MINIREVIEW

The Pancreatic Polypeptide (PP-Fold) Family: Gastrointestinal, Vascular, and Feeding Behavioral Implications (43511G)

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Historical Background. Of the four recognized peptide hormones secreted by the endocrine pancreas, pancreatic polypeptide (PP) was the third to be discovered, isolated, and characterized. Insulin (1921–22) and glucagon (1923) preceded and somatostatin (1975) followed the isolation of PP in 1968. Despite this sequence of events—and the time interval since its discovery—PP remains somewhat of an enigma both in name and in function. The reluctance to label this polypeptide with a more descriptive title than the lackluster PP it bears stems largely from the fact that while broad actions may be attributed to it, no outstanding or dominating effect has been identified with its actions. Thus, the only characteristic carried by use of the phrase PP is to signify the species of PP one is discussing, such as avian, bovine, rat, porcine, ovine, and human, for example. The avian structure has 15 identities (of a total of 36) with that of bovine PP, and the latter differs by one or two residues from most other mammalian PP at any of three or four positions within the molecule. Thus, strong structural conservatism exists from an evolutionary point of view.

The consideration that a family of pancreatic polypeptides exists is not unique within the field of physiologic chemistry, as it has been well recognized that many other polypeptides of interest are structurally related, are well conserved, and have overlapping physiological actions, and yet also may express distinctly unrelated effects in different environments. One need merely mention the "gastrin family," the "secretin family," the "insulin family," to name but a few examples (1). The PP family (or PP-fold family) has been recognized to exist only since 1980–82. Pancreatic polypeptide was first isolated by Kimmel and associates (2) in 1968 while they were characterizing chicken insulin. PP was a constant and persistent proteinaceous containment of the avian preparations and, when it received full attention as a possible biologically active peptide, it was found to be a tyrosinated 36-amino acid structure, with a suggested mol wt of 4240 (3). Early studies on the biological activity of this PP preparation did not reveal any single outstanding feature and the tentative name given to this putative hormone was simply avian pancreatic polypeptide (APP).

Twelve years passed before another peptide with strong structural homology to that of PP was isolated, this from the pig intestine by Tatemoto and Mutt (4) in Sweden. Using a new isolation technique that was highly sensitive to recognizing exposed terminal tyrosine groups, these workers characterized a 36-amino acid polypeptide possessing a Tyr at both the N- and the C-terminal segments (4). Using the (then new) single-letter codification of amino acids, they simply called it peptide YY, or PYY. Structural homology of PYY with APP and mammalian PP was found to be approximately 50–54%. At first, biological studies with PYY lagged behind as search for other possibly related peptides continued, as well as due to the ongoing efforts of 10 years of work with PP that dominated the PP literature. The fact that PYY was isolated from the intestine, and had been identified early in the human lower digestive tract, implied that the polypeptide was possibly of importance in the regulation of gastrointestinal function in mammals. The first described of PP's

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biological actions, too, had been those concerning effects on the gastrointestinal tract of birds, dogs, and humans. But in addition to the gastrointestinal and (fewer) metabolic studies with PP, interest grew and intensified as to the significance of reports that indicated APP-like peptides existed in the central nervous system of most animals studied. In fact, in those organisms lacking a discrete pancreas (the major source of PP in mammals and Aves), APP-like material was detected by immunocytochemical techniques mainly in the central nervous system, and to a lesser degree in the peripheral nervous system (5, 6). Use of the blowfly was common in such studies.

Thus, it may not be surprising that in 1982 the same Swedish workers (4) announced the isolation from pig brain of another 36-amino acid residue structure, one that was tyrosinated at both termini as was PYY and that possessed 70% structural homology with PYY, 56% homology with APP, and 47–50% homology with various mammalian PP. Because YY had already been assigned to PYY 2 years earlier, neuropeptide-Y, as the new polypeptide was called, assumed the acronym of NPY. Clearly, then, PP, isolated from the discrete pancreas, is not a unique polypeptide, despite its lack of structural relationship to any and all other pancreatic and gut peptides. Rather, structurally it is closely related to both PYY and NPY, even though the latter two are localized to the lower gut, and brainstem and certain peripheral neural elements, respectively. One may conclude that a pancreatic polypeptide family exists, one that may well have additional members added to it as the search for their significance in physiologic systems continues.

Chemistry of Pancreatic Polypeptides and Their Receptor Structures

The first of the PP family to be characterized as to primary, secondary, and tertiary structures was that of APP by Kimmel and associates (3) in 1975, which was quickly followed by Lin and Chance's (7) laboratory on various mammalian PP. Isolation of PYY in 1980 demonstrated the class homology with that of the various PP discovered previously, and in 1982 NPY was reported to be approximately 70% homologous with PYY. As seen in Figure 1, all three peptides are 36-amino acid residue structures, all have a tyrosinated 36th position, and all have a very highly conserved C-terminal hexapeptide. The tyrosine amide on the carboxyl terminus is absolutely essential for biological activity. The similarity among the mammalian PP and the two NPY presented in this figure are striking. Only the rat PP stands out among PP structures as deviant, with eight residue substitutions, which probably explains the dearth of literature on rat PP due to immunoassay limitations when employing nonhomologous species antiserum. Various structural comparisons of the family are shown in Table I.

What Table I and Figure 1 do not show is the tertiary conformation of the peptides as has been established by a number of workers in several laboratories. Human PP (HPP) is a very stable, tightly packed, globular peptide containing a polyproline (collagen-like) helix involving residues 2, 5, and 8, closely packed against an amphiphilic α-helical region of residues 14 through 32. The folded structure results in a close association of the N- and C-terminal ends of the molecule, a feature important to receptor recognition (9). Because of the fold, many workers suggest that the term PP-fold replace that of PP family. Data available at the present time indicate that all PP have virtually the conformation thus described and, in fact, the peptide backbones of porcine PYY, porcine NPY, and human PP are almost identical. The structural organization of precursors of PYY, NPY, and PP is almost identical, with a 28- or 29-amino acid signal peptide followed by a hormone-coding region containing a Gly-Lys-Arg amidation cleavage sequence, and then a carboxyl-terminal extension peptide of approximately 30 residues (10, 11). The tertiary structure of the three peptides may be even more highly conserved than the individual amino acid sequences (10).

Rat nucleotide and amino acid sequences of the signal peptide are highly homologous to those of the human (12). However, prepropancreatic polypeptide may be a unique prohormone in that the rat and the human amino-terminal two thirds are highly homologous, yet the carboxyl-terminal one third (distal to the complete PP structure) exhibits a high degree of divergence (12). It is possible that the two terminals of PP have evolved at different rates because they may have faced different selective constraints. Sequence homology in the rat and human C-terminal regions of prepro-

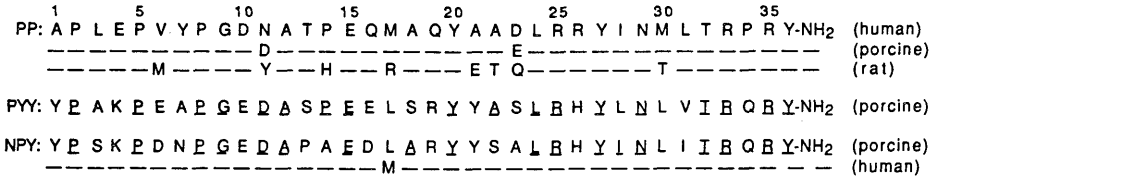

Figure 1. Structures of pancreatic polypeptide, peptide-YY, and neuropeptide-Y. One-letter code for amino acids is used. A dash indicates identity with the human form for PP or the porcine form for NPY. An underlined residue in PYY or NPY is identical with porcine PP.

Table I. Sequence Homology of the Pancreatic Polypeptide Family[a]

Peptides	Residues common to each	Sequence homology (%)
NPY vs PYY	25	70
NPY vs APP	20	56
NPY vs BPP	17	47
PYY vs APP	19	53
PYY vs PPP	18	50
PYY vs BPP	18	50

[a] This table was modified with permission from Kuenzel *et al.* (8).

pancreatic polypeptide is much lower than in any domains of other prohormones, including preproinsulin C-peptide, which so far has been considered the most variable (13). Thus, prepropancreatic polypeptide may be an example of mosaic evolution at the molecular level (12). Figure 2 presents the structural organization of precursors of PYY, NPY, and PP as described by Leiter *et al.* (11).

Of note is a feature common to all three family members, namely the C-terminal region of residues 31–36. This hexapeptide region apparently projects "as an arm extension" out from the tight globular structures, forming an appendage that is highly conserved for PP throughout phyla as well as between and among various family members, as shown for HPP, PYY, and NPY in Table II. Such an appendage carries considerable biological significance as a probable site for interaction with an appropriate receptor.

The identities at positions 32, 33, 35, and 36 are striking, as is the fact that what substitutions that have occurred are modest, indeed. As indicated in Figure 2, the biosynthetic precursor of the amidated peptide is a C-terminal glycine-extended form. A specific carboxypeptidase separates the amidated carboxyl hormone from its associated basic amino acid cleavage sequence. Thus, the amidation at position C-36 of various pancreatic polypeptides is one of the final steps in a series of posttranslational modifications of peptide precursors (14, 15). At least half of all biologically important neuropeptides, as well as peptide hormones known today, are amidated at the carboxyl terminus. It was this fact, more than any other, that spurred the first work on APP in the late 1960s in terms of assessing the molecule's biological activity. APP_{1-36} has full biological activity; APP_{1-35} (or any other PP_{1-35}) has none.

Receptors

Recent work has been directed at PP, PYY, and NPY-receptor interaction, mainly by characterizing the various receptors in a variety of tissues. Such work is of obvious importance in initiating the next step, namely, identifying the nature of cellular expression of hormone activity. APP receptors in chicken brain have been studied in detail, as PP receptors have been in the porcine brain (16). Results of such studies indicate that the binding of PP and related peptides is strongly dependent upon the presence of certain functional groups, especially the guanidinium group of Arg-35 and the C-terminal aromatic amide function (17). The arginyl residue at position 33 is also conserved in the various peptides of the family, but it is not essential for binding. Chicken brain and spleen specifically bind labeled APP, regardless of whether or not the label is at the N terminus or the C terminus. Gastrointestinal tissues do not. Liver membranes, however, bind with low affinity to APP labeled at the C terminus but not the N terminus. Collectively, these observations would indicate that avian liver and brain receptors/binding sites are distinct from each other and, also, that both termini of the APP molecule interact with the membrane receptors (18). These data also indicate that gastrointestinal tissues may not be direct targets for APP actions. Characterization of liver and brain binding sites in chickens indicates that free thiol groups are essential for the latter but not the former in the binding of APP. Both sites are proteinaceous and possess disulfide bonds that are important to ligand binding (19).

