus of the receptor. Rats that received this treatment displayed marked signs of anxiety, indicating the disruption of an endogenous pathway involving stimulation of (anxiolytic) Y1 receptors (16). Food intake was not affected in the Y1 AS D-oligo-treated rats, lending further support to the view (see above) that feeding, unlike anxiolysis, is not mediated through typical Y1 receptors.

COCAINE WITHDRAWAL Behaviorally, the end of a cocaine binge is often associated with intense anxiety and an episode of a syndrome that may be indistinguishable from major depression (see 210 for a review). The dysphoria resulting from cocaine withdrawal can be lengthy and of importance in cocaine craving and recidivism (210). Conceivably, the behavioral changes associated with prolonged use of and withdrawal from cocaine may reflect the unmasking of slow-onset, long-lasting central adaptive processes that act to counteract the immediate stimulatory actions of the drug (211). Some time ago, we hypothesized that NPY might be a candidate neurotransmitter related to long-term cocaine use and withdrawal (211). We showed that rats treated with cocaine for 1 week exhibit substantial, long-lasting, but reversible reductions of NPY and NPY mRNA in the cerebral cortex and nucleus accumbens (211).

These reductions were assumed to be elicited by transneuronal changes in synaptic dopamine associated with mesolimbic and mesocortical dopamine neurons. Thus, although the initial effects of cocaine may be to activate dopaminergic mechanisms, NPY may be a sensitive marker for long-term cocaine use and withdrawal (211).

It has been known for some time that NPY biosynthesis can be modulated by dopamine (212–214); conversely, NPY has been shown to accelerate dopamine turnover (215), indicating the reciprocal feedback regulation of NPY-ergic and dopaminergic mechanisms. Finally, amphetamine is also thought to affect NPY biosynthesis (216, 217).

NPY RECEPTOR PHARMACOLOGY

NPY Receptor Agonists

As described above, NPY, PYY, and PP, as well as various C-terminal NPY and PYY fragments, have different abilities to bind to and activate the defined receptor subtypes (Y1, Y2, and Y3). PYY and NPY are about equipotent at Y1 and Y2 receptors. Recent data have suggested that NPY, but not PYY, recognizes a receptor which is referred to here as Y3. PP is essentially inactive at either receptor subtype but may have a specific receptor(s) of its own (28). The C-terminal fragment NPY 13–36 is a Y2 receptor agonist, being about 1 order of magnitude less potent than NPY but being virtually inactive at the Y1 receptor.

The substituted analogs [Pro34]NPY and [Leu31, Pro34]NPY seem to be full Y1 receptor agonists while being virtually inactive at Y2 receptors (68). In fact, a single-amino acid substitution, introducing proline in position 34, confers Y1 receptor selectivity. These Y1 receptor-specific ligands have been useful in recent attempts to identify binding sites and to delineate the physiological significance of Y1 receptors.

Several truncated and/or substituted NPY analogs, e.g. C2-NPY, have been

Table 2 Proposed NPY antagonists

Proposed antagonist	Comments
NPY 18–36	Partial to full agonist
PYX-1 and PYX-2	Low potency
Benextramine	Low potency and specificity
He90481	Low potency and specificity (vs histamine)
R-116	Not documented
$Ins[1,2,6]_3$ (PP56)	Noncompetitive, Ca^{2+} signaling interference, selected effectors
AS D-oligos to the Y1 receptor	High specificity

developed and have been found to be Y2 selective (28, 103, 104, 218). The Y2 receptor efficacies and potencies of these agents have not been found to exceed those of the long C-terminal fragments (e.g. NPY 13–36), which thus remain the agents of choice for pharmacological studies of the Y2 receptor (19).

The most frequently reported orders of potency of agonists to Y1, Y2, and Y3 receptors are shown in Table 1.

