Stimulatory effect of β -adrenergic agonists on ileal L cell secretion and modulation by α -adrenergic activation

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Abstract

Postprandial release of peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) from L cells results from both nutrient transit in the ileal lumen and neural drive of endocrine cells. The adenosympathetic system and its effectors have been shown to induce secretion of L cells *in vivo* or *in vitro*. Because these transmitters act through three receptors, β , α_1 , α_2 , coupled to different intracellular pathways, we evaluated the responses of L cells to specific agonists, using the model of isolated vascularly perfused rat ileum. General stimulation of adrenergic receptors with epinephrine (10^{-7} M) induced significant GLP-1 and PYY secretions (94 ± 38 and 257 ± 59 fmol/8 min respectively) which were abolished upon propranolol (10^{-7} M) pretreatment and strongly decreased upon infusion with 10^{-8} M prazosin. Blockade of α_2 -receptors with idazoxan (10^{-8} M) did not alter epinephrine-induced peptide secretion. The β -adrenergic agonist isoproterenol (10^{-6} M) infused for 30 min induced a transient release of GLP-1 and PYY (integrated release over the 8 min of the peak secretion: 38 ± 16 and 214 ± 69 fmol for GLP-1 and PYY respectively, $P < 0.05$). Because terbutaline but not dobutamine or BRL 37,344

(10^{-5} M) induced significant GLP-1 and PYY secretions (135 ± 30 and 305 ± 39 fmol/8 min respectively), isoproterenol-induced secretions are suggested to result mainly from stimulation of the β_2 -isoreceptor type. In contrast, the α_1 -agonist phenylephrine (10^{-7} M) did not stimulate peptide release. When co-infused with 10^{-6} M or 10^{-7} M isoproterenol, 10^{-7} M phenylephrine raised GLP-1 release to 174 ± 53 and 108 ± 28 fmol/8 min respectively (vs 38 ± 16 and 35 ± 10 fmol/8 min for isoproterenol alone, $P < 0.05$) whereas PYY secretion was not significantly increased. Clonidine (10^{-7} M), an α_2 -agonist, induced a moderate and delayed increase of GLP-1 and PYY but abolished the isoproterenol-induced peptide secretion. Our results showed that general stimulation of adrenergic receptors stimulates the secretory activity of ileal endocrine L cells. The net peptide secretion results from the activation of the β_2 -isoreceptor type. Additionally, GLP-1 and PYY secretions are positively modulated by α_1 -receptor stimulation and inhibited by α_2 -receptor activation upon β -receptor occupation.

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Introduction

Intestinal endocrine cell secretion is under the control of nutrients in the lumen, and of nervous or endocrine mediators acting at the basolateral side (Walsh 1994). In the ileum, endocrine cells are primarily accounted for by enterochromaffin cells, and L cells that secrete peptide YY (PYY) and several pro-glucagon-derived peptides (Sjölund *et al.* 1983, Walsh 1994). Pro-glucagon-derived peptides are glucagon-like peptide-1 (GLP-1), GLP-2, oxyntomodulin, and glicentin. GLP-1 is a potent insulinotropic hormone (Holst & Orskov 1994). PYY, which is co-stored with GLP-1 in L cells, induces vasoconstriction, inhibits intestinal motility and secretion, and inhibits pancreatic and gastric secretions (Walsh 1994). PYY has thus been proposed to participate in the ileal brake (Wen *et al.* 1995).

Whereas the effects of nutrients on PYY and GLP-1 secretions have been extensively studied *in vivo* and *in vitro* (Fu-Cheng *et al.* 1995, Wen *et al.* 1995), the modulation of these endocrine secretions by the nervous system has been poorly investigated. The sympathetic nervous system is known to control mucosal functions (Cooke 1986), and mucosal glands are surrounded by putative noradrenergic processes (Newson *et al.* 1979). Electrical stimulation of the splanchnic nerves induced PYY release in anaesthetized dogs and pigs (Sheikh *et al.* 1989, Zhang *et al.* 1993). Epinephrine was shown to induce GLP-1 release in the isolated vascularly perfused rat ileum (Herrmann-Rinke *et al.* 1995), and to stimulate the release of PYY and enteroglucagon from enteric endocrine cells in primary short-term culture (Barber *et al.* 1987, Aponte *et al.* 1988, Buchan *et al.* 1987). In agreement with these results, isoproterenol, a β -adrenergic agonist, was shown to

stimulate the release of the two peptides from the isolated rat ileum (Dumoulin *et al.* 1995). The effects of α -adrenergic agonists on GLP-1 secretion have been studied in cell cultures only (Buchan *et al.* 1987). Because β - and α -adrenoceptors are coupled to different intracellular transduction pathways (Summers & McMartin 1993), we assessed the effects of epinephrine in the presence of specific adrenergic blockers and of β -, α 1- and α 2-adrenergic agonists alone or associated on L endocrine cell secretions.