PP receptors have been identified and characterized in the basolateral membranes prepared from the canine

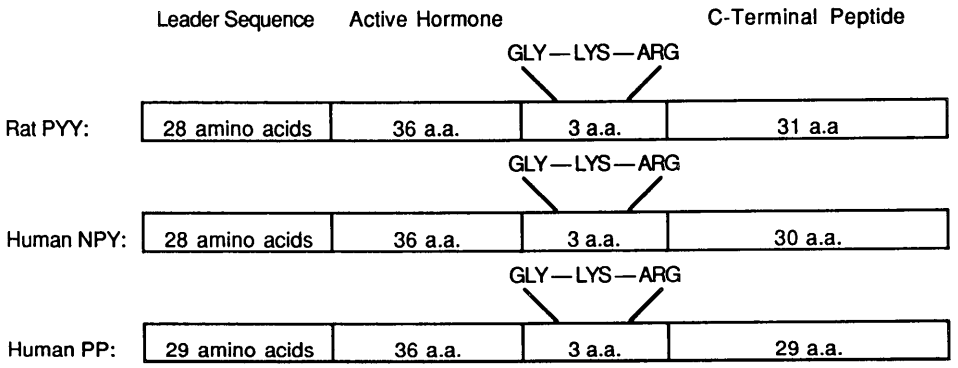

Figure 2. Structural organization of pancreatic polypeptide family precursors as determined by isolation of cDNA encoding the precursors. Reproduced with permission from Leiter *et al.* (11).

Table II. Comparison of C-Terminal Hexapeptides

	- 31 -	32 -	33 -	34 -	35 -	36
Human PP	- LEU -	THR -	ARG -	PRO -	ARG -	TYR·NH_2
Porcine PYY	- VAL -	THR -	ARG -	GLN -	ARG -	TYR·NH_2
Human NPY	- ILE -	THR -	ARG -	GLN -	ARG -	TYR·NH_2

small intestine. As suggested in avian studies, both ends of the bovine PP (BPP) molecule are necessary for binding to the basolateral membrane receptor, and the tyrosinated amide at C-36 also must be intact (20). With serial deletion of residues from the N terminus of BPP, there is an increased loss in binding ability. Also, BPP_{1-35} binding is <0.1% of that of BPP_{1-36}. One may conclude that the molecular homolog of the PP primary structure is required for full receptor binding (20). The possibility that some PP actions are mediated via a central nervous system-adrenal gland axis has been suggested after discovery of saturable, high affinity, specific binding sites in the adrenal cortex and medulla of rats (21).

Membranes prepared from pig brain possess two classes of APP-binding sites, both high affinity and low affinity components, as described by Scatchard plots (16). High specificity to APP is evident but not to NPY, PYY, or porcine PP (PPP). The highest binding is found in the pituitary gland, followed in decreasing order by the hippocampus, amygdala, cerebral cortex, hypothalamus, and cerebellum. (Interestingly, APP binding to chicken brain is highest in the cerebellum and lowest in the cerebral cortex.) Cross-linking studies indicate that both the porcine and chicken brain receptors for APP are a 67-kDa protein lacking disulfide-linked subunits. In chicken brain, both PYY and NPY cross-link to the 67-kDa receptor, even though their "normal" receptor in porcine brain is a 50-kDa receptor protein. Such observations would indicate that PP, PYY, and NPY may act at multiple sites in chicken brain, sites that are similar to and shared by the family. APP sites in porcine brain appear to be more specific than those in chicken brain, which suggests the possibility that pigs may have an endogenous APP-like peptide in the brain (16).

PYY receptors have been studied intensely, particularly in the canine upper small intestine and in the porcine hippocampus, where the receptors appear in great abundance. Studies involving the binding of PYY to rat intestinal (jejunal) plasma membranes indicate the existence of a single class of high affinity binding sites. Binding is rapid, saturable, reversible, specific, and dependent on time, temperature, ionic strength, and pH (22). Rat, human, and avian PP (with 45, 47, and 53% structural homologies, respectively) are very weak inhibitors (1/100,000 lower). NPY, with 70% structural homology to PYY, has high affinity for the PYY receptor. No PYY receptors appear to exist in the rat stomach, lower small intestine, colon, or liver. Collectively, these observations argue favorably for PYY and NPY sharing a common jejunal receptor site (22).

In the porcine brain, PYY receptors bind both PYY and NPY with high affinity. C-terminal fragments of PYY bind poorly. The N terminus of PYY appears to be the active region of the molecule in this respect and, apparently, is coupled to a guanine nucleotide regulatory protein (23). Solubilized PPY receptors maintain a rank order of potency for binding related peptides and fragments characteristic of the membrane PYY receptor, namely PYY > NPY >>> APP and PPP, also PYY > PYY_{22-36} >>> PYY_{1-22}, and PYY_{22-28}. Two classes of binding sites have been described for the solubilized receptors (24). Unlike the avian PP receptor, there is no evidence of the receptor subunits involving disulfide bonds. Again, the identity in the size of PYY and NPY receptors and similarities in their properties, including regional distribution and peptide specificity, argue well for these two members of the family acting via a common receptor (23).

Evidence is at hand indicating that brain PYY receptors are highly conserved through the process of evolution. When this 50-kDa receptor protein is compared via binding kinetics in various mammals, birds, lower vertebrates, down to the level of fish, the affinities and potencies of PYY are virtually equaled by that of NPY (25). The outstanding exception is the chicken. PYY and NPY bind to both the PYY and NPY receptors with high affinity, but porcine and avian PP do not. However, in the chicken brain, PYY, NPY, PPP, and APP all bind to the receptor with high affinity. Data obtained from Scatchard plots are curvilinear, suggesting two orders of binding sites, one with high affinity and one with low affinity characteristics (25). Overall, therefore, it would appear that for over 400 million years, brain PYY and NPY receptors have been highly conserved in all vertebrates ranging from fish to man. The divergence observed in the chicken receptor may well indicate an evolutionary change in function.

NPY receptors have received considerable attention of late, largely due to the intense interest in the probable central nervous system role the peptide plays

in vasomotor regulation, gonadotrophin release, and behavior attending the hunger-satiety cycle (26). It has been established above that gut PYY-preferring receptors recognize NPY as well, though only one eighth the potency when inhibition of PYY binding is measured. The majority of these receptors lie in the crypts as opposed to the villar regions of the mucosa (27). In the rat, at least, the sharing of a common intestinal receptor by PYY and NPY does not translate to a lack of discrimination between the two peptides; rather, there appears to be justification for calling the receptor a PYY-preferring receptor. Nonetheless, this receptor is totally distinct from the PP receptor regardless of the tissue involved.

By employing long C-terminal fragments of NPY, some of which were 23 residues long (e.g., NPY_{13-36}), two subclasses of the PYY-NPY receptor have been reported. Both are glycoproteins called Y_1 and Y_2, with the former being a 70-kDa and the latter a 50-kDa membrane component (9, 28–30). NPY_{13-36} binds well to Y_2 receptors but hardly at all to Y_1 receptors. Presynaptic NPY receptors have been reported to be of the Y_2 type, whereas postsynaptic receptors are probably both Y_1 and Y_2 types. The majority of the NPY receptors in the mammalian central nervous system are probably of the Y_2 type (9). Pharmacologic studies that first suggested the existence of these two subtypes of NPY-PYY receptor have now been confirmed by the use of affinity cross-linking techniques. Such efforts have established that the Y_1 and Y_2 subtypes of NPY and PYY receptors are structurally distinct membrane components rather than disulfide links to other subunits (9, 28). Table III presents some biological functions that are mediated by specific PYY-NPY receptor subtypes.

A human Y_1 cDNA clone (hY_{1-5}), isolated from a fetal brain library, has been described (31). This NPY/PYY receptor is of the Y_1 type, consists of 384 amino acids, and has seven putative transmembrane domains like other members of the G-protein-coupled superfamily of receptors. When activated by NPY or PYY, this receptor accelerated cellular Ca^{2+} influx and inhibited forskolin-stimulated cAMP accumulation, phenomena that are characteristic of mammalian Y_1 receptors (31).

In the chicken brain, two different classes of NPY receptors have been reported, there being regional differences in distribution. Thus, cerebellar binding of NPY appears to involve two orders of binding sites, as predicted by Scatchard plots. Conversely, cerebral cortex membranes appear to have linear Scatchard plots, indicating one order of binding components. APP inhibits the cerebellar binding of NPY but is without effect on the cerebral cortical binding (32). These observations, in conjunction with others, would indicate that the avian cerebral cortex contains only NPY receptors, whereas the cerebellum contains distinct APP and NPY receptors (16).

Clearly, then, the characterization of receptors for all three members of the pancreatic polypeptide family leads us to an appreciation that their diverse distribution, with sometimes overlapping affinities, between and among gastrointestinal, liver, vascular, and certain central nervous system areas, may be of physiologic significance stemming from a period over 400 million years ago. Certainly, such receptor work focuses our attention on the physiologic role that each plays in these tissues, and lays groundwork for investigating a gut-central nervous system-gut axis, as modulated by PP-type neuropeptides.

