Peptide YY as a growth factor for intestinal epithelium

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Abstract

Peptide YY is an abundant distal gut hormone that may play a significant role in intestinal epithelial proliferation. Gut epithelial cells express specific receptors for PYY, PYY induces proliferation in intestinal cells in vivo and in vitro, and the Y1 receptor subtype couples to mitogenic signaling pathways. In addition to proposed physiologic effects on gut mucosal maintenance, PYY proliferative effects may be hypothesized to contribute to pathophysiologic consequences of stimulated growth. © 2002 Elsevier Science Inc. All rights reserved.

1. Introduction

Peptides play a key role in activating proliferation signals within the gastrointestinal epithelium. The best known factors include ligands for classic receptor tyrosine kinases such as epidermal growth factor (EGF), transforming growth factor-α(TGF-α) and insulin-like growth factor (IGF), but gut endocrine peptides that signal through alternative families of receptors also exert growth effects, with gastrin as a well-characterized example. Gut peptides that have been proposed as epithelial growth factors include enteroglucagon [33], glucagon-like peptide-2 [35] and monitor peptide [23] as well as those with mitogenic effects on neoplastic cells such as gastrin releasing peptide, bombesin, and neurotensin. Peptide YY has been characterized primarily for its postprandial inhibitory effects on the gastrointestinal tract, inhibition of gastric emptying, gut motility, and gastrointestinal mucosal secretion, fulfilling its role as an enterogastrone factor. However, observational studies of PYY production in the gut epithelium hinted that PYY might play a role in epithelial proliferation. An association between elevated serum PYY levels and trophic responses of the small intestinal mucosa, in a model of limited enterectomy, was the first evidence suggesting a potential role for PYY in gut epithelial growth [61]. Further studies demonstrated receptors for PYY on gut epithelial cells thereby

providing a mechanism for direct proliferative effects on cells. Finally subsequent data showed that PYY and the closely related NPY induce proliferation in a variety of cell and tissue models. This biological effect is complemented by biochemical data showing that the Y1 receptor can couple to intracellular signaling molecules that are well recognized for their role in proliferative responses. This review will examine the evidence supporting a role for PYY in gut epithelial growth and propose a medical hypothesis for PYY in gut epithelial pathophysiology that has proliferation as a key component.

2. Receptors for peptide YY are localized to gut epithelial cells

In order for PYY to be able to exert a biological effect directly on a cell, that cell must express cell surface receptors that specifically bind PYY. Studies using whole gut tissue or stripped mucosa preparations showed that PYY or NPY could inhibit short circuit current or apical chloride secretion in a variety of species including the rabbit [6,21, 22,34], rat [16,60], guinea pig [49], and human [50]. However, it was not possible to tell whether PYY or NPY had direct effects on the epithelial cells, consistent with epithelial cell expression of Y receptors, or whether the PYY effects were indirectly exerted by intervening neurotransmitter or other gut peptide networks included in the tissue preparation (even if PYY/NPY responses persisted after tetrodotoxin administration). Using techniques that identify

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specific Y receptor binding activity, Y receptor mRNA production, and Y receptor protein expression within thin sections of gut tissue, it was confirmed that gut epithelial cells could indeed express Y receptors. Receptor autoradiography, a technique that allows radiolabeled peptides to specifically bind to receptors located within slide-mounted tissue sections, showed Y2 subtype receptors largely restricted to the crypt epithelium of rat small intestine with little or no receptor binding in the rat colon [71]. In contrast, receptor autoradiography also showed PYY and NPY binding sites throughout the entire epithelium of the rabbit colon [44]. In situ hybridization techniques identified mRNA for the Y1 receptor subtype within basal crypt epithelial cells of the human colon [72]. By comparison, the reverse transcription polymerase chain reaction (PCR) detected Y2 receptor mRNA in epithelial cells isolated from the rat small and large intestine and Y5 mRNA in small intestinal epithelium (this is in contrast to the receptor autoradiography results where a relative lack of Y receptor binding activity in the rat colon compared to the small intestine suggests much less functional expression of the Y receptor protein given comparable detection of mRNA) [29]. Lastly, a specific antibody to the human Y1 receptor showed Y1 receptor protein expressed throughout the colonic crypt epithelium in humans [43], suggesting that the receptor protein is rather long lived since Y1 receptor mRNA production is limited to the basal crypt cells. Confocal microscopy of Y1 receptor immunofluorescent antibody staining in human colon showed that the Y1 receptor protein was distributed along the basolateral membrane, where receptors would be accessible to PYY released into the lamina propria by surrounding PYY-containing enteroendocrine cells