Isoproterenol, as epinephrine, binds the three types of β -adrenergic isoreceptors (Giacobino 1995), and effects of catecholamines on the gastrointestinal tract have been attributed both to β 2- and β 3-adrenergic stimulation (Coruzzi *et al.* 1997, Levasseur *et al.* 1997). β -adrenergic receptors are coupled to the cAMP pathway (Emorine *et al.* 1989) but β 3-, unlike β 2-adrenoceptors, are not subject to desensitization (Nantel *et al.* 1993), which may have consequences on the pattern of response to catecholamine stimulation. We thus wished to study the effects of specific β -adrenergic agonists on GLP-1 and PYY secretion. This study was conducted in a model of isolated vascularly perfused rat ileum (Cuber *et al.* 1990).

Materials and Methods

Reagents

Chemical reagents were purchased from Merck (Darmstadt, Germany). Bovine serum albumin (BSA) was obtained from Biovalori (Cassen, France) and the amino acid mixture hyperamine 25 (25.6 g nitrogen/l) from Braun Medical (Boulogne, France). The following reagents were from Sigma (St Louis, MO, USA): clonidine, dobutamine, idazoxan, isoproterenol, phenylephrine, prazosin, terbutaline, yohimbine, and GLP-1-(7-36) amide. PYY (porcine) was purchased from Peninsula Laboratories (St Helens, Merseyside, UK). BRL 37,344 was a kind gift from SmithKline Beecham Pharmaceuticals (Welwyn Garden City, Herts, UK).

Surgical procedure

The dissection of the ileum and controls of viability in *ex vivo* conditions have been described previously (Cuber *et al.* 1990). Briefly, male 250–300 g Wistar rats (Dépré, Saint Doulchard, France) were anaesthetized with i.p. pentobarbitone (75 mg/kg), the abdomen was opened with a midline incision, viscera were exteriorized and, after ligation of vessels, the colon was removed. A first cannula was inserted 10 cm proximal to the ileo-colic junction and after emptying and rinsing the intestine a second one was inserted at the ileo-colic junction. After ligating its supplying vessels, the foregut was pulled apart, and steel and silicone elastomer cannulas were inserted in

the mesenteric artery and the portal vein respectively. Upon catheterization, the ileum was vascularly perfused (2.5 ml/min) with a medium consisting of washed bovine erythrocytes (25%), BSA (3%), glucose (5 mM), amino acid solution (1%) in Krebs–Henseleit buffer oxygenated with gaseous O₂:CO₂ (95:5%). The perfused ileum was transferred to a bath at 37 °C and luminally perfused with isotonic saline (250 µl/min).

Infusion protocol

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 assay. Drugs were infused (250 µl/min in isotonic saline containing 3% BSA) through a catheter connected close to the arterial input. Infusion of adrenergic agonists consisted of a 20-min control period, 30-min drug infusion, and a 10-min post-infusion control period. Blockers were infused 2 min before and during agonist infusion (i.e. after an 18-min control period).

RIA

GLP-1 and PYY were assayed directly in the supernatant from venous effluents, as previously described (Plaisancié *et al.* 1994, 1995). The 199D anti-GLP-1 antibody was used at a 1:250 000 dilution. This antibody reacts 100% with GLP-1-(7–36) amide, 84% with GLP-1-(1–36) amide, and less than 0.1% with GLP-1-(1–37), GLP-1-(7–37), GLP-2, and glucagon. The detection limit and ID₅₀ (half maximal inhibitory dose) were 0.4 and 4 fmol/tube. PYY was assayed with the A4D anti-porcine PYY antiserum at a 1:800 000 dilution. This antibody cross-reacts less than 1% with bovine pancreatic polypeptide and neuropeptide Y. Sensitivity and ID₅₀ were 1 and 7 fmol/tube respectively.

Statistical methods

Results are expressed as means $\pm$ S.E.M. obtained in five to eight experiments. Integrated responses were calculated for each animal from the sum of discharges measured during peak secretion (from the second to the ninth minute of infusion) minus the mean value of the 20-min control period. Comparisons were performed with Student's *t*-test for paired or unpaired data as appropriate. Differences reaching the *P* < 0.05 level were considered significant.