Distribution and General Effects: Recognition of Family by Function

Pancreatic Polypeptide. As its original name implies, PP emanates from the pancreas of all vertebrates. Specifically, this polypeptide has been traced to the endocrine type F cell, usually located in the periphery of the pancreatic islet, but also found in clusters that sometime aggregate outside the islet in the surrounding

Table III. Specific PYY-NPY Receptor Subtypes and Some of Their Reported Biological Functions[a]

Biological function	Receptor subtype	Action
Central actions		
Synaptic transmission in hippocampus	Y_2	Decrease
Feeding behavior	Y_1	Increase
Blood pressure regulation	Y_1	Vasodilation
	Y_2	Vasoconstriction
Reproductive: LHRH release	Y_1	Increase
Memory enhancement	Y_2	Increase
Peripheral actions		
Vascular smooth muscle activity	Y_1	Increase
	Y_2	Decrease
Vas deferens smooth muscle activity	Y_2	Decrease
Intestinal electrolyte secretion	Y_2	Decrease
Urine Na^+ excretion	Y_2	Increase

[a] Table was modified with permission from Sheikh (9). LHRH, luteinizing hormone-releasing hormone.

acinar tissue. PP has been extracted from other tissues such as the antrum or upper duodenal regions in dogs and monkeys, but the contribution of such PP cells to the circulating levels of PP is quite small (5). In all probability, over 95% of plasma PP may be attributed to the endocrine pancreatic F cell (33). Generally, in all vertebrates examined, F cell populations vary inversely with the A cell (glucagon) population in the same pancreas. Thus, the head of the pancreas secretes considerably more PP than does the tail region on an islet to islet basis (33). The reverse is true in the tail region, where A cells predominate. In insects and other organisms lacking a discrete pancreatic organ (below the teleostian level), PP is identified mainly within the central nervous system. Thus, migration of F cell types from the central nervous system to the discrete pancreas, and from the gut to the pancreas, probably occurred millions of years ago. (The reader is referred to the review found in Ref. 33.)

Pancreatic polypeptide circulates mainly as a dimer (>80%), has a half-life of 6–7 min *in vivo*, and is cleared mainly by the kidney in its active form. While circulating levels of PP in avian forms approximate 7–10 ng/ml plasma in the postabsorptive state (and decrease 30–50% during an overnight fast), levels in humans range from 40 to 80 p*M*/liter and may or may not decrease with fasting (33). In one of its earliest reported effects, PP was demonstrated to have prominent effects on gastrointestinal and accessory gland function. Depending on dose, PP increases gastric (proventricular) secretion in birds (34) and dogs (35), but at higher levels inhibits canine acid secretion (36). PP inhibits exocrine pancreatic secretion of enzyme, bicarbonate, and water in several mammalian forms (33, 36) and is known to decrease gall bladder contractions, thus decreasing bile secretion in mammals (33, 35, 37, 38). Gastric and small intestinal motilities also are suppressed. Thus, PP acts to pace the entry of nutrients into the circulation by slowing down the digestive process. A slow, progressive rise in plasma concentration of nutrients results. Metabolic effects of APP include glycogenolysis, hypoglycerolemia, hypocholesterolemia, and a decrease in free fatty acid levels. Further, it acts via inhibiting cAMP-directed lipolysis as an antilipolytic agent in isolated adipocytes (39). Such metabolic effects of (A)PP encouraged workers to seek out possibly more holistic mechanisms of regulating nutrient metabolism, mainly ones involving the central nervous system. Thus, as seen below, by use of chronic infusions both peripherally and via intracerebroventricular routes, investigators began to focus on a possible role of PP in acting locally as well as centrally in regulating nutrient uptake and distribution in vertebrates.

Release of PP is quick, biphasic in many mammals, and associated with meal eating. Gut distention, release of cholecystokinin (and to a lesser extent other gut peptides), as well as direct action of absorbed nutrients on the islet F cell all play a role in triggering PP secretion. Vagal elements are a major efferent path for PP release. Acetylcholine release by postganglionic cholinergic fibers in the pancreas is a primary stimulus for PP secretion (33, 40). PP also is released during insulin-induced hypoglycemia, mainly as a result of activation of muscarinic acetylcholine receptors on the islet F cell and to a lesser extent by activation of adrenergic receptors (41).

Peptide-YY. Peptide-YY, or PYY, was first discovered in 1980 and soon localized (via use of a sensitive radioimmune assay) within various tissues of many mammals, including humans. The major source of PYY in higher vertebrates is in endocrine-type cells scattered throughout the mucosa of the terminal ileum, colon, and rectum. Very few immunoreactive (to PYY antiserum) cells are found in the gastric, duodenal, or jejunal regions of the gut (42–44). This appears true of a wide variety of animal species, as is the fact that the polypeptide is colocalized with the largest form of enteroglucagon, namely glicentin, in the intestinal L cell. Small amounts of PYY have been described in secretory granules within the periphery of the pancreatic islet, again colocalized with glucagon or glucagon-like products. A small, measurable amount of PYY has been identified in the head portion of the dog pancreas and its release to the pancreatic vein is increased by vagal stimulation but blocked by the presence of atropine (45). However, this pancreatic source of PYY pales in significance next to the abundance of L cells found in the lower gastrointestinal tract. The PYY cells of the gut are of the open type, extending from the basal lamina to the gut lumen (10). In some, but not all, species, PYY and glicentin are distributed in the same secretory granules of the L cell, a finding that might suggest concomitant release (10). However, in the colon of the rabbit, colocalization of the two peptides does not translate to dual secretion. To the contrary, rabbit L cells contain different populations of secretory granules, ones that may selectively release PYY or glicentin (46). It is interesting to note that the distribution of PYY along the gastrointestinal tract, from stomach to rectum, is contrary (in its ascending concentration) to all other gut peptide hormones with the exception of glicentin. Thus, the distribution of the colocalized peptides within the L cell runs counter to the regional concentrations-distribution of gastrin, glucagon, secretin, cholecystokinin, gastric inhibitory peptide, motilin, gastrin-releasing peptide, and somatostatin, to name but a few gut hormones (47).

Release of PYY to the circulation, like that of PP, is prompted by meal eating, though on a delayed time frame. Normal PYY levels are in the range of 6–10 p*M*/liter of plasma in humans, but will rise to at least 0.5 n*M*/liter after a meal and remain high for several

hours. Both volume of intake (dinner greater than breakfast) and composition of the meal (lipid greater than protein) are important factors in PYY secretion (40, 48, 49). Unlike PP, however, vagal activity is unimportant in releasing PYY, with the slow rise after meal eating implying that endocrine and/or extramural neural mechanisms are involved (10, 50). These mechanisms probably originate in the foregut, while interruption of intramural neural pathways between the foregut and the colon is without effect on meal release of PYY (50). Communication between the two gut regions appears to be partially dependent on foregut release of CCK. Studies with cultured PYY cells *in vitro* support this suggestion (10).

Generally, the actions of PYY are similar—but more intense—to those of PP on the gastrointestinal tract. Thus, the polypeptide decreases gastric acid secretion, gastric motility, exocrine pancreatic secretion, gall bladder activity, and intestinal motility. Its action (feedback?) at the level of the stomach makes PYY the most likely candidate to fill the role of entergastrone, the putative inhibitory hormone described—but never found—over 50 years ago (10, 51, 52). Unlike PP, hormone PPY has an intense vascular component in that it is a powerful constrictor of vessels, particularly intestinal blood vessels. PYY deprives some gut regions of blood flow and simultaneously redistributes blood flow within and between gut layers. It has least effect on duodenal blood flow (10). Once again, PYY would appear to act as an endocrine modulator, as evidenced by the observation that its receptors are mainly in the upper intestinal region although its site of secretion is almost exclusively within the lower ileum-colon-rectum region. These effects, along with those of PYY on brain structures, once again, raise the question of whether or not another member of the PP family plays a modulating-regulating role in gastrointestinal function in vertebrates through a gut-central nervous system-gut axis. (Excellent reviews on PYY actions are available to the reader, e.g., Refs. 10 and 40).

Neuropeptide-Y. NPY is the most recent of the PP family to be described (1982), yet more is known about it than either of the other two PP-fold polypeptides. Of all neuropeptides in the brain, NPY is the most widely distributed. Of all areas within the central nervous system, the highest concentration of NPY resides in the hypothalamus. And of all hypothalamic areas, the highest concentration of NPY appears to be in the paraventricular, dorsal medial, lateral, amygdala, and arcuate nuclei and their related pathways (26). What does this focal concentration of NPY suggest? It may well imply that this peptide plays a significant physiologic role in modulating activities involving feeding, drinking, vascular control, and endocrine secretion (see below). Unlike PP and PYY, NPY is strictly a neuropeptide found not only within the central nervous system but also in the peripheral and autonomic nervous system. Some of the perikarya of NPY neuronal elements also synthesize catecholamines. Thus, it is not surprising that NPY is known to coexist with norepinephrine in brainstem neurons as well as in peripheral postganglionic sympathetic fibers. NPY, therefore, may exert significant effects by influencing catecholaminergic input to neurons within the brainstem, median eminence, and hypothalamus, or at the periphery (26). Among the widely distributed NPY fibers at the periphery, one finds NPY-immunoreactive neurons in both exocrine and endocrine pancreas, thyroid, adrenal, and gonadal tissue. Of much recent interest, however, is the vast display of NPY elements to the vasculature, mainly the cerebral, coronary, and intestinal beds (26, 53, 54).

Once released to the plasma, where in humans it circulates at levels ranging from 20 to 30 pM/liter of plasma, NPY has a half-life similar to that of PP, namely 6–8 min. Since the majority of NPY is associated with neuronal elements, much attention has been devoted to its neuropeptide role within the central nervous system and innervated vasculature. However, the wide distribution of NPY receptors throughout various organ systems (renal, respiratory, and gastrointestinal, to name but a few) implies a widespread area of actions for the peptide. NPY has been identified in the pancreas of many mammals (but not humans) and the angler fish, and is usually associated with the vasculature, ducts, acinar cells, and islets. Vagotomy is without effect on the NPY-renal elements, but treatment with 6-OH dopamine (chemical sympathectomy) causes a marked reduction—but not total elimination—of NPY (26). The peptide probably acts as a vasoconstrictor in renal tissue.

By 1985, it was established that NPY is fully capable of exerting effects at presynaptic junctions where it can block release of transmitter substances at postsynaptic junctions as in vasoconstriction and/or act as a potentiator by recruiting α_2-binding sites without an effect on the affinity of such sites. Although diversity of action is not to be confused with significance of action, the widespread nature of NPY both within and outside the central nervous system suggests strongly that alone or with its peptide cousins (especially PYY, since they frequently share a common receptor), it may be a modulator of significant stature. Certainly, there exists, in addition to structural homology among this family of peptides, overlapping areas of action, invariably oriented toward the same end point. Thus, the three polypeptides may be considered "family" both by structure and by function.

