Review

Peptide YY: A neuroendocrine neighbor of note

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1. Introduction

Peptide YY (PYY) and its degradation product, PYY(3–36) are both paracrine and hormonal members of the pancreatic polypeptide (PP) family whose levels increase significantly in tissue and plasma after a meal. This article is a summary of an invited lecture given at the eighth International Neuropeptide Y (NPY) meeting (St. Petersburg, FL, April 2006) and presents a "gut-centric" overview of PYY’s paracrine effects, specifically, (i) its cellular distribution, (ii) mechanisms of release.
and, (iii) functional consequences highlighting the specific Y receptors activated in normal human and mouse intestine (comparing these response profiles with the phenotypes exhibited by tissues from specific knockout (KO) models). Finally (iv), a summary of intestinal symptoms observed following either surgical manipulation or specific gastrointestinal (GI) diseases will provide a basis for final conclusions regarding the potential for Y-based agonists as mimics of endogenous PYY, etc., and to act therefore as novel anti-diarrheal drugs of the future.

How physiologically important are PYY and PYY(3–36) as defenders against diarrhea-causing stimuli? In vivo studies have shown that PYY or NPY infusion to healthy human subjects attenuate hypersecretion precipitated by either prostaglandin E2 or vasoactive intestinal polypeptide (VIP) [30,53]. Recent studies utilizing isolated colon mucosa have shown that PYY, PYY(3-36), NPY and PP are anti-secretory and these peptides stimulate the same repertoire of Y receptors (Y₁, Y₂ and Y₄) in human and mouse tissue [11,13,32]. However, the identity of the Y receptor type(s) that are responsible for the in vivo effects remain unknown, through lack of clinical experimentation with selective Y receptor agonists. Since diarrhea is a symptom of increased intestinal electrolyte and fluid secretion as well as increased motility, we have been interested in ascribing specific functions to exogenous and endogenous Y peptides and their cognate receptors given the background of different peptide distributions in the GI tract.

### 2. Cellular distribution of PYY compared with related peptides

PYY is expressed in ~50% of colorectal endocrine L-cells in mouse [3] and human large bowel [21] and is co-localized with pro-glucagon products, glicentin and glucagon-like peptide 1 (GLP-1) and GLP-2 [19]. Sundler et al. have shown that these unrelated peptides are co-packaged in the same secretory granules [7,19] and they are co-released, at least initially when food arrives in the duodenum [2]. PYY-positive endocrine cells in human colon are often surrounded by Y₁-immunoreactive epithelia and nerve fibers, but are themselves Y₁-negative [39] thus an autocrine action for PYY is unlikely in this tissue. PYY is additionally expressed in a very sparse population of gastric neurons discrete from those expressing NPY and only found in certain species [6] notably not in human or mouse stomach. PYY-positive neurons are also found in specific regions of the central nervous system, e.g. hypothalamus, pons, medulla and spinal cord [19] and these are discrete from those expressing NPY. The peptide is also found in pancreatic endocrine cells, more often co-localized with PP and less frequently with glucagon (depending again on the species studied).

PP-positive F-cells constitute the fourth group (~10%) of pancreatic islet endocrine cells, after insulin (in beta cells) glucagon (alpha) and somatostatin (delta) cells. PP is also present in a sparse, minor population of intestinal endocrine cells, throughout the GI tract of most species studied to date [19,21]. In contrast, NPY is present in >50% of enteric submucous neurons the majority of which innervate targets in the lamina propria including the epithelial lining of all mammalian models studied to date, including rat, mouse and human colon [18,20,49,57]. NPY is a non-adrenergic, non-cholinergic neurotransmitter in the mammalian enteric nervous system and is more commonly co-localized with vasoactive intestinal polypeptide (VIP) than with any other neuropeptide, although there are variations between species and between areas of intestine in the same species [18,20,24].