Results

Effects of epinephrine alone or with specific blockers on GLP-1 and PYY secretion

Epinephrine infusion (10^{–7} M) induced GLP-1 and PYY secretions (Fig. 1, *n* = 6, *P* < 0.05) to maximal secretion rates

Figure 1 Effects of 10^{-7} M epinephrine alone (●), and in the presence of 10^{-8} M prazosin (▽), or 10^{-7} M propranolol (▼), on GLP-1 (A) and PYY (B) release by the isolated rat ileum. Values are means $\pm$ S.E.M.

of 65 ± 17 fmol/2 min (controls: 25 ± 4 fmol/2 min) and 104 ± 17 fmol/2 min (controls: 27 ± 2 fmol/2 min) respectively. Corresponding integrated secretions over the 8 min of peak secretion were respectively 94 ± 38 and 257 ± 59 fmol/8 min.

Under infusion of the non-specific β -adrenergic antagonist propranolol (10^{-7} M), 2 min before and during the epinephrine (10^{-7} M) infusion, the GLP-1 and PYY secretions were nearly abolished (Fig. 1A and B).

When prazosin (10^{-8} M) was co-infused with 10^{-7} M epinephrine the resulting GLP-1 secretion was decreased (Fig. 1A) to a level of 41 ± 5 fmol/2 min (controls: 29 ± 7 fmol/2 min, $n=4$). The net integrated release over 8 min was then 4 ± 12 fmol (to be compared with 94 ± 38 fmol with epinephrine alone, $P<0.05$). PYY secretion was decreased (Fig. 1B) to a value of 63 ± 15 fmol/2 min (controls: 24 ± 3 fmol/2 min, $n=4$). The integrated release over 8 min was decreased to 94 ± 55 fmol to be compared with 257 ± 59 fmol with epinephrine alone ($P<0.05$).

Blockade of α_2 -adrenoreceptors with idazoxan (10^{-8} M, Fig. 2), infused 2 min before and during epinephrine (10^{-7} M), failed to modify significantly GLP-1 or PYY secretions induced by epinephrine alone.

Epinephrine and epinephrine plus propranolol increased arterial pressure from 51 ± 3 to 66 ± 4 and from 50 ± 1 to 73 ± 6 mmHg respectively. Under infusion of prazosin or idazoxan, epinephrine did not alter arterial pressure any more.

Effects of isoproterenol on GLP-1 and PYY secretions

Isoproterenol, a non-specific β -agonist (10^{-6} M), induced a transient increase of the output of the two peptides in the portal effluent. GLP-1 release rose within 2 min of infusion (Fig. 3A) and reached a maximal concentration of 37 ± 12 fmol/2 min at 6 min (vs basal 19 ± 7 fmol/2 min, $n=8$). The net integrated release over the 8 min of the peak was 38 ± 16 fmol ($P<0.05$). The 10^{-7} M concentration resulted in a quite similar pattern with a maximal concentration of 32 ± 7 fmol/2 min (net integrated release: 35 ± 10 fmol/8 min, $n=7$, $P<0.05$).

Isoproterenol induced a stronger, dose-dependent release of PYY in the portal effluent to 111 ± 31 (10^{-6} M, $n=8$, Fig. 3B) and 54 ± 14 (10^{-7} M, $n=5$) fmol/2 min ($P<0.05$, basal values: 34 ± 7 and 22 ± 4 fmol/2 min respectively). The corresponding integrated releases were 214 ± 69 and 77 ± 38 fmol/8 min for the 10^{-6} M and 10^{-7} M concentrations.

Isoproterenol (10^{-6} M, 10^{-7} M) decreased very moderately the arterial pressure during the entire perfusion period (from 60 ± 2 to 55 ± 3 mmHg, and from 65 ± 6 to 58 ± 4 mmHg, $P<0.05$ respectively).

Stimulation of GLP-1 and PYY secretions by specific β -adrenergic agonists

The β_1 -adrenergic agonist dobutamine (10^{-5} M) did not alter GLP-1 or PYY secretion (Fig. 4). In contrast, terbutaline (10^{-5} M), a β_2 -agonist, strongly and transiently stimulated ($P<0.05$) GLP-1 and PYY secretions (Fig. 4), to maximal secretions of 76 ± 10 fmol/2 min (control values: 19 ± 2 fmol/2 min) and 120 ± 20 fmol/2 min (control values: 25 ± 4 fmol/2 min). The corresponding integrated releases during the 8-min peak were

Figure 2 GLP-1 (A) and PYY (B) release by the isolated rat ileum during infusion of 10^{-7} M epinephrine (●), alone and in the presence of 10^{-8} M idazoxan (▽). Values are means $\pm$ S.E.M.