Current Perspectives of Pancreatic Polypeptide Family Function

From the above general comments relative to various effects of the three polypeptides, three areas of

overlapping action are obvious and have attracted a considerable body of research relative to the elucidation of what role each member of the family plays in these arenas. Reference is made, of course, to the areas of gastrointestinal, vasomotor, and feeding behavior physiology. Other areas of interest, especially that of the reproductive actions of NPY, are covered by excellent, available reviews (e.g., 26).

Gastrointestinal Physiology and the Pancreatic Polypeptide Family. The very first biological effect of any PP member was that demonstrated in chickens by APP (34) where bolus injections of the peptide increased protein and volume secretion by the proventriculus. These observations were confirmed with low doses of BPP in dogs, whereas higher doses perfused into fistula-cannulated dogs caused a marked inhibition of cholecystokinin-stimulated pancreatic juice (36). Several laboratories verified these inhibitory observations with doses of BPP, PPP, or HPP that were said to be physiologic in concentration relative to levels induced by those eating meals, but attempts to demonstrate such an action on isolated pancreatic acinar cells *in vitro* failed. Such data, along with the failure of labeled PP—regardless of which terminus was radiolabeled—to bind to gastrointestinal receptor sites, may well indicate that these gastrointestinal effects of PP are of an indirect nature (19, 55). Workers have been successful in demonstrating the localization of highly specific PP receptors in the rat area postrema, an area closely associated with the dorsal motor nucleus of the vagus (56). This finding may implicate PP in a feedback loop whereby the hormone's pancreatic inhibitory action is mediated by decreased vagal activity.

In studies comparing the equimolar activity of PP, PYY, and NPY on biliary-exocrine pancreatic secretion in rats, Louie *et al.* (38) demonstrated clearly that PP, PYY, and NPY are equipotent in inhibiting water, enzyme, and electrolyte secretion by the exocrine pancreas, as well as in inhibiting bile secretion. More recent studies have emphasized this inhibition of the exocrine pancreas effect. Evidence presented above (see Distribution and General Effects: Recognition of Family by Function, Peptide-YY) indicted PYY as a probably significant circulating gut hormone based on its known (until 1985) effects, its high concentration in gut epithelial cells, and its release to the circulation after a normal meal (48). PP's similar effects, especially on the exocrine pancreas, were established even earlier (see Distribution and General Effects: Recognition of Family by Function, Pancreatic Polypeptide). Currently, a reasonable amount of data suggests that release of PYY from its L cell in the lower gastrointestinal tract is prompted by meal eating, though not necessarily by a direct action of nutrients on the L cell. Rather, it appears that the foregut plays a significant role in stimulating the release of PYY. Such a consideration is based on the observation that blockade of chyme moving from duodenal to colon regions does not prevent the release of PYY, nor does interruption of the intramural neural elements along the small bowel prevent PYY secretion (50). Thus, we are led to conclude that meal eating prompts PYY release by an endocrine mechanism from the foregut, and this action may be augmented by an extramural neural mechanism.

Once released, PYY plays an important, maybe more important than PP, inhibitory role at the level of the exocrine pancreas (40, 51, 57–59). In rats, this role would appear to be one of completing a negative feedback loop involving exocrine pancreatic secretion (51). Thus, while the delayed response to meal eating (increased PYY levels) occurs as seen in other mammalian species, the levels of PYY attained are capable of inhibiting cholecystokinin-stimulated bile-pancreatic juice volume, pancreatic amylase, and protein secretion (9, 59). Meal-induced release of PYY, therefore, is sufficient to overpower the release of cholecystokinin, emphasizing in part the endocrine role of PYY. Furthermore, as indicated by Putnam *et al.* (58), PYY has a central site of action, probably one involving the dorsal motor vagal nuclei. Using bethanechol (to stimulate pancreatic muscarinic-cholinergic receptors), direct vagal stimulation (presynaptic activity), and 2-deoxyglucose (to stimulate central vagal centers), these workers demonstrated in rats that although both PP and PYY were inhibitory by acting at several sites (40–83% inhibition), only PYY was 100% effective in totally blocking the 2-deoxyglucose-stimulating effect on the exocrine pancreas (58). This was accomplished at a PYY dose that was 1/25th that required to achieve only partial blocking in the bethanechol-treated or cervical-vagal-stimulated animal (Fig. 3). PYY affects only the stimulated pancreas, not the basal secretion (9). Such observations emphasize, therefore, that PYY has both central and peripheral inhibiting effects as part of a feedback loop involving the exocrine pancreas. Inhibition is via suppression of cholecystokinin release and by neural mediation at a preacinar cell site, probably in the brainstem. This central effect of PYY would be similar to that described for PP's inhibition of the pancreas (56). Could PYY be the long sought after enterogastrone hormone described well over 50 years ago? At present, this pancreatic peptide appears to fill just such a role, as its temporal and end effects are very much in accord with both older and more recent literature (40, 59). PP may act similarly, but generally it is about one fifth the potency of PYY in inhibiting pancreatic secretion (57, 58). In all probability, NPY also acts via a central vagal locus to exert its inhibitory effect on the pancreatic-biliary secretory volume.

In addition to suppressing the exocrine pancreas, PYY also inhibits gallbladder and gastric activity. Like PP, PYY infusion into cholecystokinin-primed animals

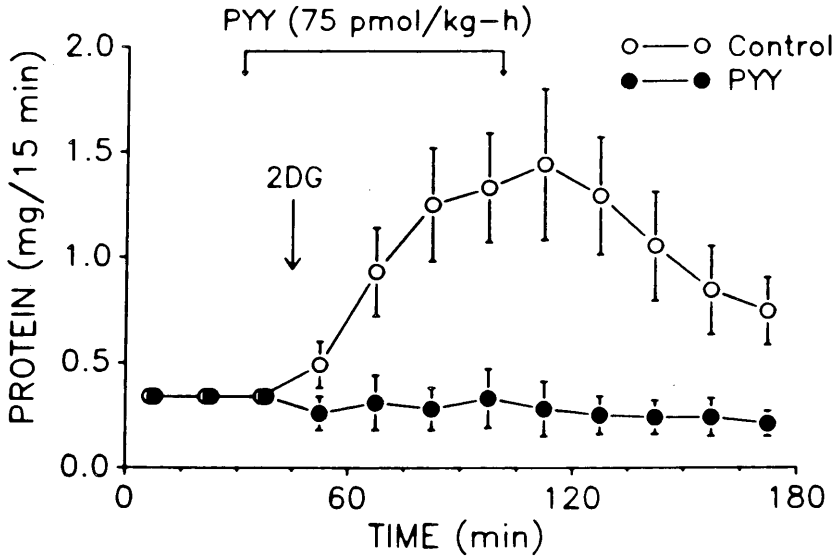

Figure 3. Effect of PYY on 2-deoxyglucose-stimulated pancreatic protein secretion. Reproduced with permission from Putnam *et al.* (58).

results in increased volume and filling of the gallbladder, but with a significant decrease in intragallbladder pressure (60). Again, these observations, along with PYY's effects on the exocrine pancreas, may qualify it to be labeled the anticholecystokinin hormone, or entergastrone, of the past. Postprandial and interdigestive activity of the gallbladder may well fall under the aegis of PYY, assisted by PP and NPY (38, 40, 51). Gastric motility and acid secretion, also, may come under the influence of PYY during mealtime or shortly thereafter. PYY significantly inhibits gastric acid secretion in mammals, and does so by a means other than by releasing gastric somatostatin. At the dose of PYY that inhibits acid secretion, the peptide fails to trigger somatostatin release from the gastric mucosa (52).

Neuropeptide-Y's role as a peripheral regulator of gastrointestinal function in mammals is largely associated with the intestine per se, notwithstanding its effects (already cited) on pancreatic-biliary volume flow. The finding of a potent antisecretory effect on the small intestine, as well as the fact that NPY and PYY appear to share a common receptor on the intestinal mucosa, has prompted intensive research on the intestinal action of these peptides (e.g., 9, 10). The presence of neural elements containing the neuropeptide is well distributed along the entire intestinal length, but the heaviest concentration appears to be in the upper small intestine. Nerve cell bodies in the submucosal and myenteric ganglia indicate an intrinsic origin of the NPY fibers. Yet, extrinsic fibers containing NPY are in abundance also, and originate from sympathetic ganglia (10) and are mainly intertwined with blood vessels. The distribution of NPY fibers in all layers of the gut wall suggests multiple actions of NPY, including a role in the regulation of intramural neuronal activities, smooth muscle tone, local blood flow, and epithelial transport (9, 10). NPY coexists with norepinephrine in some, if not all, sympathetic neurons that supply the intestinal vascular system (61). The submucosal and myenteric plexuses, however, are more likely to contain only NPY, or two other peptides that are related to each other, namely peptide histidine isoleucine and vasoactive intestinal peptide. Species variation as to transmitter colocalization in the gut is well recognized.

The C-terminal region of NPY is critical to binding to its intestinal receptor (62). The first 21 amino acid residues from the N terminus of NPY are virtually inactive in the binding process. This is to be contrasted with this peptide's receptor in the brain, where both the N and the C terminus are required for binding, and with the binding of BPP to intestinal basolateral membrane receptors, where, again, both ends of the molecule are important to binding (see Distribution and General Effects: Recognition of Family by Function, Pancreatic Polypeptide and Neuropeptide-Y, above). The large intestine does not appear to contain NPY receptors, whereas a moderate number of receptors reside in the ileum and a high density of them are found in the jejunum and duodenum (10). (It is to be recalled that regional differences in the location of PYY-secreting cells and of PYY receptors exist, which suggests an endocrine role for PYY.) Various inhibitory effects of both PYY and NPY have been reported in the small and large intestine. Depression of motility in the colon occurs and is attributed to the peptides, not to mediation by adrenergic receptors (9). *In vitro*, NPY causes relaxation of the longitudinal smooth muscle of the colon via a release of norepinephrine (10). This inhibits release of acetylcholine by α_2-receptors located on postganglionic nerves (63). In the small intestine, however,

NPY inhibits the enteric ascending reflex contraction of circular smooth muscle by inhibition of both cholinergic and noncholinergic components. Finally, NPY abolishes peristaltic movements in the small intestine, as well as nerve-mediated phasic contractions of circular gut smooth muscle. Collectively, therefore, these observations would indicate that the site of NPY inhibitory action is via an interference with the enteric neural excitatory events, rather than a direct effect on smooth muscle per se (9, 10). Species differences are numerous but the general, underlying theme is that both PYY and NPY exert inhibitory modulation of intestinal motility by multiple actions that are indirect in nature and that usually involve interactions with the enteric nervous system.