### 3. Mechanisms of PYY release from intestinal endocrine cells

Nutrients stimulate PYY plasma levels within 30 min of ingestion of a high caloric meal (and reaching a maximum within 60 min), long before the luminal contents have reached the terminal intestine where there is a high density of PYY-positive endocrine cells [1]. Dietary fat, fatty acids, bile salts, carbohydrates and proteins all stimulate PYY release but to different degrees and with different time-courses and there is a suggestion that ileal PYY-positive endocrine cells are responsive to fewer luminal cues (oleic acid and bile salts in particular) than those in the large bowel (for full review see [58]). PYY release is also regulated by and in turn regulates, parasympathetic nerves, specifically vagal nerve activity [63] thereby indirectly modulating intestinal activity and is also sensitive to neurohormonal influences (for review see [58]).

Of the total PYY released, ~40% is thought to be converted to PYY(3-36) [26,43] which can activate Y₂ and/or Y₅ receptors in peripheral and central targets. This hydrolysis occurs relatively rapidly via dipeptidyl peptidase IV (DPP-IV; EC3.4.14.5) [16]. Thus, the biological activity of PYY is not abolished by the actions of this enzyme, rather a subtle change in pharmacology occurs from potentially three (Y₁,Y₂ and Y₅) receptors being activated by full length PYY, to co-activation of two, Y₂ and Y₅ receptors by PYY(3-36). This change in target activity may be important in modulating digestive and feeding behavior, as well as initiating satiety after eating a meal consequent to post-prandial increases in plasma PYY and PYY(3-36). DPP-IV inhibitors are of particular current interest as they are undergoing trials as new treatments for type 2 diabetes. In addition to the focus of their proposed clinical benefit, i.e. to prolong the half life on incretin hormones such as GLP-1 and GLP-2 that will lower blood glucose in a glucose-dependent manner, some of the DPP-IV inhibitor effects (i.e. promoting satiety, reducing food intake and subsequently body weight) are likely to be mediated by unrelated peptides/hormones that are also substrates for the enzyme, such as PYY. If future studies with DPP-IV inhibitors show PYY stability and plasma levels are enhanced, then potential GI side effects may include constipation and this disturbance could additionally be amplified by increased stability of other pro-absorptive DPP-IV substrates such as enkephalins.

While fat and protein ingestion produce sustained release of PYY, carbohydrates elicit a transient release profile in human subjects (maximal within 30 min) [1]. In perfused rat colon luminal amino acids, bile salts, glucose and oleate varyingly stimulate PYY release and largely by as yet uncharacterized mechanisms. Short chain fatty acids (SCFAs) that are produced through fermentation of dietary fiber by colonic microflora also cause PYY release with consequent
effects upon intestinal motility [9] via neural and non-neural mechanisms [44], and altered mucosal activity, butyrate being the most potent SCFA that causes epithelial ion secretion [15]. One SCFA receptor, namely GPR43 (a recently discovered G protein-coupled receptor) is co-localized with PYY (but not 5-HT) in endocrine cells and is also expressed by enterocytes of the rat ileum and colon [34]. Whether this endocrine co-localization is emulated in other species remains to be determined.

In the small intestine the presence of digestive products of fat, i.e. free fatty acids (FFA) of a particular length (C12, but not C10) increase plasma levels of PYY and GLP-2 (while both FFA’s stimulated smaller increases in PP) in healthy volunteers, and these elevated levels were in marked contrast to the predicted decline in plasma ghrelin (an appetite stimulator) [23]. Luminal sensing of digested nutrients or products of bacterial fermentation (SCFAs) is an area of growing interest and it is clear that different neuroendocrine peptides (including PYY) link these stimuli, via different receptor populations, to subsequent modulation of energy intake. FFAs have also recently been shown to stimulate GLP-1 release via an orphan receptor, GPR120 [28] that is expressed abundantly in the large bowel. Whether PYY release is similarly affected remains to be seen, but this is likely. Hallden and Aponte [27] have also shown that PYY and FFAs act in a concerted manner to change mucosal cell differentiation. Continuous replacement of mucosal cells, including endocrine cells, by differentiating crypt stem cells is a process essential for maintaining normal intestinal function [61]. Ultimately, whatever the stimulus, PYY release is likely to result in multiple actions (hormonal and paracrine) that occur over the longer term, i.e. cell adhesion and differentiation [35], as well as the shorter term, i.e. altering the patterns of motility and ion transport (see below).