135 ± 30 and 305 ± 39 fmol for GLP-1 and PYY respectively. As shown in Fig. 4, the β_3 -agonist BRL 37,344 (10^{-5} M) did not significantly alter GLP-1 secretion and moderately increased PYY secretion to a maximal secretion of 67 ± 13 fmol/2 min (integrated release: 105 ± 36 fmol/8 min, $n=4$, $P<0.05$).

Of the three β -specific adrenergic agonists only BRL 37,344 induced a slight decrease of the arterial pressure (from 65 ± 1 to 60 ± 1 mmHg, $P<0.05$).

Figure 3 Release of GLP-1 (A) and PYY (B) by the isolated rat ileum (means $\pm$ S.E.M.) under the influence of 10^{-6} M isoproterenol (●), 10^{-7} M phenylephrine (▽) and 10^{-6} M isoproterenol plus 10^{-7} M phenylephrine (▼).

Effects of phenylephrine alone or combined with isoproterenol

Infusion of the α_1 -agonist phenylephrine (10^{-7} M) did not alter GLP-1 or PYY secretions (Fig. 3). Peptide release progressively rose however by the end of the infusion and during the post-infusion period. Phenylephrine moderately increased the arterial pressure in the preparation, from 64 ± 6 to 72 ± 5 mmHg ($n=8$, $P<0.05$).

Figure 4 Effects of 10^{-5} M dobutamine (●), 10^{-5} M terbutaline (▽) and 10^{-5} M BRL 37,344 (▼) on GLP-1 (A) and PYY (B) release by the rat ileum. Values are means $\pm$ S.E.M.

Infusion of 10^{-7} M phenylephrine plus isoproterenol (10^{-6} M) resulted in a transient peak of GLP-1 (maximum release of 91 ± 18 fmol/2 min, Fig. 3A). The integrated peak release (174 ± 53 fmol/8 min, $n=8$) was significantly greater than under isoproterenol alone ($P<0.05$). Similarly, 10^{-7} M phenylephrine significantly increased 10^{-7} M isoproterenol-induced GLP-1 release (from 35 ± 11 fmol/8 min, $n=7$ to 108 ± 24 fmol/8 min, $n=6$, $P<0.05$).

The effect of phenylephrine (10^{-7} M) on 10^{-6} M isoproterenol-induced GLP-1 secretion could be reversed by infusion of the α_1 -antagonist prazosin (maximum release: 58 ± 16 fmol/2 min, integrated peak release: 55 ± 6 fmol/8 min $n=4$).

Phenylephrine (10^{-7} M) plus isoproterenol (10^{-6} M) infusion did not raise the integrated peak PYY response (246 ± 71 fmol/8 min, $n=6$) over isoproterenol alone (214 ± 69 fmol/8 min, $n=8$, Fig. 3B). Accordingly, blockade of α_1 -adrenoreceptors with prazosin had little effect on phenylephrine plus isoproterenol-induced PYY secretion. Likewise, 10^{-7} M isoproterenol-induced PYY release was not altered by 10^{-7} M phenylephrine (107 ± 16 , $n=8$ vs 77 ± 38 fmol/8 min, $n=5$, not significant).

The phenylephrine and isoproterenol co-infusions did not alter the arterial pressure. Additional infusion of prazosin decreased arterial pressure from 56 ± 1 to 51 ± 1 mmHg ($P<0.05$).

Effects of clonidine, alone or with isoproterenol

The α_2 -adrenergic agonist clonidine (10^{-7} M) did not result in significant GLP-1 and PYY secretions, but only elicited a sluggish rise of secretion of the peptides by the end and after the drug infusion (Fig. 5). When co-infused with isoproterenol, clonidine abolished isoproterenol-induced GLP-1 and PYY secretion peaks (Fig. 5). Blockade of α_2 -adrenoreceptors with 10^{-7} M yohimbine reversed the inhibitory effect of clonidine on isoproterenol-induced peptide release (Fig. 5).

Under clonidine and clonidine plus isoproterenol infusion, blood pressure increased progressively to 92 ± 7 and 76 ± 9 mmHg (vs controls: 61 ± 9 and 63 ± 4 mmHg). Upon additional infusion of yohimbine, blood pressure decreased from 59 ± 2 to 54 ± 1 mmHg.

