Involvement of β1- and β2- but not β3-adrenoceptor activation in adrenergic PYY secretion from the isolated colon

S Brechet, P Plaisancié, V Dumoulin, J A Chayvialle, J C Cuber and J Claustre

U 45 INSERM, Hôpital Edouard Herriot, Pav H bis, 69437 Lyon Cédex 3, France
(Requests for offprints should be addressed to J Claustre; Email: claustre@lyon151.inserm.fr)

Abstract

The secretion of PYY by endocrine L cells of the terminal gut is under the control of nutrients, the autonomic nervous system and hormones. Catecholamines, and the non-specific β-adrenergic agonist isoproterenol induce PYY secretion from rat isolated colon or ileum. Because β3-adrenergic receptors now appear to mediate many of the effects of catecholamines in the gastrointestinal tract, we investigated the involvement of β1-, β2-, and β3-adrenoceptor stimulation in PYY secretion from the isolated, vascularly perfused rat colon. Infusion of 10⁻⁶ M isoproterenol induced a transient increase in PYY secretion (from 36 ± 4 to 87 ± 20 fmol/2 min; n=7, P<0·05), that was abolished by a previous infusion of the β1- and β2-adrenergic blocker (and partial β3-agonist) alprenolol (10⁻⁶ M). The β1-adrenergic agonist dobutamine and the β2 agonist terbutaline also (both at 10⁻⁵ M) significantly stimulated PYY secretion, from 29 ± 1 to 79 ± 12 fmol/2 min and from 19 ± 1 to 73 ± 13 fmol/2 min respectively (n=7, P<0·05). Neither of the β3-adrenoceptor agonists tested (BRL 37 344 (10⁻⁵, 10⁻⁶ M) and SR 58 611A (10⁻⁶ M)) significantly stimulated PYY secretion, thus confirming the exclusive involvement of β1- and β2-receptors in β-adrenergic agonist induced hormone secretion.


Introduction

Endocrine intestinal L cells are scattered predominantly in the epithelium of the ileum and the colon. These cells co-secrete peptide YY (PYY) and glucagon-gene-derived peptides. PYY has a crucial role in the regulation of digestion, through inhibition of intestinal motility and of gastric, pancreatic and intestinal secretions. The secretion from L cells is controlled by nutrients present in the lumen and by transmitters and hormones acting at the basolateral side of the cells (Walsh 1994). Electrical stimulation of the splanchnic nerve in the dog induced a strong PYY release (Zhang et al. 1993), and the effect of catecholamines and of adrenergic agonists on colonic PYY secretion is also well documented (Plaisancié et al. 1995, Walsh 1994), but the type of β-adrenergic receptor that triggers this secretion is unknown. The effects of β-adrenergic stimulation were ascribed to the β2- or β1-adrenergic receptors (Lands et al. 1967) until the existence of another type of adrenergic receptor was proposed (Bond & Clarke 1987) and the β3-adrenergic receptor was cloned (Emorine et al. 1989). From a pharmacological point of view, receptors of the β3-adrenergic type differ from β1- or β2-receptors in that they are not subject to desensitization, and they bind catecholamines with a lower affinity (Lafontan & Berlan 1993, Nantel et al. 1993). This may have important physiological consequences for the responsiveness of targets to sympathetic activation. β3-Adrenoceptors are chiefly localized to adipose tissue and gut (Giacobino 1995). Low levels of β3-adrenoceptor mRNA have been found in rat ileum (Granneman et al. 1991) and human colon (Krief et al. 1993), and these receptors were found to mediate most of the isoproterenol-induced ileal and colonic relaxation (Nokes et al. 1996a, Kelly & Houston 1996). In the rat, β3-adrenoceptors were demonstrated by autoradiography in the ileal mucosa, and then by RT-PCR in the ileal and colonic mucosae (Evans et al. 1996, Roberts et al. 1995). In humans, in contrast, the latter approach evidenced predominantly β2-adrenoceptor mRNA but failed to demonstrate β3-adrenoceptor mRNA in the colonic mucosa (Roberts et al. 1997). Little information is available on the involvement of β-adrenoceptor subtypes in endocrine secretion from the gastrointestinal tract. The β2-agonist terbutaline was shown to release PYY in the dog in vivo, before the β3-subtype was investigated (Kogire et al. 1990). In the isolated ileum, terbutaline, and the β3-adrenergic agonist BRL 37 344 to a minor extent, stimulated PYY release (Claustre et al. 1999). In contrast, the secretion of gastrin and somatostatin from isolated rat gastric cells has been
shown to be mediated by β3-receptors, although a minor participation of β2-receptors could not be ruled out (Levasseur et al. 1997). Ligands of the β1- and β2-adrenergic receptors have been used in pathology for a long time, but β3-adrenergic agonists might also provide new drugs in several therapeutic domains such as the energetic metabolism and disorders of intestinal motility (Howe 1993, Roberts et al. 1999). Thus we found it important to assess the responsiveness of endocrine PYY secretion to the different β-adrenergic receptor subtypes and to examine their respective importance in the stimulation of intestinal L cell secretion. For that purpose, we determined the effects of β1-, β2-, and β3-adrenergic receptor stimulation or blockade on PYY secretion from the isolated, vascularly perfused rat colon. This model permits study of the effects of drugs on enteroendocrine secretions from L cells maintained in their natural environment: the organ has all its intrinsic (nervous, endocrine, paracrine) regulatory systems still functional, but the pitfall of uncontrolled effects through interaction with the extrinsic nervous system or other extraintestinal hormonal systems is avoided (Plaisancié et al. 1995).