4. Functional consequences of PYY: its paracrine actions in the intestine

PYY is a major mediator of both ileal and colonic brakes, mechanisms that ultimately slow gastric emptying and promote digestive activities to increase nutrient absorption, particularly when fat reaches the distal gut [36,47,52,58,65]. In patients with a surgically shortened bowel (but with preserved colon) gastric emptying is normal and the high transit observed in patients without a colon, is reduced. PYY is most likely to be the “rescuing” hormone [47]. Together with NPY, the C-terminal products and PP, these peptides are all potent, broad-spectrum inhibitors of electrolyte secretion in human [13,53] and mouse [11,32] intestine. In addition, we have recently discovered that endogenous Y-absorptive tone exists in human and mouse colon mucosae, this being revealed initially by the use of selective, competitive Y1 and Y2 antagonists (Y2 (ant)agonists having no effect) [11,13,32].

Full length and shorter versions of PYY and NPY will therefore activate Y1 or Y2 receptors, in preference to Y4 receptors and functional studies in human and mouse isolated colon predict that Y4 receptors are epithelial, Y2 are neuronal and Y1 are predominantly, but not exclusively, epithelial (Fig. 1). Irrespective of these differences in cellular localization any of the three Y receptor populations activated by PYY, NPY or PP is predicted to lead consistently to prolonged anti-secretory effects. This conclusion has been achieved primarily by functional studies utilizing isolated tissues from genetically modified mice lacking either a single Y receptor (Y1−/− [50], Y2−/− [55] or Y4−/− [56], with selective Y1 [72] or Y2 antagonists [17] where appropriate) or single peptide KO tissues (i.e. NPY−/− [22]).

Immunohistochemical studies have also provided much needed cellular resolution, and the localization of Y1 receptors in pediatric and adult human colon reveal extensive labeling on epithelial basolateral membranes and discrete labeling of intrinsic neurons (some co-stain for NPY) in the lamina propria [39,49]. In the rat intestine numerous Y1-positive cell bodies were observed in myenteric but not submucous ganglia, implicating a neuromodulatory role in smooth muscle activity [41]. Human colon morphological [39,49] and functional studies [13] show that Y1 receptors are predominantly epithelial, but they are also present on myenteric neurons.

Fig. 1 – Schematic diagram depicting the sites of action of endocrine PYY and enteric neuron NPY upon different targets in normal mouse colon mucosa. Direct activation of epithelial Y1 receptors by PYY and NPY (or Y4 receptors by hormonal PP, not shown) will inhibit epithelial anion (Cl−) secretion. Veratridine nonselectively depolarizes intrinsic submucous neurons. NPY released from submucosal secretomotor neurons can auto-inhibit NPY release (LHS, a Y2 receptor-mediated effect) and also, when released from interneurons can inhibit (again via Y2 receptors) other NANC secretomotor (e.g. VIP-ergic) neurons. Endocrine PYY may co-activate neuronal Y2 receptors as well as predominant epithelial Y1 receptors (in mouse and human colon) and both mechanisms result in a sustained inhibition of epithelial Cl− secretion. The NANC neurotransmitter in the final secretomotor neuron (RHS) has not yet been positively identified but is most likely to be VIP that then causes epithelial CAMP-dependent Cl− secretion.
Functional studies of colon mucosa from adult Y2−/− mice are predictably insensitive to Y1 agonists and lack Y1-absorptive tone, while Y2-tone (and agonist sensitivity) is unaltered [31]. Chemical stimulation of submucosal neurons (with depolarizing agent, veratridine) causes sustained secretory responses that are unaltered by either Y1 antagonist (BIBO3304) or ablation (i.e. in Y1−/− tissue, Ref. [31]). Y1−/− mucosa is also significantly more sensitive to choleran toxin (CTX)-induced hypersecretion and this effect can be mimicked by pretreatment of WT tissue with Y1 antagonist (BIBO3304, Cox et al., unpublished data). CTX elicits a well characterized intramural reflex that is initiated by endocrine 5-HT stimulation of intrinsic sensory enteric nerves ultimately resulting in prolonged VIP-mediated epithelial secretion of electrolytes and fluid [37,51]. While the absence of Y1 receptors predisposes the mucosa towards hypersecretion, the absence of NPY from NPY−/− colon [22] has no effect upon CTX responses, nor does NPY loss apparently alter Y1 absorptive tone (Cox et al., unpublished observations). The absence of NPY does however abolish colonic Y2-tone, indicating a specific functional link between NPY and Y2 receptors in submucous secretomotor neurons (a mechanism also revealed using veratridine, see below and Fig. 1).