Discussion

The peptide responses to the three adrenergic agonists, epinephrine, isoproterenol and terbutaline, were transient and lasted for about 8 min. This pattern recalls the 3-min spike of insulin release from isolated perfused canine pancreas upon isoproterenol stimulation (Iversen 1973). Whether the swift fading of adrenergic stimulation of endocrine cells results from receptor desensitization or from interference by adrenergic-sensitive modulators of hormone release remains to be established. It is noteworthy that bombesin and calcitonin in gene related peptide, two enteric neuropeptides, induced a transient increase of GLP-1 and PYY secretion from the isolated rat ileum (Dumoulin *et al.* 1995). These observations favour the hypothesis that neurotransmitters and neuropeptides are scheduled to modulate intestinal endocrine cell secretion for brief time-periods only. Actually, the

Figure 5 Effects of 10^{-6} M isoproterenol (●), 10^{-7} M clonidine (▽), and 10^{-6} M isoproterenol plus 10^{-7} M clonidine (▼) on GLP-1 (A) and PYY (B) release by the rat ileum (means $\pm$ S.E.M.). Restoration of the isoproterenol-induced peptide secretion peak by 10^{-7} M yohimbine after its suppression by clonidine (open circles with broken lines).

adrenosympathetic system is primarily devoted to short-term adaptations (Chrousos & Gold 1992).

Under infusion of the β -adrenergic blocker propranolol, epinephrine-induced peptide secretion was abolished (GLP-1) or nearly abolished (PYY). Propranolol was previously found to inhibit epinephrine-induced enteroglucagon secretion from canine isolated endocrine cells in

short-term culture (Buchan *et al.* 1987). Isoproterenol induced the same pattern of secretion of GLP-1 and PYY as epinephrine, in agreement with a prominent β -adrenergic effect in epinephrine-induced peptide secretion.

β -receptor stimulation may proceed through the β_1 -, β_2 - and β_3 -isoreceptors (Emorine *et al.* 1989). The β_1 -adrenergic agonist dobutamine failed to induce a significant peptide secretion. β_1 -receptors are not strongly involved in β -adrenergic activation in the gastrointestinal tract, and are located to ganglia only (Ek *et al.* 1986). Our results suggest that β -adrenergic stimulation-induced GLP-1 and PYY secretions result from a predominant β_2 -receptor stimulation, although β_3 -adrenoceptors could be involved to a minor extent. The concentration of the β_3 -adrenergic agonist BRL 37,344 that we used was unable to meaningfully stimulate peptide secretion, but was sufficient, in contrast, to decrease arterial pressure. Ileal smooth muscle relaxation is predominantly mediated by β_3 -adrenoceptor stimulation (Manara *et al.* 1995) but few studies have dealt with the involvement of β -adrenoceptor (sub)types in endocrine secretion. Secretion of gastrin and somatostatin from isolated gastric antral cells of rat was shown to result from β_3 -adrenergic stimulation (Levasseur *et al.* 1997), whereas inhibition of gastric acid secretion in the cat (putatively through somatostatin secretion) could be mediated through stimulation of both β_2 - and β_3 -adrenoceptors (Coruzzi *et al.* 1997). Our results are in agreement with a binding study showing that in the ileal mucosa of old Brattleboro rats, β -adrenergic receptors are accounted for by β_2 -receptors (Yu & Ouyang 1997). These results would require further experiments with specific blockers, however.

Specific stimulation of α_1 -receptors did not evoke peptide release. In contrast, when epinephrine was infused with the α_1 -adrenergic blocker prazosin, the GLP-1 as well as the PYY secretion peaks were strongly decreased, thus showing the involvement of α_1 -adrenoceptor stimulation in GLP-1 and PYY secretions. At variance with our results, prazosin did not decrease epinephrine-induced enteroglucagon secretion from canine isolated endocrine cells (Buchan *et al.* 1987). A likely hypothesis is that the peptide secretion was already maximal under the remaining β -adrenergic stimulation alone in this latter study. Actually, the epinephrine concentration was 100-fold higher than the concentration presently used. Species to species variations could also explain these differences.