Materials and Methods

Reagents

Chemical reagents were purchased from Merck (Darmstadt, Germany). The pharmacological reagents: isoproterenol, terbutaline, (+/-)dobutamine and alprenolol were from Sigma (St Louis, MO, USA). Bovine serum albumin was provided by Biovalori (Cassen, France) and the amino acid mixture Hyperamine 25 (nitrogen 25·6 g/l), by Braun Medical (Boulogne, France). PYY (porcine) was purchased from Peninsula Laboratories (St Helens, Merseyside, UK). SR 58 611A was generously provided by Sanofi Recherche, (Milan, Italy) and BRL 37 344 was a kind gift of SmithKline Beecham (Welwyn Garden City, Herts, UK).

Surgical procedure

The dissection of the colon and controls of viability in ex vivo conditions were as described previously (Plaisancié et al. 1995). Male 250–300 g Wistar rats (Dépré, Saint Doulchard, France) were anesthetized with i.p. pentobarbitone (75 mg/kg). The abdomen was opened with a midline incision, viscera were exteriorized and the colon was dissected free from its visceral fixations. An incision was made at the ceco-colic junction and a first cannula was inserted. An incision was then made at the distal end of the colon. After the intestine had been emptied of its contents and rinsed with saline, a second cannula was inserted at the distal end of the colon. The cecum and small bowel were dissected after ligation of their supplying vessels. A steel cannula was inserted in the mesenteric artery and the colon was perfused immediately (2·5 ml/min) in order to avoid anoxia. The perfusion medium consisted of washed bovine erythrocytes (25%), bovine serum albumin (3%), glucose (5 mM) and amino acid solution (1%) in Krebs–Henseleit buffer oxygenated with gaseous O2:CO2 (95:5%). A silastic cannula was then inserted in the portal vein. The perfused colon was isolated by dissecting all its vascular, nervous and connective bonds, transferred to a bath at 37 °C and luminally perfused with isotonic saline (250 µl/min). After equilibration (5 min), venous effluents were collected on EDTA (final concentration 10 mM) every 2 min and rapidly centrifuged. Supernatants were stored frozen at −20 °C until required for assay.

Experimental procedure

β-Adrenergic agonist drugs were infused (250 µl/min in isotonic saline containing 3% bovine serum albumin) through a catheter connected close to the arterial input. Infusion experiments consisted of a 20-min control period, 30-min drug infusion, and a 10-min post-infusion control period.

Infusion of the adrenergic blocker alprenolol was started after a 16-min control period. Four minutes later, isoproterenol infusion was added for the subsequent 30 min, followed by the 10-min post-infusion control period.

Radioimmunoassay

PYY was assayed as described previously (Plaisancié et al. 1995). The A4D antiserum raised against porcine PYY was used at a 1:800 000 dilution. This antibody cross-reacts less than 1% with bovine pancreatic polypeptide and NPY. PYY was iodinated with [125I]NaI using the chloramine T method and purified by reverse-phase HPLC. Sensitivity and ID50 were 1 and 7 fmol/tube respectively.

Statistical methods

Results are expressed as means ± s.e.m. obtained in three to seven experiments. Significance was tested with the non-parametric analysis of variance of Kruskal–Wallis, followed where appropriate by the Wilcoxon test. Differences reaching the P<0·05 level were considered significant.

Results

Effect of the non-specific adrenergic agonist isoproterenol (β1+β2+β3) on PYY secretion from the isolated rat colon

PYY secretion increased within 2 min of the 10−6 M isoproterenol infusion (Fig. 1A) and reached a maximal
increase of 140% over the basal secretion 2 min later (87 ± 20 fmol/2 min compared with a mean basal secretion of 36 ± 4 fmol/2 min; n=7, P<0·05). Despite sustained agonist infusion, the PYY secretion returned to control by the 10th min of infusion.