3. Proliferative effects of peptide YY: observations in animals

Initial suggestions that PYY might be associated with a proliferative effect in the gut epithelium came from observations of elevated PYY serum levels following intestinal resection [27,61]. Within days of intestinal resection PYY serum levels exceeded peak postprandial levels, coinciding with peak epithelial mitotic activity. Similar results of PYY serum elevations were noted after resection of 75% of the small intestine in dogs and resection of 40–200 cm of ileum humans [4,5,7]. Apropos of PYY serum elevations in the setting of a proliferative epithelial state, several studies showed that classic peptide growth factors can also stimulate PYY release. Following TPN therapy, administration of EGF stimulates significant elevations in PYY serum levels which may or may not be accompanied by elevations in gastrin or enteroglucagon levels [25,52]; EGF, insulin-like growth factor-I and TGFα induce elevated PYY serum levels in a mouse and dog model [38]. Keratinocyte growth factor can also increase blood PYY, enteroglucagon, gastrin, and GLP-1 levels [54]. Lastly, in an experiment of

nature, small intestinal hypertrophy was seen in the setting of a metastatic neuroendocrine tumor found to be secreting high levels of PYY (along with glucagon-like peptides 1 and 2) [12]. The primary tumor likely derived from a hindgut enteroendocrine L cell, a subset of PYY-secreting cells that co-release enteroglucagon peptides [20]. These observations establish an association of PYY with clinical settings characterized by proliferative responses and an association with proliferative factors themselves but do not prove PYY-caused proliferation

Controlled experimental studies went further to demonstrate a causal role for PYY inducing a proliferative effect on the gut epithelium: intraperitoneal (i.p.) administration of PYY (t.i.d. for 10–14 days) was associated with significant increases in gut wet weight, DNA, and protein content in adult mice (nursing rats had more modest results and only in the small intestine, consistent with the functional gut receptor distribution in rats [71]) [24]. Also noteworthy is a report of PYY infusion preventing mucosal atrophy during total parenteral nutrition. When PYY was co-infused with TPN solutions in rats for 7 days, compared to rats who got TPN alone there was significant amelioration of the small and large bowel protein and mass reductions with near total preservation of villus height [15]. These findings are in contrast to other studies that were not able to detect significant proliferative effects for PYY during parenteral administration even at levels mimicking post-enterectomy states. In one study, rats were treated with PYY-containing mini-osmotic pumps subcutaneously (s.c.) for 12 days and allowed to feed ad libitum [61]. It is possible that exogenous proliferative effects could not be detected against the background activity of endogenous PYY was released by feeding, similar to the increased efficacy of keratinocyte growth factor in TPN-fed compared to orally fed rats [28]. Another study treated rats on TPN for three days with or without co-infusion of PYY; during this time the rats had no oral food intake and crypt cell proliferation was measured between groups [26]. The duration of treatment may not have been long enough to see a difference, and only the highest PYY dose used in this study was in the range of proliferation-inducing doses used in similar experimental designs. Furthermore, in the setting of TPN-induced gut atrophy, perhaps the action of PYY is less proliferative and more anti-apoptotic as reflected by the preservation of gut mass and architecture. Regardless it is important to take into account the details of experimental design when comparing conflicting results in apparently similar studies.

4. Proliferative effects of peptide YY: observations in vitro

Peptide YY and NPY can induce proliferative effects in a variety of cell types. Activation of the Y2 receptor subtype by NPY on rat renal epithelial cells stimulates DNA synthesis and increases cell numbers [70]. Both PYY and NPY

increase radiolabeled thymidine incorporation into DNA after binding to the Y1 receptor subtype in human vascular smooth muscle cells [18], and NPY has similar mitogenic effects on rat vascular smooth muscle cells [75]. When the human Y1 receptor is expressed in a non-transformed intestinal epithelial cell line derived from rat small intestine (IEC-6 cells), PYY treatment increases cell numbers [45], confirming the ability to stimulate proliferation in a gut epithelial cell. The Y1 receptor is also a subtype that is characteristic of human colonic epithelium, being expressed endogenously in the human colonic adenocarcinoma cell line HT-29 following differentiation [46] as well as constitutively expressed in normal human colon epithelium [43, 72].