No information is currently available concerning Y2 receptor localization in the intestine of any species, however RT-PCR analysis of rat jejenum and colon shows Y2 expression in epithelial and muscle layers [25]. In the same tissues our functional studies have shown Y2 responses in jejunum to be solely epithelial [10,12], while a combination of epithelial and neuronal Y2 responses are involved in the colon of the rat [69]. Interestingly there is no evidence of Y2- or Y1-absorptive tone in either of these rat GI tissues [12]. In human and mouse colon mucosae however, there is significant endogenous Y2-absorptive tone, which is exclusively neurogenic [11,32] and we propose is NPY-, rather than PYY-mediated [31].

Predictably Y2−/− mucosa is Y2 agonist-insensitive and lacking in Y2-absorptive tone but Y1-tone is unchanged [32]. Interestingly, veratridine’s neurogenic responses in WT colon are amplified by Y2 antagonist and increased in Y2−/− colon [32]. We conclude, that activation of presynaptic Y2 receptors inhibits secretomotor nerves (most probably VIP-ergic) and provides a mechanism by which Y2 antagonism can cause acute diarrhea in vivo. Local NPY, NPY(3–36), PYY and PYY(3–36) can co-activate neuronal Y2 receptors in WT mouse (and human) colon, thus inhibiting ongoing mucosal electrolyte secretion (Fig. 1).

Y4 receptor mediated electrolyte absorption is solely epithelial, in human tissue and monolayers [12,14], and mouse colon [11] and in all these preparations, PK is active. Although low levels of Y4 mRNA expression have been reported in the rat intestine [25], rat PK is inactive here [10]. A single report shows Y4 receptor immuno-labeling of rat duodenal goblet cells and basal lamina (with discrete CNS Y4-labeling, Ref. [8]) with no labeling of epithelial cells. No other Y4 receptor immunohistochemical studies have been published to date using either human or mouse intestine. Functional studies have however been revealing, especially as Y4 antagonists have yet to become available. We have recently found Y4−/− mucosa to be insensitive to Y4 agonist (tPP, but not hPP which co-activates murine Y1 and Y4 receptors) and slightly more sensitive to CTX-induced secretion at 60 min (Cox et al., unpublished data; Ref. [70]). Otherwise normal levels of Y1− and Y2-absorptive tone were observed in Y4−/− (Cox et al., unpublished data) and the sensitivity to any added Y agonist was abolished in this KO tissue pretreated with Y1 and Y2 antagonists [71].

Dependent upon the cell type expressing any one of the pertussis toxin-sensitive Gs/Go coupled Y receptors different signaling pathways can be stimulated. In intestinal epithelium Y receptor-mediated electrolyte absorption is Gs-coupled and epithelial cAMP levels reduced [62] and for review see [29] this providing the main signaling pathway that results in epithelial Cl− secretion being reduced. In addition, both PYY and NPY can cause proliferation of intestinal epithelia, via MAP kinase [40], for review see [38]). PKC-dependent (Y3) as well as PKC-independent pathways (Y1, Y2 and Y4) can be stimulated in transfected CHO cells [46]. In vascular smooth muscle cells, Y receptors can couple to phospholipase C and cause intracellular Ca2+ release as well as activate neuronal GIRK channels (causing hyperpolarization [65]) and block Ca2+ channels (Y1, Y2 and Y4), thereby inhibiting neurotransmitter release [54,68]. The latter two mechanisms are most probably involved in Y2-mediated enteric neuron inhibition.