Epithelial effects of PYY and NPY, also, are of considerable interest, largely because through study of these polypeptides and their mechanisms of action, gut antisecretory drug design may evolve. PYY and NPY are potent inhibitors of secretion of fluid and electrolytes within the small intestine. Actually, they stimulate net absorption. Functional studies support the findings of basal lateral membrane receptors in mammals, namely the availability of the natural ligands NPY and PYY at this site, the negative coupling with adenylyl cyclase (which is localized in this membrane), and the fact that only application of either PYY or NPY at this site produces electrogenic ion transport in the (rat) intestinal epithelium (10, 64). The inhibitory effects of PYY and NPY on intestinal secretion are both direct (mainly) and indirect (lesser so) in nature, as both reduce the transmural electrical potential difference and short circuit current, and thereby increase the total ionic conductance in the ileum (10). PYY is much more potent in this regard than NPY. Tetrodotoxin is without effect on this action of NPY, which suggests that this peptide does not exert its transmural electrical effects by liberating a second transmitter. An inhibition of Cl^- secretion is probably the mechanism of the direct action of PYY and NPY on the intestinal epithelium (64). The indirect inhibitory effects of PYY and NPY are attributed to their ability to suppress the release of the powerful stimulator of intestinal secretion, vasoactive intestinal peptide. The peptides also are potent inhibitors of prostaglandin E_1- and E_2-stimulated cAMP in epithelial cells of the small intestine. Since the common receptor to PYY/NPY resides in the intestinal crypt region, where it is negatively coupled to production of cAMP, and both peptides are inhibitory to the production of this nucleotide, which acts as an intracellular mediator of ionic secretion, particularly Cl^-, it follows that expression of direct inhibition of intestinal secretion of water and electrolytes probably occurs at the level of the cryptic elements (10). Other ions and/or second messenger systems may also be involved in this inhibitory response to the PP-like peptides. Clearly, therefore, PYY and NPY, acting through multiple receptors most prominent in the upper small intestine, are among the most potent known physiologic inhibitors of gut fluid and electrolyte secretion (10, 26). Such a statement is supported by considerable *in vitro* and *in vivo* data. Also, such a statement emphasizes the very significant role which agents that could be designed to mimic either of these two members of the pancreatic polypeptide family may play as antidiarrheal tools (10). Both peptides control intestinal motility and blood flow, both reduce secretion of vasoactive intestinal peptide, and both suppress hydroelectrolytic secretion through an action on epithelial receptors. Such actions decrease propulsion of the digesta, allowing more time for absorption of fluid and nutrients, inhibit mucosal secretion, and lower the intestinal hydrostatic pressure to favor water absorption. Collectively, all three pancreatic polypeptides have significant inhibitory effects on gastrointestinal motility and secretion, actions that are extensions of the very first report on APP in chickens. Currently, however, the possible value of PYY-like and/or NPY-like drugs as antidiarrheal agents looks promising.

Vascular Physiology (Central and Peripheral) and the Pancreatic Polypeptide Family. Early studies with APP in chickens indicated that PP was without effect on heart rate, blood pressure, electrocardiogram, blood volume, or hematocrit. These observations, however, were made after a bolus injection of APP and in a species considered less than ideal for cardiovascular evaluation. One of the earliest cardiovascular effects of PP reported was that infusion of the peptide in cats causes a vasoconstriction that is slow in onset, protracted in duration, and persistent, even in the presence of simultaneous α- and β-adrenergic receptor blockade (65). Such actions imply that the peptide acts directly on vascular smooth muscle. This vasoconstriction response is not duplicated by infusion of norepinephrine. PYY and NPY, also, are active in this regard. PYY exerts a direct action on vascular smooth muscle, even in the presence of adrenergic antagonists or sympathectomy (5). On a molar basis, PYY's constricting action parallels—if not exceeds—that of angiotensin II in humans. Combined local infusions of norepinephrine and NPY cause a vascular response virtually indistinguishable from that of direct stimulation of sympathetic elements to the same area (65). Systemic infusion of PYY and NPY elevates systemic blood pressure, which indicates a general vasoconstrictive action, and usually is accompanied by bradycardia. The latter is due to inhibition of sympathetic tone. Of significance, therefore, is the demonstration that vasoconstriction as commonly observed in mammals may well be the result of release of NPY, or NPY colocalized with norepinephrine, and that normal vasomotor tone is under dual control. Infusion of norepinephrine in the absence of

an NPY component, therefore, will not totally mimic the effect of direct sympathetic stimulation. Dual control of pressor responses may be the offsetting equal to the dual control of vasodilation by acetylcholine and vasoactive intestinal peptide, now generally accepted for parasympathetic tone.

The presence of APP-like material in the brain and/or neural elements of vertebrates, as well as those organisms lacking a discrete pancreas (reported in the 1970s), in all likelihood can be traced to cross-reaction of NPY with antisera to APP. Structural homology between NPY and PP allows for cross-reaction with the very high concentrations of antibody (1,000 to 100,000 times greater than that employed with radioimmunoassay) that are commonly used in such studies. It is to be recalled that no PP-like material was extracted from neural tissue until Tatemoto and Mutt's (4) isolation of NPY in the early 1980s. Colocalization of NPY and norepinephrine in the brain is frequently associated with arteriolar innervation (53, 54). Such colocalization has been described for the medulla and involves centers important to regulation of respiration and cardiovascular function. When NPY is administered intracisternally, the peptide causes bradycardia, hypotension, reduced respiration, and synchronization of electroencephalogram patterns (66). These effects are similar to those of intracisternal administration of epinephrine or clonidine (53, 54). Furthermore, *in vitro* incubation of medullary membranes with NPY selectively increases the number—but not affinity—of α_2-binding sites for the peptide. No effect of NPY on α_1- or β-binding sites has been reported (67). Potentiation of norepinephrine's pressor effects by NPY has been reported many times (e.g., 26), and subsequent studies have clearly demonstrated that NPY and the catecholamine exert their pressor effects via distinctly separate receptors. (Note, however, that in many reported studies, species differences cloud the picture of vascular involvement of NPY to some extent.)

Splanchnic nerve stimulation in the calf is known to release NPY to the circulation. This may be of importance in human subjects who suffer from pheochromocytoma, a condition attended by plasma NPY levels that are elevated manyfold above normal. Studies on NPY labeled with iodine-125 at the molecule's N terminus (Bolton-Hunter procedure) indicate the existence of NPY-PYY shared receptors on pure smooth muscle fibers (aorta) in culture (68). These receptors do not recognize PP and, in fact, demonstrate characteristics quite different from those of hippocampal membranes. Perfusion of coronary vessels with graded levels of NPY results in graded reduction in blood flow in the isolated rabbit heart (Langendorff) preparation. The very high concentrations of NPY reported in the heart (atria higher than ventricles) and the dense innervation of coronary vessels by NPY neural elements argue for consideration of the peptide as a significant regulator of intracardiac blood flow (26). Not all cardiac NPY fibers contain catecholamines. Again, α- and/or β-adrenoreceptor blockade is without effect on the vasoconstrictive activity of NPY. Calcium channel blockers, however, reduce, if not abolish, NPY pressor effects in the heart. NPY has been shown to inhibit β-receptor-induced vasodilation in coronary vessels, an action thought by some workers to be more important than NPY's direct pressor effect (26). The current use of antihypertensive drugs may need to be modified to allow for the possible NPY component in coronary disease.

Collectively, available data on the systemic effects of NPY in mammals indicate that at least three different mechanisms are probable routes of action, none of which is mutually exclusive. NPY exerts its direct effects on vasoconstriction, potentiates norepinephrine-, and histamine-, induced vasoconstriction through different receptors, and also inhibits relaxation of coronary vessels (26). The peptide and norepinephrine also probably modulate their own release from sympathetic nerves in the heart. Again, the prominence of NPY in the heart vessels and muscle is striking.

That NPY is released in response to splanchnic nerve stimulation has already been noted. In addition, the stimulation of vagal elements in the isolated perfused pancreas causes a marked release of NPY to the effluent, in fact a 7-fold greater release of the peptide than that observed with sympathetic stimulation (67). Such observations indicate a probable dual control of pancreatic secretion by sympathetic and postganglionic parasympathetic neurons containing NPY as a transmitter. These observations do not imply that the pancreas per se is the source of NPY. In addition to its effect on central regulation of blood pressure, NPY contributes to regulation of gastrointestinal function by direct effects on smooth muscle tone of vascular elements within the gut. NPY and PYY are potent inhibitors of both intestinal and pancreatic blood flow (9). Within the splanchnic circulation, NPY has been estimated to be at least 20–25 times more potent on an equimolar basis than norepinephrine in producing vasoconstriction. Unlike norepinephrine, however, the vasoconstrictive response to NPY is not followed by a period of vasodilation. Sheikh (9) suggests that three different actions of PYY and NPY occur at the sympathetic neuroeffector junction in the gut to regulate intestinal function by regulating local blood flow (9): First, the aforementioned direct vasoconstriction of slow onset and protracted duration occurs independently of α- and β-adrenergic blockade but is reduced by calcium channel blocker. Considerable variability exists in the sensitivity of vessels to this direct action, with cerebral vessels appearing most sensitive. Second, prejunctional effects of PYY and NPY may inhibit elec-

trically stimulated release of norepinephrine from adrenergic nerve endings. Such an action may be mediated through the Y_2 receptor in the gut, an action that has been shown in rats to reduce systemic blood pressure. And third, NPY may act as a potentiator of other vasoconstrictors in the gut vasculature, an action observed *in vivo* as well as on isolated vessels *in vitro* from a wide variety of species including human preparations. A considerable body of evidence exists supporting this potentiator role of the peptides, particularly in relation to norepinephrine, angiotensin II, and histamine-induced vasoconstriction. While it is unlikely that all three of the above are effected at each sympathetic neuroeffector junction, the peptides do appear to be intimately involved in nonadrenergic components of sympathetic vascular control in gastrointestinal tissues (9).