That the Y1 receptor could activate a mitogenic response is not unexpected given that it belongs to the G protein-coupled superfamily of receptors (GPCRs), and specifically is coupled primarily through the G_i and G_o subtypes. G_i- and G_o-coupled receptors can activate mitogen-activated protein kinase (MAPK) and cell proliferation [14,30,31,39, 55,68,69]. The G_i- and G_o–protein subtypes are pertussis toxin-sensitive and inhibit adenylate cyclase upon receptor activation [1,32,36,47,62,67]. This signal is responsible for PYY/NPY inhibitory effects on apical chloride secretion in gut epithelial cells.

In recent years general mechanisms for coupling G protein-associated receptors to mitogenic pathways have been demonstrated. Although GPCRs offer a potential alternative pathway to classic receptor tyrosine kinases (RTKs), such as the EGFR, for transducing extracellular growth stimuli, many examples exist of GPCRs actually transactivating RTKs as part of their own mitogenic signaling pathway. Activation of GPCRs have lead to the phosphorylation of the EGFR [17,66], PDGFR [40], IGF-1R [57], and p185^{neu}(HER-2/Erb-2) [17] in the absence of the classic receptor tyrosine kinase cognate ligands. The mechanisms by which GPCRs transactivate RTKs are varied and involve intracellular, and even extracellular [56], signals. One well-characterized mechanism for GPCR transactivation of the EGFR involves the G protein $\beta\gamma$-subunit activating other non-receptor tyrosine kinases, such as Src, either through the PI3-kinase [41] or the proline-rich tyrosine kinase (Pyk2) [11,63], but in other circumstances the way $\beta\gamma$-subunits couple to Src is not clear [42]. In the pathway that couples the human Y1 receptor to MAPK phosphorylation, $\beta\gamma$-subunits clearly couple the Y1 receptor to EGF receptor involvement in the MAPK pathway. Furthermore Src kinase activity is active in the Y1R-MAPK pathway but it is unclear if it is acting between the Y1R and the EGFR, the EGFR and MAPK or in both locations. Finally, protein kinase C (PKC) plays a major role in the signaling pathway between the Y1 receptor and MAPK, clearly acting between the EGFR and MAPK (Fig. 1). PKC may activate MAPK in either a Ras-dependent [47,48] or Ras-independent [2,13, 37] manner, as seen in other models; in Chinese hamster ovary fibroblasts, the transfected human Y1 receptor needs
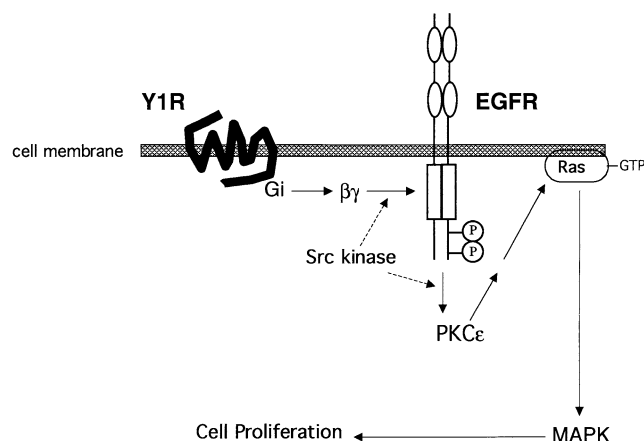

Fig. 1. Proposed signaling pathway for mitogen-activated protein kinase (MAPK) phosphorylation by PYY-activated Y1 receptors in IEC-6 cells. In rat intestinal epithelial cells (IEC-6 cells) transfected with the human Y1 receptor, PYY binding stimulates MAPK phosphorylation. This pathway requires $\beta\gamma$ subunits, EGF receptor tyrosine kinase activity, non-receptor tyrosine kinase activity (Src kinase), and protein kinase C (PKCε). Src kinase may act either proximally or distally to the EGFR, and PKC likely links the pathway to Ras/Raf activation upstream of MAPK.

both PKC and Ras for activation of MAPK [47]. Of interest to gastrointestinal cell-specific growth pathways is that of all the isoforms detected in the IEC-6 cell model, only the PKCε isoform was activated by PYY in the Y1 receptor-expressing IEC-6 cells. PKCε is an isoform that has been specifically linked to mitogenic effects in gut epithelium, where its over-expression can induce unregulated growth in non-tumorigenic colonic epithelial cells [51]. The EGFR is a crucial component in PYY-induced growth in the IEC-6 cells as inhibition of EGFR kinase activity blocks the growth response to both EGF and PYY [45].