Proposed NPY Receptor Antagonists

No antagonists, in the classical pharmacological meaning, with reasonable potency and/or receptor selectivity have yet gained full acceptance. Thus, most of the agents dealt with below (and listed in Table 2), which have all been proposed to be NPY receptor antagonists, definitely do not fulfil requirements for general use by pharmacologists with intentions to block presumed NPY responses. In the following discussion they are dealt with individually (as in Table 2).

NPY 18–36 This C-terminal fragment, dealt with in the above discussion as a Y2 receptor agonist, was found to inhibit ^{125}I-NPY binding and NPY-evoked adenylate cyclase activity in rat cardiac ventricular membranes. Although the data presented by Balasubramaniam & Sheriff (219) are clear-cut, the possibility remains that NPY 18–36 in this cardiac preparation, like other long C-terminal fragments in several other experimental settings, behaves like an antagonist by virtue of being a partial agonist at Y1 receptors. Since NPY 18–36 has been found to be a full Y2 agonist (as well as a reasonable Y3 agonist) in other systems, this (unmodified) fragment will be of little use as an antagonist (see also 19).

PYX-1 AND PYX-2 Tatemoto (220) has introduced two C-terminally based benzyl analogs of NPY with antagonistic properties in human erythroleukemia (HEL) cells (a Y1 receptor model system). They are Ac-[3-(2,6-dichlorobenzyl)-Tyr27, D-Thr32]NPY$_{27-36}$ (PYX-1) and Ac-[3-(2,6-dichlorobenzyl)-Tyr27–36, D-Thr32]NPY$_{27-36}$ (PYX-2) and show promise but are themselves of limited use because of their low potencies. Their receptor subtype selectivity is not elucidated.

BENEXTRAMINE The irreversible α-adrenoceptor antagonist benextramine seems to evoke a weak antagonism on NPY binding and to attenuate NPY-evoked hypertension. However, the potency seems to be very low, and the concentration used exceeds those generally used for inactivation of α-adrenoceptors (221).

HE90481 The nonpeptide He90481, which is known to affect histamine H1 and H2 receptors, blocked NPY-evoked Ca^{2+} mobilization and NPY binding to HEL cells, but did so with low potency. Its lack of specificity and low potency invalidate its use in physiological assays (17).

R-116 Computer modeling work has yielded a nonpeptide, R-116, which appears to be a selective Y1 antagonist (M. R. Brown, personal communication). The K_i of R-116 at Y1 receptors in SK-N-MC is 60 nM, but it is much higher (30 μM) at Y2 receptors in SK-N-BE(2). Interestingly, the compound lowers blood pressure in conscious spontaneously hypertensive rats and inhibits exogenous NPY but not norepinephrine or angiotensin II induced elevation of arterial pressure. (M. R. Brown, personal communication)

INS[1,2,6]P_3 An inositol phosphate (Ins[1,2,6]P_3, PP56) has been reported to suppress the amplitude of Y1 receptor-mediated vasoconstriction without causing a parallel right-shift of the concentration-response curve (81, 82, 222), reflecting noncompetitive antagonism. Although it does not interact with the Y1 receptor-binding site itself or with other NPY-binding sites (81, 82, 118), Ins[1,2,6]P_3 appears to inhibit NPY-evoked elevations of the Ca^{2+} level in vascular smooth muscle cells (81, 82). The agent does not affect responses associated with α_1-adrenergic or other PI-coupled receptors. Besides NPY, ATP is the only vasoconstrictor shown to be affected by Ins[1,2,6]P_3, although not to the same degree as NPY is (81), NPY-evoked pressor responses are also effectively and selectively inhibited by Ins[1,2,6]P_3 in vivo in the pithed rat (82, 223). Interestingly, the agent blocks a portion of the nonadrenergic pressor response to preganglionic sympathetic nerve stimulation in the pithed rat, possibly reflecting inhibition of the actions of neuronally released NPY and/or ATP (81, 82).

Ins[1,2,6]P_3 was found to inhibit a portion of the feeding response elicited by icv injection of NPY. Its usefulness as a specific NPY antagonist may, however, be limited, since in the feeding experiments the response to another neuropeptide, galanin, was affected as well as NPY (118).

