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Functional characterization of receptors with affinity for PYY, NPY, [Leu^{31},Pro^{34}]NPY and PP in a human colonic epithelial cell line

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1 Confluent epithelial layers of a human adenocarcinoma cell line called Colony-6 have been shown to respond to nanomolar concentrations of vasoactive intestinal polypeptide (VIP), peptide YY (PYY), neuropeptide Y (NPY) and somatostatin (Som).

2 The VIP-induced increase in basal short-circuit current (SCC) was attenuated by basolateral application of Som, PYY or NPY, and also by the Y_1-receptor agonist [Leu^{31},Pro^{34}]NPY, as well as pancreatic polypeptide (PP). High concentrations (0.1-3.0 μM) of NPY(2-36) were effective but the C-terminal fragment NPY(13-36) (0.1-1.0 μM) and desamidoNPY (0.6 μM) were not active. A rank order of agonist EC_{50} values was: PYY > NPY >[Leu^{31},Pro^{34}]NPY > PP > NPY(2-36) >> NPY (13-36).

3 Receptors for all these peptides were preferentially located within the basolateral domain. Apical addition of PP (1 μM) and Som (100 nM) had no effect upon basal SCC while apical VIP (10 nM) responses were 18%, and apical PYY (100 nM) were 27% the size of respective basolateral controls (100%).

4 Cross-desensitization was observed between [Leu^{31},Pro^{34}]NPY (1 μM) and both PYY (100 nM) and PP (1 μM) and between PYY and NPY(2-36) (1 μM), but was not significant between PYY (100 nM) and PP (1 μM). We suggest that either these cells express a single new Y-receptor with an unusual phenotype or that two Y-receptor populations exist in Colony-6 cells.

Keywords: Pancreatic poylpeptides; NPY receptors; epithelial ion transport

Introduction

The pancreatic polypeptide family of regulatory peptides includes neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) and all exert distinct effects upon gastrointestinal function (Cox, 1993). To date, three G-protein-coupled NPY receptor subtypes have been characterized (for reviews, see Michel, 1991 and Grundemar & Håkanson, 1994) each exhibiting a different rank order of agonist potency. Y_1 receptors are PYY-preferring and are selectively stimulated by Pro^{34} substituted NPY, though not by C-terminal fragments of NPY or PYY. Y_2 receptors are also PYY-preferring but are stimulated by C-terminal fragments, whereas the Y_3 receptor is NPY-preferring and PYY is much less potent. In addition, specific receptors have been described in plasma membranes of canine small intestine epithelia, with high affinity for PP but relatively low affinity for both NPY and PYY (Gilbert *et al.*, 1988). The NPY receptor subtype most commonly expressed by epithelial cells whether renal or gastrointestinal in origin, is Y_2-like (Sheikh *et al.*, 1989; Cox & Cuthbert, 1990) activation of which results in a reduction in prestimulated adenosine 3': 5' cyclic monophosphate (cyclic AMP) accumulation (Servin *et al.*, 1989; Edwards *et al.*, 1990) and as a consequence a reduction in electrolyte secretion.

In vivo (Saria & Beubler, 1985; Bilchik *et al.*, 1993) and *in vitro* studies (Hubel & Renquist, 1986; Cox *et al.*, 1988) have characterized NPY and PYY as major antisecretory peptides. Clinical studies have shown that PYY will reduce VIP-stimulated fluid loss (Playford *et al.*, 1990) and that NPY can attenuate both basal and prostaglandin E_2 (PGE_2)-stimulated fluid and electrolyte secretion from the human intestine (Holzer-Petsche *et al.*, 1991). A common feature of many investigations with mammalian intestine *in vitro*, is the relative inactivity of PP. However, an unusual Y-receptor phenotype was observed in the rabbit distal colon (Ballantyne *et al.*, 1993) where PP was equipotent with PYY and NPY, and the Y_1 receptor agonist, [Leu^{31},Pro^{34}]NPY, was only slightly less potent than the other three agonists. Our own *in vitro* studies with rat jejunum have shown that nanomolar concentrations of NPY and PYY but not PP, will produce prolonged reductions in basal and stimulated electrogenic chloride secretion (Cox *et al.*, 1988; Cox & Cuthbert, 1988) and until recently we have not been able to find an epithelial cell line responsive to any members of the PP family. The present study is the first functional characterization of Y-receptors exhibiting sensitivity to PYY and NPY (and surprisingly also to [Leu^{31},Pro^{34}]-NPY and PP) in a human epithelial cell line.

Methods

Cell culture and short-circuit current (SCC) measurement

Colony-6 (Col-6) epithelia, one of a number of subpopulations derived from a human colonic adenocarcinoma cell line called HCA-7, were obtained from Dr S. Kirkland (Hammersmith Hospital, London, U.K.). This cell line was isolated by seeding HCA-7 cells sparsely and trypsinizing colonies of 50-100 cells using cloning cylinders (Marsh *et al.*, 1993). Cells were maintained in Dulbecco's modified Eagle medium (DMEM, Gibco, Paisley, U.K.) supplemented with foetal calf serum (10%) kanamycin (100 μg ml^{-1}) and amphotericin (1.2 μg ml^{-1}). Once confluent in culture flasks (25 cm^2) epithelia were trypsinized (0.5% trypsin in versene, w/v) and seeded onto collagen-coated millipore filters as described previously (Cox & Tough, 1994). Cells covered the 0.2 cm^2 area of filter within 10 days and once confluent, filters were used within 3 days.

Epithelia on filters were placed between the two halves of Ussing chambers and immediately bathed in oxygenated (95% O_2/ 5% CO_2) Krebs-Henseleit (KH) solution at 37°C, pH 7.4.