Effect of infusion of β1-, β2- and β3-agonists on PYY secretion from the isolated colon

When infused in a concentration of 10⁻⁵ M, the β1-adrenergic agonist dobutamine readily increased the PYY secretion from the isolated colon (Fig. 2A). A peak secretion rate of 79 ± 12 fmol/2 min was obtained after 6 min of infusion (mean basal secretion 29 ± 1 fmol/2 min; n=7, P<0·05), followed by a lower rebound. Infusion of the β2-adrenergic agonist terbutaline (10⁻⁵ M) induced an increase in PYY secretion (Fig. 2B), with a pattern quite similar to that observed with the isoproterenol infusion. A maximum secretion of 73 ± 13 fmol/2 min (mean basal concentration 19 ± 1 fmol/2 min; n=7, P<0·05) was reached after 4 min of drug infusion. Neither dobutamine (n=3) nor terbutaline (n=6) significantly increased PYY secretion when infused at the lower 10⁻⁶ M concentration.

The β3-adrenergic agonists BRL 37 344 infused in concentrations of 10⁻⁵ M (n=7) and 10⁻⁶ M (n=4) and SR 58 611A infused at a concentration of 10⁻⁶ M (n=4) did not elicit any PYY secretion (Fig. 3).

Discussion

Isoproterenol and epinephrine have been shown to induce PYY secretion from the isolated colon or ileum (Claustre et al. 1999, Plaisancié et al. 1995). This effect probably does not result from a change in blood flow to the gut, because infusion of the vasodilatory neuropeptide vasointestinal polypeptide (VIP), in contrast, did not alter PYY release. Furthermore, isoproterenol induced a PYY secretion from native cells isolated from canine colon, thus indicating a direct effect of the stimulant on the endocrine cell (Aponte et al. 1988). Despite sustained infusion, isoproterenol and the other β-adrenergic agonists only induced a transient increase in PYY secretion from the isolated colon. This short-lasting response may well result from desensitization, as has been described for β1- and β2-, but not for β3-, adrenergic receptors (Nantel et al. 1993, Summers et al. 1997). Other possibilities must be considered, however, such as the activation of inhibitory systems, either endocrine or nervous. β3-Receptors were demonstrated on duodenal D cells that secrete the inhibitory hormone somatostatin (Anthony et al. 2000), and activation of these same receptors released somatostatin from rat antrum (Levasseur et al. 1997).
The profile of receptors present on endocrine L cells has not been determined, because these cells represent only a small proportion of epithelial cells (Sjolund et al. 1983) and are difficult to isolate to purity (Aponte et al. 1988, Saija et al. 1998), thus making binding studies quite impossible. β3-Adrenergic receptors have recently been found on human duodenal D cells (Anthony et al. 2000), but to date no such data on β-adrenergic receptors are available concerning colonic L cells.

In our experiments, alprenolol abolished isoproterenol-induced PYY secretion, thus showing that the PYY secretion occurred through stimulation of β1- or β2-receptors (or both) exclusively. The existence of atypical or β3-adrenoceptors was, indeed, originally proposed because
of the inability of β1- and β2- (or ‘classical’) adrenergic antagonists to block some effects of isoproterenol (Bond & Clarke 1987). After molecular cloning of the receptor, several of these antagonists, including alprenolol, were shown to behave as partial agonists at β3-receptors (Blin et al. 1993, Granneman et al. 1991). Partial agonism of alprenolol was shown with human β3-adrenoceptors cloned in CHO cells, in which an intrinsic activity of 0.97 relative to isoproterenol (intrinsic activity 1.0) was found (Blin et al. 1993), and was confirmed in rat ileum after blockade of β1- and β2-receptors (Hoey et al. 1996b). This property of alprenolol at β3-adrenoceptors affords the possibility of checking the involvement of β2- and β1-receptor activation in isoproterenol-induced secretion, with little risk of β3-receptor blockade. Incidentally, alprenolol did not induce any PYY secretion when infused alone (before the isoproterenol infusion), despite its proposed high intrinsic activity, consistent with the idea that β3-receptor activation is not a stimulus for PYY secretion. However, alprenolol (alone) has been shown to induce lipolysis in rat adipose tissue, putatively through β3-receptor activation (Rosenbaum et al. 1993).