Despite its shortcomings as a specific NPY antagonist, Ins[1,2,6]P_3 is likely to be useful in studies of NPY signal transduction, notably Ca^{2+} signaling. It should be noted that Ins[1,2,6]P_3 is also an anti-inflammatory agent (224), a property that can be attributed to inhibition of NPY action only with great difficulty.

THE ANTISENSE APPROACH Recently, we have used AS D-oligos to achieve down-regulation of Y1 receptors in vitro (in cultured neurons) as well as in vivo (16). These AS D-oligos showed selectivity in that Y2 receptor densities were unaffected. It remains to be seen whether the AS approach will be widely

used. Its major drawback is the relative instability of D-oligos in the circulation, which can be partly circumvented by use of modified nucleotides. On the other hand, nucleic acids appear to be quite stable in the brain, a fact likely to have allowed our study with the Y1 AS D-oligos that produced marked signs of anxiety in rats (see the section on affective disorders above).

CONCLUDING REMARKS: ARE NPY RECEPTORS POTENTIAL THERAPEUTIC TARGETS?

NPY is thus arguably the most abundant neuropeptide discovered to date, with a wide distribution in the central and peripheral nervous systems. NPY forms a family of peptides together with PYY (approximately 70% homology) and PP (approximately 50% homology); both NPY and PYY are extremely bioactive, whereas PP is generally much less active.

The existence of Y1 and Y2 receptors (for NPY and PYY) and Y3 receptor (for NPY only) appears to be firmly established. The Y1 receptor in particular has emerged as a potential drug target. In our view it is unlikely that NPY specificity in potential drugs will be achieved if one seeks to affect NPY gene expression or peptide processing, because no present evidence points to the uniqueness of NPY in these respects. Instead, NPY drug development should involve identification of compounds displaying affinity to NPY receptors.

Very much like classical neurotransmitters, NPY and PYY have a wide range of effects on exogenous application in various experimental settings. Many of these reported effects may be of limited interest from a drug development viewpoint since they represent examples in which NPY is only one among many messenger molecules with similar effect profiles. In this review we have singled out three actions of NPY, i.e. as a vasoconstrictor, as a stimulant of food intake, and as an anxiolytic, in which the peptide is extraordinarily potent and is likely to be of both physiological and pathophysiological significance.

Thus, a peripherally acting Y1 receptor antagonist should lower blood pressure and, perhaps uniquely, inhibit pressor responses to (stress-induced) increments in sympathetic nerve activity. Two agents, Ins[1,2,6]P_3 and R-116, have showed promise as possible lead compounds for the development of antihypertensive agents acting by way of suppressing NPY evoked vasoconstriction.

Second, many investigators at universities and pharmaceutical companies appear to be aiming for the development of a centrally acting NPY antagonist effectively suppressing feeding elicited by (endogenous) NPY. Interestingly, several lines of evidence have recently pointed to the possibility that an "atypical" (not Y1, Y2 or Y3) hypothalamic receptor is involved in this robust feeding response. Hence, in theory it should be possible to find a drug with

specific affinity to the atypical hypothalamic NPY receptor and thereby to avoid interference with functions associated with the defined NPY and PYY receptors.

Third, a centrally acting Y1 agonist will have anxiolytic properties. Conversely, a Y1 receptor AS D-oligo, administered directly into the brain of rats, was shown to be markedly anxiogenic, implying that endogenous NPY mechanisms (perhaps in the amygdala) act to tonically relieve anxiety. A nonpeptide (and nonbenzodiazepine) anxiolytic drug that mimics NPY at Y1 receptors might find a place in the treatment of affective disorders, including both major depression and the clinically similar condition that often follows psychostimulant (e.g. cocaine) withdrawal. The latter syndromes have been associated with reduced brain NPY synthesis and, quite often, with severe anxiety.

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