In minisummary, therefore, the powerful vasoconstricting actions of PYY and NPY, less so with PP, appear to be executed via both central and local (peripheral) mechanisms, acting via various mechanisms and probably through multiple receptors. Of particular note is that locally the effects of these peptides are quite prominent within gastrointestinal tissues and, along with their direct action on hormone-secreting and enteric-electrolyte-secreting cells (see above), they very likely exert considerable inhibitory control over gastric, small intestine, and exocrine pancreatic secretions by regulating blood flow to these areas. Such actions are largely independent of the well-established catecholamine system.

Hunger-Satiety Behavioral Patterns in Health and Disease. *Normal ingestive behavior.* In previous sections, it was mentioned that early observations indicated that members of the PP-fold family probably were involved with various central nervous system functions, not only those of a cardiovascular nature but also some of a metabolic nature as well. First suggested in the late 1970s by reports that APP-like material was concentrated in the central nervous system of lower forms, the possibility that a pancreatic-central nervous system functional axis existed in higher forms was given a large boost by the observation that specific binding of ^{125}I-APP was highest to plasma membranes prepared from brain tissue, more so than from any other tissue, including stomach, intestine, pancreas, spleen, and muscle (69). At about the same time, other reports appeared linking the PP-family to various forms of metabolic-feeding behavioral patterns, some including pathophysiologic conditions (33). Thus, APP's antilipolytic action on isolated adipocytes (5) was followed by the observation that in congenitally obese (*ob/ob*) mice, meal eating does not foster the normally observed immediate release of PP, as it does in lean littermates (70, 71). Yet the *ob/ob* pancreas contains at least twice as much PP as does the pancreata of lean control mice. If such an obese mouse is parabiosed with a lean littermate, or receives transplanted normal pancreatic islet tissue, the usual characteristics attending this form of obesity, such as hyperphagia, glycosuria, hyperglycemia, obesity, and glucose intolerance, disappear (33, 70, 72). These symptoms also disappear if the *ob/ob* animal receives chronic injections of APP or BPP (72, 73). Such observations strongly suggest a defective trigger-release mechanism in the experimental animal model. Furthermore, humans that are considered patho-obese (that is, >30% of their ideal body weight) also do not respond to the postprandial increase in HPP. Finally, Prader-Willi subjects, who frequently exhibit obesity, hyperglycemia, and glucose intolerance, fail to respond to meal eating with a release of PP (33). Collectively, therefore, these observations, reported over a period of several years, focused investigators' attention on the possible roles that the central nervous system and PP play in the hunger-satiety sequence, a sequence that had been documented by earlier studies to be dominated by certain hypothalamic nuclei. Certainly, the *ob/ob* data cited above indicate that such an obese animal possesses a functional satiety center, one that can be stimulated by a blood-borne factor issued by its lean littermate. And also evident is the fact that this "satiety factor" in all probability originated from a non-B or A cell component of the pancreas because, alloxanized pancreatic transplants are equally effective in correcting the hyperphagic obesity as are normal pancreatic transplants to which antiglucagon antibody is added (72).

Fundamental differences in the regulation of hypothalamic NPY have been reported in Zucker (*fa/fa*) obese rats (74). Such obese rats have increased NPY concentrations in areas of the hypothalamus that are considered important to feeding behavior. Food restriction does not alter these high NPY concentrations, nor does refeeding (after a fast) to regain weight loss. The latter regimen increases nuclear levels of NPY in lean control rats. When injected into the paraventricular nucleus (PVN), NPY causes hyperphagia, obesity, and increased insulin and glucagon secretion (74). The *fa/fa* report strongly suggests that NPY acts on certain hypothalamic nuclei as an orexigenic agent. With the realization that the APP-like material identified within the central nervous system of mammals, including humans, was in fact the result of cross-reactivity of anti-APP sera with the homologous NPY neuropeptide, interest steamrollered toward elucidation of the physiologic role such a peptide would play in terms of feeding behavior. Also, the demonstration of PYY-NPY receptors within the mammalian brain, as well as that of a distinct brain PP receptor (see Chemistry of Pancreatic Polypeptides and Their Receptor Structures), encouraged investigators to focus on studies that would elucidate the purposefulness of a putative (at that time, circa

early 1980s) pancreatic-central nervous system axis. Should PP be named by the more descriptive, though precise, term satietin?

Of all known neuropeptides, NPY is the most heavily concentrated within the region of the hypothalamus (75). Of all hypothalamic microregions, NPY is the most densely present in neural endings associated with the PVN, the ventromedial hypothalamic nucleus, the dorsal medial hypothalamic nucleus, and the arcuate-median eminence tract (afferent to the pituitary gland and to the dorsal motor vagal nuclei) (33, 74, 76). These areas are hypothalamic regions associated with feeding behavioral patterns. Also, the fact that norepinephrine and epinephrine are frequently (40–50%) colocalized with NPY in presynaptic neural elements in the central nervous system and the fact that both catecholamines stimulate the feeding process by an action on the PVN, would suggest that NPY may be a significant peptide in terms of the regulation of the hunger-satiety sequence of events.

Clark *et al.* (77) were the first to demonstrate that the intracerebroventricular injection of NPY causes robust feeding in rats. This report was quickly confirmed by other investigators (76, 78, 79), and certain experimental design embellishments allowed for a fuller understanding of the range of NPY effects, as well as a possible cooperative role that other family peptides may play in hunger-satiety. The likely importance of NPYergic activity at the PVN may be evaluated in light of the finding that NPY levels increase in the PVN in rats during fasting periods, and are returned to normal upon refeeding (80). Microinjection of NPY onto the PVN of rats results in an increase in food intake (measured as early as 30 min after injection) that is long lasting. In fact, if food is not presented until 4 hr after injection of NPY, a significant increase in intake is still observed (76). Water intake is also significantly increased, though the imbibition is not as dramatic. Of considerable interest is the fact that these results can be produced day or night (normally, rats do not eat meals during the daytime), in fasted or satiated animals, and even in the hibernating squirrel during its prehibernation anorectic period (76, 81). That these central effects of NPY are not singular in action is seen by the fact that when food is absent, NPY injection onto the PVN causes alterations in behavioral patterns associated with feeding in addition to the increased water intake. Such animals consistently increase their physical ("searching"?) activity, while decreasing their sleep time and grooming periods (76, 77, 81). PVN administration of NPY in quantities equal to those given intracerebroventricularly invariably produces much stronger feeding responses. The behavioral effects of NPY appear to involve its action on the region of anterior ventromedial hypothalamic nucleus and/or the PVN via the arcuate nucleus. When feeding follows the PVN injection of NPY, grooming is accentuated, as is sleep time, and there is a reduction in physical activity. Such behavioral patterns are expected with the onset of satiation and argue against a role for norepinephrine (in the induction of this pattern) as would be seen in general activation or stress.

In nonmammalian vertebrates such as chickens, the application of either PYY or NPY via the cerebral ventricles increases early and robust feeding (8). Increased food intake is approximately twice as much in NPY-injected chicks as it is in controls, crop size is significantly larger, and less time is spent standing. While NPY and PYY are equally effective in increasing food intake, PYY has a demonstrable effect in increasing crop size to twice that observed in NPY-injected chicks, which suggests that in addition to the orexigenic effect of PYY, the peptide exerts an additional effect, that of inhibition of passage of food along the GI tract (8). Rats given multiple injections of PYY exhibit a gross distention of the gastrointestinal tract (82), also possibly due to a slowing of digesta movement.

At present, NPY and PYY are considered to be the most potent endogenous orexigenic agents known. Opioids in equimolar concentrations pale beside the PP-peptides when considering enhancement of food intake. Studies carried out subsequent to those cited above with rats indicate that when administered intracerebroventricularly, NPY has a reasonably long latency until onset of action, as well as a relatively long-lasting effect (81). When two boluses of NPY are given 4 hr apart to rats intracerebroventricularly, the second injection increases food intake to even higher levels of intake than promoted by the first injection. Saline injections are without effect on food and water intake. When given a choice of diet, NPY-injected rats will preferentially select a diet high in carbohydrate over one high in protein or fat. By contrast, norepinephrine, even at concentrations 1000 times greater than that employed with NPY, exerts a modest but significant increase in food intake, but such rats are ambivalent in their choice between high carbohydrate and high fat diets (81). These comparative observations between NPY and norepinephrine may bear considerable significance in some disease states. The effects of the catecholamines on the feeding behavior patterns just described are prevented by adrenalectomy, double vagotomy, and α-adrenergic blockade, procedures that are without effect on the ingestive behavior induced by NPY. Additionally, norepinephrine is without effect on water intake. Thus, it would appear that the ingestive behavior induced by NPY is completely distinct from that induced by norepinephrine, and in no way is the result of altering the latter's activity within the hypothalamus, particularly at the PVN. In all studies reported, the effect of NPY on water intake appears to be less robust than this peptide's effect on food intake.

By contrast, PYY effects increased water intake at peptide levels far lower than that required to cause an increase in food intake. Morley *et al.* (81) suggest that during evolution, these two closely related peptides may have diverged so that PYY became a regulator of water intake, while NPY evolved as a regulator of food intake. The critical experiment in this arena awaits to be carried out.

Corticotropin-releasing factor is a known powerful inhibitor of food intake, probably by acting on the PVN, and has been suggested as a significant factor in the anorexia associated with stress and/or certain psychoemotional states in humans. Both corticotropin-releasing factor and calcitonin, another central inhibitor, block the effects of NPY on food intake. Interestingly, cholecystokinin, a well-known and accepted satiety-type gut peptide, is without effect on NPY's orexigenic action (81). This is of significance when considering the report that repeated injections of PYY (over 48 hr) distend the rat stomach greatly due to accumulated food. Thus, the central orexigenic effects of the two PP overpower peripheral signals normally sent to initiate satiety (82).