5. Proposed role for PYY gut epithelial growth effects in human health and disease

We can only speculate at present on the physiologic relevance of the proliferative effects of PYY in the absence of studies done in mutant animal models, for instance, and without precise knowledge of the hierarchical and redundant networks that regulate epithelial growth. However, the accumulated data do support a role for PYY eliciting a proliferative response from gut epithelia. Perhaps an additional action to its role as the "enterogastrone," high PYY serum levels seen in malabsorptive states sustain an adaptive response for the intestinal mucosa to maximize surface area in an effort to optimize nutrient absorption. Similarly the quotidian nutrient-stimulated PYY release signals the mucosa to maintain itself for efficient digestion and absorption of ingested energy sources (and conserving resources directed toward gut mucosal maintenance during periods of starvation). However, a more intriguing question is whether the proliferative effect exerted by PYY can contribute to pathophysiologic outcomes as well.

Peptide YY is released in response to oral nutrient ingestion. Characteristically it is released in proportion to both the calorie content of a meal and its energy source composition. In a study of human volunteers, increasing ingested calories of an identical meal lead to larger and longer lived serum levels of PYY [3]. Isocaloric meals of only fat, carbohydrate, or protein induced the highest levels of serum PYY after the fat meal, followed by carbohydrates and then protein [3]. Additionally it has been shown that direct intracolonic instillation of digestion products of fats, and to a lesser degree carbohydrates and proteins, stimulates PYY release [64]. In settings of acute or chronic fat malabsorption (infectious enteritis or pancreatic insufficiency, e.g.) basal PYY serum levels are higher compared to controls [53]. In trying to draw a line between PYY release and unintended consequences of stimulated proliferation, it is worth noting that the two factors that determine the largest release of PYY, namely high calorie and high fat meals, are also implicated as environmental risk factors for colon cancer [59,73,74]. We hypothesize that PYY actions on the gut epithelial receptor could contribute to the biological links between dietary exposure and colon cancer risk.

The accepted model of sporadic colon cancer pathogenesis is based on the proposed accumulation of mutations in tumor suppressor and growth-promoting genes resulting in a cell with transformed phenotype and unregulated growth [19]. In the absence of inherited risk factors such as mismatch repair gene defects, the susceptibility to mutation largely depends on the proliferation rate (which determines the probability of mutation risk by certain classes of mutagen), the apoptosis rate (which determines the interval that cells are susceptible to accumulate mutations), and exposure to mutagens (dose, potency, and the ability to neutralize their effects before DNA damage occurs). In this setting, we hypothesize that PYY could increase the colonic crypt cell proliferation rate, on average, in persons consuming a high calorie, high fat diet [8–10,65]. The rationale for this is that a higher proliferation rate in the colonic crypt, assuming a dose-response effect to meal-induced PYY release, would increase the probability of acquiring mutations in epithelial cells during exposure to mutagens encountered in the gut lumen. In experimental models of colon cancer induced by mutagen administration, the rate of adenomatous polyp and colon cancer is accelerated when animals are placed concurrently on a high fat diet [58]. Even administration of oral fat alone increases the proliferation index in colonic crypts in rodents and humans [8–10,65]. These data lend support to the hypothesis that PYY could potentially modulate risk of colon cancer in response to diet exposures by positively influencing the proliferation rate in colonic crypts.

In summary, we have reviewed animal data demonstrating PYY growth effects on gut epithelium, Y receptors expressed by gut epithelial cells in many species including humans, and Y receptor coupling to MAPK kinase activation and signaling molecules involved in gut epithelial growth. Future studies of the actual role PYY plays in normal gut proliferative responses will need to use models such as in vivo immunoneutralization of PYY or PYY knockout animals to show that the proliferative response to ingested fats can be blocked, for instance. The proportional contributions of PYY and co-released peptide hormones such as enteroglucagon and glicentin to proliferative responses will need to be defined. In addition the pharmacology of the PYY receptor will need to be elucidated particularly relating to the modulation of PYY activity by receptor desensitization and downregulation in the setting of constant (as in malabsorption states) or repeated (as in daily feeding cycles) PYY exposure. This is particularly important when PYY receptor transactivation of the EGFR can be expected to modulate its activity as well. Results of such studies will establish a position for PYY in the hierarchy of growth and provide models for human PYY responses. Such studies could also provide the rationale for disease prevention strategies and studies of PYY/Y receptor gene-environment interactions.

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