The isoproterenol-induced PYY secretion we obtained here was reproducible by the β1-adrenergic agonist dobutamine. Because this drug has been shown not to alter blood flow to the intestinal mucosa, it can be inferred that this effect does not result from a circulatory artefact (Secchi et al. 1997). In agreement with the lower affinity of dobutamine than isoproterenol for β1-receptors (Germack et al. 1997), only the greater concentration of agonist induced a significant release of PYY. In the colon mucosa of 4-month-old rats, β1-binding could not be demonstrated, but it reached levels similar to those of β2-binding with aging of the animal (Yu & Ouyang 1997). It should be remembered, however, that intestinal endocrine cells are scattered in the mucosa, and therefore a binding specific to these cells may well be quite undetectable under usual conditions. β1-Adrenoceptors have been demonstrated in rat ileal and colonic smooth muscle, in which they induce relaxation (Roberts et al. 1999). In the human colon, β1-adrenoceptors are the predominant β-adrenoceptor type for relaxing thin longitudinal smooth muscle, whereas β3-adrenoceptors are implicated in the relaxation of taenia coli and circular muscle (Roberts et al. 1997, de Ponti et al. 1999). In contrast to the present findings, others found that dobutamine did not induce any PYY secretion from the isolated rat ileum (Claustre et al. 1999), thus pointing to a differential regulation of L cell secretion according to anatomical location. The observed difference of response of the ileum and the colon might result from different receptor mechanisms according to location. In the human colon, relaxation is mediated primarily by β1-receptors (Roberts et al. 1997), whereas in the ileum of rat these receptors have been found to produce only a small relaxation (Roberts et al. 1999) or not to be involved at all (Hoey et al. 1996a). Also, because β1-adrenergic receptors were proposed to be located mainly on nerve terminals (Ek et al. 1986), this differential response might result from a difference in enteric innervation.

β2-Adrenoceptors have been found to be the prominent type of β-adrenergic receptor in the colonic mucosa of the rat (Yu & Ouyang 1997). The β2-agonist terbutaline stimulated PYY release from the isolated colon, although with a lower potency than isoproterenol, as a 10⁻⁵ M concentration was required in order to induce a significant secretion. This lower potency of terbutaline than isoproterenol is in agreement with its lower potency in mediating the relaxation of smooth muscle from rat colon (Ek et al. 1986). In the rat ileum also, β2-adrenoceptor activation was found to stimulate PYY (and GLP-1) secretion (Claustre et al. 1999), and infusion of terbutaline in the awake dog was shown to induce PYY secretion (Kogire et al. 1990).

The pharmacology of β3-adrenoceptors is not completely established, and discrepant potencies for receptor binding or for colonic relaxation have been reported for β3-agonists (Manara et al. 1995, Roberts et al. 1995, Kelly & Houston 1996). Thus we wished to study the effects of two classical β3-adrenoceptor agonists, BRL 37 344 and SR 58 611A, on PYY secretion. BRL 37 344 is a rodent-specific β3-adrenergic agonist (Fletcher et al. 1998) and SR 58 611A is considered to be highly specific for β3-receptors (Manara et al. 1990, Coruzzi et al. 1997). Neither BRL 37 344 nor SR 58 611A infusion elicited a significant PYY secretion from the isolated colon, thus arguing against a significant involvement of β3-receptors in adrenergic PYY secretion. The lower concentration of BRL 37 344 that we used here (10⁻⁶ M) was found by others to induce a moderate but significant release of PYY from the isolated rat ileum (Claustre et al. 1999). In the rat distal colon, this concentration of agonist fully exerted its relaxant effects on smooth muscle (Kelly & Houston 1996). The other β3-adrenergic agonist, SR 58 611A was found to be equipotent to isoproterenol for releasing gastrin from isolated rat antral cells, through exclusive β3-adrenergic stimulation (Levasseur et al. 1997). Because SR 58 611A may be active after hydrolysis of its ester bond only (Manara et al. 1995), there remains a possibility that, in our model, hydrolysis had not occurred to a sufficient extent. SR 58 611A has been found, however, to be active in vivo, but also in vitro, in intestinal loops or smooth muscle strips, and in cellular models (Levasseur et al. 1997, Manara et al. 1995).

Contrasting with the prominent role devoted to β3-adrenergic receptors and the minor role, if any, of β1-receptors in β-adrenoceptor-induced smooth muscle relaxation (Manara et al. 1995, Roberts et al. 1995, 1999), our results show that colonic secretion of PYY is stimulated by β1- and β2-adrenergic activation only. This difference of reactivity between intestine smooth muscle and endocrine L cells may have physiological
consequences for their respective pattern of response to adrenosympathetic activation: because catecholamines have a better affinity for β1- and β2- than for β3-adrenoceptors (Lafontan & Berlan 1993), endocrine L cells could be more sensitive to sympathetic activation. Conversely, because β3- but not β1- and β2-receptors are resistant to desensitization (Nantel et al. 1993), long-lasting responses may be expected for smooth muscle relaxation but not for endocrine secretion of PYY upon adrenergic stimulation. The findings of the present experiments suggest the involvement of β1- and β2-adrenoceptors, as the effects of agonist on PYY secretion were transitory despite sustained infusion of agonist.

Acknowledgements

The skilful technical assistance of Mrs A Desvignes and G Burlet is gratefully acknowledged.

References


Received 12 January 2000
Revised manuscript received 28 July 2000
Accepted 31 August 2000