The first report on the biological effects of any PP molecule was that of APP on avian liver glycogen, blood glucose, blood cholesterol and glycerol, and triglyceride levels (34). APP had no effect on blood glucose levels, either in fasted or nonfasted chickens. In more recent studies, NPY has also been found to be without effect on rat plasma glucose levels, an observation that would exclude hypoglycemia as a driving force for food ingestion (81). However, focal restriction of glucose delivery to certain hypothalamic nuclei cannot be ruled out, considering NPY's powerful pressor effects (66). Such a vascular reduction in glucose delivery may be "perceived" by the nuclei in the affected hypothalamic areas as hypoglycemia or "central starvation," thereby initiating the orexigenic activity and associated behavioral patterns just described (81). Again, the critical experiment to test this suggestion has yet to be carried out.

Much of the work described above was carried out by microinjection of NPY (or PYY) onto specific hypothalamic nuclei; some of the work was accomplished by the less precise, but still useful, technique of injection into the cerebrospinal fluid within the lateral or third cerebral ventricles. In the latter situation, data evaluation would be based largely on those brain structures known to be perfused by cerebrospinal fluid flow in that region. Of course, cerebrospinal fluid flow is rostral-caudal, that is lateral ventricles to fourth ventricle, in direction. While several areas of the hypothalamus have been shown to be sensitive to NPY's induction of robust feeding and drinking, extrahypothalamic and other forebrain areas are not. Recently, the hindbrain was investigated as a possible neural substrate for PYY and NPY's effects on ingestive behavior (78, 79). A fourth cerebroventricular injection of PYY and/or NPY very likely perfuses hindbrain structures, structures that could very well have been stimulated in earlier studies by intracerebroventricular administration of the peptides upstream, that is, into the lateral or third ventricles. In addition to its heavy localization in the lower gut in mammals, PYY also has been localized to the cervical spinal cord and medulla oblongata. PYY-like immunoreactive sites therein appear to be distinct from NPY-containing presynaptic elements (83, 84). This area also contains high affinity binding sites that cross-react with both PYY and NPY. Thus, it is not surprising to find that PYY and NPY both, when injected in microgram quantities into the fourth cerebroventricle, induce dose-dependent, robust feeding concomitant with a reduced latency in time to the onset of eating (78). Doses of NPY too low to stimulate feeding, frequently are potent enough to reduce the feeding time latency period, nonetheless. Also, water presented with food was imbibed more vigorously when the peptides were injected into the fourth ventricle, when compared with control, vehicle-injected rats. PYY is considerably more potent in inducing feeding behavior as compared with NPY, but the two peptides appear to be equally effective in inducing water intake (78). Other behavioral patterns affected by hindbrain perfusion with NPY or PYY include total time feeding (increased), resting (no effect), and sleeping (PYY only, decreased), exploratory behavior (PYY only, increased), grooming behavior (reduced 50%), total time standing (no effect), and percentage of time drinking (no effect). Overall, these ingestive behavioral effects—including the feeding and drinking activity—are very similar in quantitative and qualitative terms to those reported earlier for lateral and/or third ventricle placement of either peptide, though slightly higher peptide doses are necessary at the hindbrain than at the PVN. It could be concluded that the PYY-preferring receptor of the hindbrain plays a significant physiologic role in modulation of ingestive behavior in the rat. The possibility of heterogeneity in the distribution of NPY- and PYY-binding sites in the hindbrain (85) may be supported by the above litany of behavioral effects (other than ingestive), wherein PYY exerts a positive modulatory effect but NPY does not (decreased sleeping, increased exploratory activity, etc.). NPY-containing neural pathways extend from the hindbrain region forward to various hypothalamic nuclei, and experimental transsection at various points between the two areas has been found to block the ingestive action of NPY when placed in the fourth cerebroventricle (86–88). Thus, the argument is strengthened that a functional PP-like tract of the hindbrain may well activate anterior hypothalamic centers that in turn direct mammalian feeding behavior. Certainly, more work is needed in this area.

Pathophysiologic ingestive behavior. If it is ac-

cepted that the PP family is an important modulator of gastrointestinal activity, if it is accepted that at least two of the family peptides are potent pressor agents, thereby capable of producing highly focal areas of vasoconstriction, and if it is agreed that NPY and PYY are potent endogenous orexigenic peptides, then it would seem reasonable to expand our knowledge of these peptides in terms of their possible role in certain pathophysiologic states. For instance, could highly variable peaks and valleys of central NPY be responsible for the binge eating associated with bulimia? This condition is found most frequently in young adult women, previously normal, who develop an abnormal fear of becoming fat. Excessive use of laxatives and forced vomiting follow massive periodic (binge) eating. The amount of food ingested can be enormous, up to 50,000 kcal/binge eating episode. The eating pattern has been described as dietary chaos, but one underlying characteristic worth mentioning is the fact that bulimic subjects have a strong preference for carbohydrate-rich foods! Dental caries are common in the subjects (89). Again, could an accumulation of NPY at the site of certain hypothalamic nuclei be responsible for the binge eating syndrome and, with vascular removal, the hiatus that occurs between eating periods? Or could the increased buildup of NPY centrally invoke strong peripheral satietin release to offset the distended bowel associated with NPY action? Studies in this area are called for and certainly should be rewarding.

Anorexia nervosa is thought by some workers to be a form of bulimia, but by others it is thought to be a separate disease because a number of distinct differences between the two can be cited. Anorexia nervosa, too, is usually found in young women of middle to upper class backgrounds, and is characterized (like bulimia) by a paralyzing fear of obesity (89). A radical restriction of caloric intake results, leading to emaciation. Primary hypothalamic disorders have been postulated in anorexia nervosa, but whether they are truly causal or secondary to other factors is yet to be established. There appears to be a preoccupation with food, even in the preparation of it for others. (Changes in the luteinizing hormone response to luteinizing hormone-releasing hormone also occur, and are qualitatively similar to those seen in aged rats where NPY levels are decreased.) The question may be asked whether a "valley" of plasma NPY is a contributing factor to anorexia nervosa? If so, then the episodic binge eating that spells the prolonged anoretic period in the anorexic patient may be attributed to a central buildup of NPY. Again, definitive answers to these questions are yet to be put forth, but the questions indeed encourage investigation.

Diabetes mellitus is a metabolic condition characterized by hyperglycemia, glycosuria, polyuria, polydipsia, and hyperphagia. Frequently, a craving for carbohydrate can be added to the aforementioned. Other characteristics exist but differ according to the etiology and course of the illness, if untreated. Whereas bulimia and anorexia nervosa present intriguing postulations of possible aberrant cerebral NPY/PYY levels, a considerable amount of evidence implicating hypothalamic NPY as causal to various symptoms of diabetes mellitus has been reported. The questions could be asked: is the hyperphagia observed in diabetes mellitus due solely to failure of intracellular transport of glucose? Is the craving for high carbohydrate foodstuffs merely a reflection of "glucose starvation"? And is the attending polydipsia so frequently observed solely related to the induced osmotic diuresis as the patient excretes glucose and electrolytes? Alternatively, could abnormal central levels of NPY/PPY be a significant etiologic factor in producing the aforementioned symptoms?

Since the early studies on PP, shortly after its discovery, experimental diabetes (type I) in rodents has been known to cause an increase in the population of F cells in the periphery of the pancreatic islet. Also, in a large cross-section of humans, it was reported that diabetics (types I and II) most frequently had higher plasma levels of HPP than age-matched control subjects (90). However, no evidence could be found that indicated PP as a significant causative factor in the onset, or the course, of diabetes in humans or animals. Only recently has investigation focused on other members of the PP-family as possible contributors to the expression of this disease. In the case of NPY, results have been quite rewarding. Thus, rats injected with the β-cell cytotoxin, streptozotocin, have elevated central and lateral hypothalamic NPY levels as early as 2 weeks after induction of diabetes. These elevated NPY levels (detected by radioimmunoassay techniques) continue to increase and remain 100–200% above control levels for at least 14 weeks (91). The central hypothalamic region in this study contained the ventromedial and paraventricular nuclei, nuclei that are considered very important to food and water ingestion. Intense NPY staining was found in swollen cell bodies using immunostaining techniques. It is interesting to note that NPY levels increased only after hypoinsulinemia was permanently established, and thus weeks after the induction of hyperglycemia. Hypoinsulinemia, therefore, could reduce glucose entry to the hypothalamic glucose sensors, cells that are thought to assist in the regulation of appetite in mammals (91, 92). Increased eating would be a natural homeostatic compensatory response. Findings of increased NPY staining in the area of the supraoptic nucleus in diabetic rats may indicate an important role of the peptide in regulating water metabolism via modulation of antidiuretic hormone secretion. Certainly, the increased water intake (polydipsia) observed in diabetes would be aided by the antidiuretic effect of antidiuretic hormone in an effort to conserve water. Further attempts to right the metabolic wrong

of diabetes may well include the stimulation of the damaged B cell (due to streptozotocin) because NPY administered intracerebroventricularly causes insulin release (93). Thus, a possible corrective role can be suggested for hypothalamic NPY in diabetes, one that favors intake of nutrients (especially carbohydrate) to replenish "starved" cells (hyperphagia), conservation of water to offset loss via diuresis (polydipsia), and attempts to achieve normoglycemia (β-cytotropic action of NPY).