Interestingly, the α_1 -agonist phenylephrine co-infused with isoproterenol induced a threefold increase of GLP-1 release over isoproterenol alone. The specificity of this effect is demonstrated by its disappearance after blockade of α_1 -adrenoceptors with prazosin. The PYY response to isoproterenol was not enhanced by phenylephrine to the same extent as the GLP-1 response. This is surprising because PYY and GLP-1 are considered to be co-stored in and released by the same population of ileal L cells (Walsh

1994). Contrasting with this belief, a portion (15%) of L cell granules from rabbit colon was found to be labelled with PYY but not enteroglucagon antibodies, thus putatively explaining specific PYY or enteroglucagon secretions upon luminal stimulations (Nilsson *et al.* 1991). Conversely, in human ileum, some granules from L cells were found to contain GLP-1 but not PYY (Eissele *et al.* 1992). The specific mobilization of GLP-1-containing granules by our phenylephrine plus isoproterenol infusion might be responsible for the potentiation of GLP-1, but not PYY release, under these circumstances. Nevertheless, blockade of α_1 -adrenergic receptors with prazosin strongly decreased the epinephrine-induced secretion of both peptides. Thus, the possibility of a potentiating effect of α_1 -receptor stimulation on β -receptor stimulation-induced secretion of PYY should also not be rejected. Such a potentiation has been observed in other cell systems. In the pineal gland, β -adrenergic stimulation of N-acetyl transferase activity is potentiated by α_1 -agonist stimulation (Klein *et al.* 1983). In adipocytes, α_1 - and β -adrenergic agonists synergistically increase the expression of the uncoupling protein thermogenin, whereas α_1 -agonists alone have very limited effects (Rehmark *et al.* 1990). β - and α_1 -adrenergic agonists act via the adenylate cyclase and the phospholipase C-protein kinase C-calcium pathways respectively (Summers & McMartin 1993). Activation of both pathways was shown to induce the release of PYY and GLP-1 or enteroglucagon from isolated intestinal rat cells (Brubaker 1988, Saifia *et al.* 1994, 1998), but the direct stimulation of second messenger systems did not show any evidence of synergism between these pathways on GLP-1 release (Brubaker 1988).

The α_2 -adrenergic receptor antagonist idazoxan (10^{-8} M) did not alter epinephrine-induced GLP-1 or PYY secretion. In agreement with our findings, the α_2 -antagonist yohimbine did not significantly alter the enteroglucagon responses to epinephrine in isolated ileal canine cells (Buchan *et al.* 1987). Thus, the stimulation of α_2 -adrenergic receptors does not appear to be significantly involved in epinephrine-induced peptide secretion. When infused alone, clonidine induced a modest and delayed rise of GLP-1 and PYY concentrations in venous effluent. As compared with the increase of perfusion pressure, this time-course suggests that the present effects on peptide release resulted from alteration of mucosal blood flow. In agreement with this statement, when the clonidine-induced rise of blood pressure was blocked by a simultaneous infusion of the vasodilator hydralazine, peptide secretion was no longer observed (authors' unpublished results). Such a mechanism could obviously not be at work in ileal cell cultures, in which clonidine did not alter enteroglucagon release (Buchan *et al.* 1987). Clonidine abolished the isoproterenol-induced GLP-1 and PYY secretion. This effect was reversed by blockade of α_2 -adrenergic receptors with yohimbine, thus showing that

activation of α_2 -receptors may exert an inhibitory modulation of β -adrenergic receptor-induced peptide secretion. We failed, however, to disclose this effect in our epinephrine experiments. The potency of clonidine is greater than that of epinephrine or norepinephrine upon α_2 -receptor occupation (Langer 1981). Thus, the inhibitory effect of clonidine as here recorded was a true but probably amplified image of the impact of α_2 -receptor occupation by natural transmitters in control conditions. α_2 -receptors are negatively coupled to adenylate cyclase (Watson & Girdlestone 1996). Inhibition of cyclic adenosine monophosphate production is also induced by somatostatin, which was found to inhibit epinephrine-induced enteroglucagon release from isolated canine cells (Barber *et al.* 1987). The effect of α_2 -adrenergic receptor stimulation that we observed here is in agreement with the inhibitory function of α_2 -adrenoceptors in the gastrointestinal tract (Tanila *et al.* 1993).

Our results show that adrenergic activation of endocrine ileal L cells in rats may result from combined activation of the three types of receptors. The level of secretion of GLP-1 and PYY thus appears to be controlled very accurately. Activation of β -receptors, most probably of the β_2 -subtype, induces a rapid, short-lived GLP-1 and PYY secretion. This response is synergistically increased by α_1 -receptor stimulation. α_2 -adrenergic stimulation is liable to modulate the peptide release induced by β -receptor stimulation.

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