The recent generation of PYY−/− mice now provides the opportunity for detailed, selective assessment of functional losses in germline and tissue-specific knockdown models. Germline PYY−/− mice develop late-onset obesity, particularly when fed a high fat diet [4] and it will be especially interesting to establish how their GI function is altered compared with transgenic mice that over-express PYY in their colorectal L-cells. A second PYY−/− mouse surprisingly exhibits normal weight gain [60] but this KO apparently also lacks the PP gene, that is only 8 kb downstream from the PYY gene locus. Despite this, it appears that PYY (and PP) gene deletion is not lethal and that endocrine cell differentiation and morphology are normal.

5. Surgical manipulations of PYY and specific diseases of the GI tract

Surgical removal of the large intestine as a treatment for particular intestinal diseases such as colorectal carcinoma or chronic slow transit constipation remove the main source of PYY from the body (PYY is thought to be the primary cause of the latter disorder). It is notable that removal of PYY-rich terminal colon (colectomy) results in diarrhea that can last for months post-surgery [33]. Colonic PYY levels also decrease in rodents with infective diarrhea [67] and in distal bowel of patients with inflammatory bowel disease (IBD) where a selective 30% drop in inflamed tissue PYY has recently been observed [59]. Thus, the removal of colonic PYY will contribute to the increased transit and electrolyte secretion observed following surgery and therefore Y agonists may be useful in alleviating the diarrheal symptoms often experienced by patients post-operatively.

Conversely, patients with PYY-expressing carcinomas can suffer severe constipation [42,45]. While elevated plasma PYY levels have been reported in other GI diseases including steatorrhea and acute infective diarrhea, it is more likely that
these changes in PYY are an adaptive response to alterations in the patho-physiology of gut function and that rises in PYY levels can promote satiety [5]. For example, abnormal levels of PYY and NPY have been observed in biopsies from patients with diarrheal-associated irritable bowel syndrome (IBS) [64]. NPY levels were lower in diarrheal-associated IBS colonic tissue compared with constipation-associated IBS, and PYY levels were also reduced in the distal large bowel of diarrheal-associated IBS patients. The reduced levels of these pro-absorptive peptides in diarrhea are predictable, given the functional background described above.

Globally, diarrhea still kills ~2.2 million people per year, mostly children, and can cause death in previously healthy adults within hours, e.g. cholera infection. In the US, diarrhea due to rotavirus affects ~3 million children annually but good medical care ensures less than 50 cases are fatal. Effective treatments that restore both normal electrolyte and fluid absorption and bowel motility are still required to complement oral re-hydration therapy (which alone does not halt diarrhea) and to treat altered intestinal function following for example, bowel resection surgery that can result in protracted diarrhea. Moreover, acute loss of Y receptor-absorptive tone is likely to result in diarrhea. Our functional studies, where \( Y_1 \) and \( Y_2 \) (and to a lesser degree, \( Y_4 \)) colon is significantly more susceptible to hypersecretion stimulated by CTX than \( Y_1 \) and \( Y_2 \) and that PYY and NPY tissues are unchanged, indicates that PYY is a prime candidate defender of colonic absorption. Future studies will determine whether PYY (and PYY(3–36)) or NPY afford absorptive protection in the small intestine and the relative roles these peptides play in vivo in coordinating intestinal reflexes.

6. Conclusions

In conclusion, PYY exhibits multiple actions, many of them inhibitory and mediated by one of three Y receptor types (Y1, Y2 and Y4), each most likely with different cellular localization. The PYY-synthesizing endocrine L-cells of the large bowel play a pivotal role, not only in the regulation of digestive processes, but also in the adaptive responses to a range of luminal cues and changing dietary components. The functional observations described above present a timely rational basis for developing stable synthetic Y receptor agonists: (i) to replace missing PYY post-resection surgery, (ii) as novel anti-diarrheals in patients refractory to loperamide/other currently available anti-diarrheals, and possibly also, (iii) for Y receptor antagonists as novel anti-constipatory agents for a different set of GI disorders.

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References


[70] Tough IR, Cox HM. Mucosal cholera toxin responses are partially and differentially mediated by SHY4 and Y4 receptors in the mouse descending colon. Br J Pharmacol 2003;138:91P.