Further work (via microdissection) along these lines indicates, indeed, that increased synthesis of NPY occurs within nuclear areas of the rat hypothalamus, and that stored NPY is readily released by an appropriate stimulus. Studies on prepro-NPY mRNA in the hypothalamus reveal that up to a 500% increase in the messenger occurs soon after the induction of streptozotocin diabetes in rats (94). Insulin treatment of streptozotocin-diabetic rats returns these elevated mRNA levels to normal. Elevated levels of the polypeptide are readily released from hypothalamic slices by depolarizing techniques (95). While most hypothalamic nuclei contain elevated levels of NPY 3–5 weeks after streptozotocin-induction of diabetes, the most consistent and prominent are the arcuate nucleus, the ventromedial hypothalamic nucleus, dorsal medial hypothalamic nucleus, and PVN nuclei (96). Insulin therapy in these streptozotocin-diabetic rats completely prevents the NPY accumulation otherwise seen (95). Those areas of the diabetic hypothalamus with high levels of NPY that are not associated with feeding and drinking are structures associated with efferent regulation of anterior pituitary secretion. Diabetes is known to be associated with impaired secretion of luteinizing hormone (97), thyroid-stimulating hormone (98), growth hormone (99), and prolactin (100).

Could the persistent high concentration of NPY at various hypothalamic centers play a significant role in the expression of diabetes in mammals? From the above review, serious consideration must be given to this possibility. The orexigenic nature of NPY is complemented by the peptide's effect on water intake and the preferential intake of diet high in carbohydrate content. Furthermore, NPY's increased release at the supraoptic nucleus implies a homeostatic role in correcting an imbalance in water metabolism. The fact that centrally administered NPY stimulates insulin release in normal rats and that NPY levels do not increase in streptozotocin-treated animals until a clear hypoinsulinemia is established presents a scenario enmeshed in homeostatic, compensatory considerations. And finally, the fact that the diabetic hypothalamus, in the absence of adequate plasma insulin, synthesizes, stores, and releases NPY at levels far in excess of normal hypothalami would indicate a significant functional role for this member of the PP family in the altered feeding, drinking, and impaired reproductive functions associated with human diabetes mellitus. While this field of PP activity is no longer virgin, it certainly is pregnant with experimental possibility.

Overview

Clearly, a family of pancreatic polypeptide hormones exists. Others may well be added to this list based upon structural homology. This polypeptide family possesses overlapping structural similarities, as well as overlapping physiologic expressions, though the origin of each member appears to be distinct from each other. Thus, PP originates in the pancreatic islet F cell and shares the quality of PYY (and to a lesser extent that of NPY) as a suppressor of exocrine pancreatic secretion and gallbladder activity. Additionally, it is a weak pressor agent. PYY, originating in the lower intestinal tract and rectum, acts formidably as a suppressor of gastrointestinal activity (is it the long sought after enterogasterone?), is an effective vasoconstricting agent, and peripherally may be part of the battery of agents that act as satietins. Centrally, PYY is an extremely powerful orexigenic agent as well as an inhibitor of vagal-induced gut secretion. Its action on the gut stands out as a model of efficiency because it has direct inhibitory effects on enteric cells, indirect inhibiting effects on myenteric vasculature by causing vasoconstriction, and antihormonogenic secretion effects on cholecystokinin-secreting cells. End result? PYY, alone or with other family members, is a powerful antidiarrhetic hormone. NPY appears to be strictly a neuroendocrine product, mainly of central nervous system-hypothalamic and peripheral sympathetic neuronal elements. Its frequent colocalization with norepinephrine in neural terminals and its very powerful pressor capability encourage workers to re-evaluate their understanding of sympathetic control over the vascular system. Could its vascular effects at the pancreas level explain the failure of meal eating to release PP in obese rodents and humans? Furthermore, NPY's powerful orexigenic effects at anterior hypothalamic sites is complemented by its equally powerful effects on behavioral patterns associated with hunger-satiety. Excursion or pulsating levels of NPY may play a role in the binge-eating syndromes in humans or in the protracted high levels on the aberrant feeding and drinking habits of diabetics. Peripherally, NPY shares PYY's and PP's gut, gallbladder, and pancreatic antisecretory activity, partially by its direct enteric cell action and partly by an indirect vascular pressor effect. Despite the various powerful satietins that bombard the central nervous system during meal eating, the central (hypothalamic) effect of NPY appears to be overpowering.

When taken holistically, the substance of this Minireview has a singular, common theme. Thus, despite the diverse properties of each of the three polypeptides

discussed, there emerges the simple fact that all three are gut oriented. The artificiality in subdividing the major actions of the PP family (as suggested by the title to this paper) becomes evident when one considers that virtually all actions discussed, normal or pathophysiologic, have fundamental involvement with gastrointestinal phenomena. Central regulation of food and water intake (NPY, PYY), central regulation of exocrine secretion (PYY), central (NPY) and peripheral (NPY, PYY, PP) control of gut vascular smooth muscle, peripheral inhibition of gut peptide release (PYY), direct inhibition of crypt enteric secretion (PYY, NPY), and inhibition of gut motility (PP, PYY, NPY), and gut accessory organ secretion (PP, PYY, NPY) collectively emphasize the very probable significant role that the PP family plays in regulation of ingestive and digestive behavior. Other significant effects of some members of the family, particularly NPY, fall outside this review, especially those actions related to gonadotrophin release.

Finally, it should be noted that over the last 5–7 years, excellent review articles have appeared from laboratories actively engaged in elucidating the physiologic significance of one or more of the pancreatic polypeptide family. Each is presented in technical detail to a greater extent than space permits here. Thus, for further information on various aspects of PP family physiology, the reader is referred to: receptors (12, 16, 23, 28), ingestive behavior (25, 66, 76, 78), gut circulation (9, 26), biochemical evolution of the PP family (12, 25); cerebral circulation (54), gonadotrophin release (26), and a comparative overview (40).

Fertile Fields Favorable for Future Funding

Our understanding of the physiologic significance of the PP family relative to the gastrointestinal-vascular-feeding observation described is limited to those major reports covered herein. Obvious voids in our knowledge still exist in these areas, as well as in other areas of PP family function, ones that should be experimentally attacked in the near future not only for the information gained but also because in certain cases, corrective measures of treatment in certain diseases may result.

Actually, very little is known relative to the mechanism of action of any of the family members, especially that of NPY. Although APP has been implicated to be antilipolytic via a cAMP mechanism and PYY has been linked negatively to intestinal (enteric) cellular cAMP, little else is known relative to mechanisms of action. As for NPY, it is known that this polypeptide inhibits voltage sensitive Ca^{2+} channels in dorsal root neurons as well as stimulates adenylate cyclase activity in cultured rat atrial cells (101, 102). Both of these actions are blocked by a pertussis toxin-sensitive G-protein. Furthermore, the polypeptide stimulates the synthesis of inositol triphosphate and diacylglycerol in dorsal root ganglion neurons (101). We are still ignorant of what postreceptor mechanisms carry out the cellular messages of these three polypeptides.

Another area of considerable need—and interest—is that of the vascular importance of NPY in cerebral, coronary, and general systemic arteries. What is the function of NPY-only neural elements? How does (mechanism of action) NPY potentiate norepinephrine action in the autonomic nervous system? Is normal vasomotor tone truly under the dual pressor control of norepinephrine and NPY? Does NPY play a significant role in cerebral vascular spasms and/or systemic hypertension? Answers to these questions could be of considerable significance in future treatment of cardiovascular disease.

What are the major sources of NPY in the blood and cerebrospinal fluid? How is it released from the neurosecretory neurons (26)? Can this release be attenuated or intensified to meet the needs of the organism?

But probably of most importance (and due to the salient fact that many of the answers sought above would come forth) is the development of highly specific and highly affine agonists and antagonists to each member of the PP family. Recently, certain "partial antagonists" were employed in receptor recognition studies. Thus, centrally truncated analogs of porcine NPY have been synthesized and tested in binding studies. Results of such studies indicate that the central region (residues 7–17) of the molecule probably serves a structural role but is not involved in direct receptor interaction (103). A fairly highly potent agonist of NPY (of reduced size) has been described as containing the N-terminal NPY segment 1–4 linked via a ϵ-aminocaproic acid to the C-terminal partially α-helical peptide segment 25–36 (104, 105). Also, studies with [Leu^{31}, Pro^{34}]neuropeptide-Y indicate that this analog of NPY is a powerful agonist *in vitro* in rats, in that it is even more potent than native NPY in increasing blood pressure (106). Apparently, this analog is specific for NPY's Y_1 receptor.

However, no true deficiency state of any of the polypeptides has been reported, except possibly for PP in the chicken. We do not know what homeostatic imbalance would occur in such a total deficiency. Could a major physiologic effect of one or another, or even one that is yet to be reported, be unearthed in a "true" deficient state and thereby give insight as to what role normally is played by the peptide in question? It would appear that use of a specific antibody in avian embryos may be a reasonable approach to this problem.

Finally, and further to the possibility of drug-designed agonists and antagonists to PP, PYY, and NPY, work is needed for the development of specific agents to act:

—on the F cell of the pancreatic islet of the path-

ologic obese patient to release PP at the time of meal eating. This may not only curb the volume of food intake via the peripheral inhibitory mechanisms described, but it very well may act as a vascularly borne satietin at the level of the central nervous system. Metabolic control of plasma glucose, cholesterol, free fatty acid, glycerol, and insulin levels could also result.

—as antivascular pressor agents, thereby reducing the potential of cerebral vascular accidents, coronary attacks, and/or general hypertension.

—in conjunction with phasic peaks and valleys of NPY or PYY (but unlikely PP) in established cases of bulimia and anorexia nervosa. More detailed study is required in such patients to establish that such plasma perturbations truly exist. If so, antagonists may greatly relieve the "drive" toward aberrant eating behavior.

—to alleviate the voracious appetite and water intake in uncontrolled, or poorly controlled, diabetes mellitus. Hypothalamic synthesis of excessive amounts of NPY appears to be an integral part of the abnormal behavior observed in diabetic rodents. A specific antagonist, possibly one acting at the PVN, may greatly improve the craving for food, and food with high carbohydrate content, in diabetics. Also, in type II diabetes, where obesity is commonly an attending symptom, if not a complication, an anti-NPY antagonist may ease the difficulty of dietary compliance so commonly seen as an obstacle to successful weight reduction. Frequently associated with diabetes in adult women, also, are faulty gonadotropin release, ovulatory failure, and infertility (96).

From the stingy litany of "fertile fields" listed above, it is obvious that there is much to do and much to learn about the pancreatic polypeptide family. And in doing so, the excitement of accomplishment could very well be accompanied by uncovering not only new members of the family, but also more effective ways by which certain disease states can be alleviated.

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