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Cell monolayers were voltage-clamped at zero potential (WP Instruments, Sarasota, FL, U.S.A.) and changes in short-circuit current (SCC) were recorded continuously on pen recorders. Each side of the epithelial layer was bathed with 12 or 15 ml of KH and peptides and other drugs were added to either reservoir as necessary. The peak change in SCC that occurred within 15 min of peptide addition was taken as the response and converted from μA 0.2 cm^{-2} to μA cm^{-2}. Where concentration-response relationships were constructed, single peptide additions were made to the basolateral domain (unless otherwise stated in the text) and the data pooled and analysed using the iterative curve-fitting programme, Graphpad Inplot (version 3.01, Graphpad Software Inc, San Diego, U.S.A.). Each best fit curve provided an EC_{50} value (with 95% confidence limits and degrees of freedom). Desensitization studies were performed without intermediate washing between addition of peptides. Statistical analysis of data groups was performed using Student's unpaired t tests and a P value less than 0.05 was considered significant.

Chemicals

All peptides were purchased from Peninsula Labs Inc. (Merseyside, U.K.) and the following abbreviations have been used throughout the manuscript: rat/porcine peptide YY (PYY), porcine neuropeptide Y (NPY), porcine NPY(2–36) and NPY(13–36), human/porcine/rat pancreatic polypeptide (PP), porcine [Leu^{31},Pro^{34}]neuropeptide Y ([Leu^{31},Pro^{34}]NPY), free acid human NPY (desamidoNPY), porcine vasoactive intestinal polypeptide (VIP) and somatostatin 14-28 (Som). All peptide stocks were diluted in sterile water and aliquots were frozen and stored at −20°C. KH constituents in mM were: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.1. Piretanide was a gift from Hoechst Pharmaceuticals and trypsin was purchased from Worthington Biochemical Corporation.

Results

Col-6 epithelial monolayers exhibited basal s.c.c. values of 3.6±0.8 μA cm^{-2} (n=30) and basal resistances which were 69.3±4.7 Ω cm^2 (n=30). Basolateral addition of VIP resulted in rapid concentration-dependent increases in SCC that reached a peak within 5 min (Figure 1). These electrogenic responses were very similar in duration and profile to VIP-mediated secretion observed in rat jejunum (Cox *et al.*, 1988) and colon mucosa (Ferrar *et al.*, 1990). The stimulated SCC remained elevated for at least 30 min and was totally inhibited by basolateral application of piretanide (Figure 1). Reductions in VIP-elevated SCC were observed following a basolateral addition of PYY, NPY and surprisingly of PP (1 nM–3 μM, Figure 1). Col-6 epithelia pretreated with VIP (10 nM) and one of the pancreatic polypeptides also showed responses to Som (100 nM, basolateral) and the latter responses were larger than those to either PYY, NPY (30 and 100 nM respectively) or PP (1 μM, Figure 1). Forskolin (10 μM, basolateral) stimulated prolonged elevations of SCC (peak increases were 8.5±0.9 μA cm^{-2}, 15 min after addition of the diterpene, n=8) and basolateral addition of PYY (100 nM) reduced these levels by −1.3±0.2 μA cm^{-2} (n=4).

The EC_{50} value for VIP-induced secretory responses was 1.6 (1.52–1.64) nM (Figure 2) and the maximally effective concentrations were 3–100 nM. When added to the basolateral reservoir VIP (10 nM) increased the SCC by 7.0± 1.3 μA cm^{-2} (n=8) whereas apical application of the peptide at the same concentration elicited an increase of only 0.6±0.07 μA cm^{-2} (n=3, Figure 3). Following pretreatment of confluent Col-6 epithelia with 10 nM basolateral VIP for 30 min, one of the following polypeptides was added to either reservoir: PYY (100 nM), PP (1 μM) or Som (100 nM) and subsequent reductions in SCC were recorded (Figures 1 and 3). The sidedness of all these peptide responses (at their respective maximally effective concentrations) was obvious (Figure 3) there being no change in SCC to either apical Som or PP, while apical PYY produced reductions in SCC that were 27.0±1.2% of those stimulated by the same concentration (100 nM, 100%) of basolateral PYY.

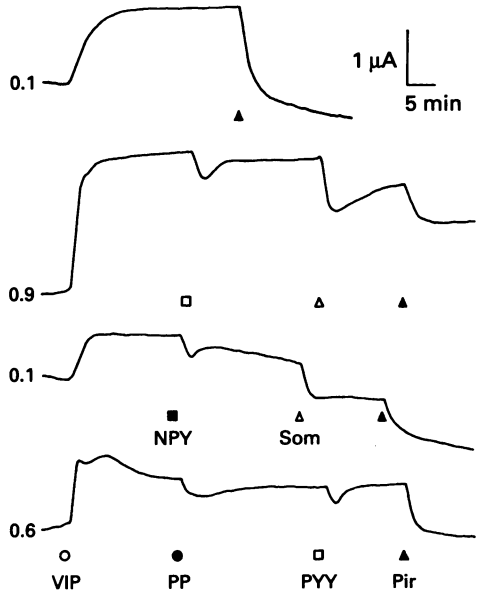

Figure 1 Reprensentative traces of changes in SCC in Col-6 epithelia to basolateral VIP (10 nM, ○) and subsequently to basolateral additions of PYY (30 nM, □); NPY (100 nM, ■); PP (1 μM, ●) or Som (100 nM, △) and finally of piretanide (Pir, 200 μM, ▲). The starting baseline currents are given to the left of each trace (in μA) and responses were obtained from epithelial areas of 0.2 cm^2.

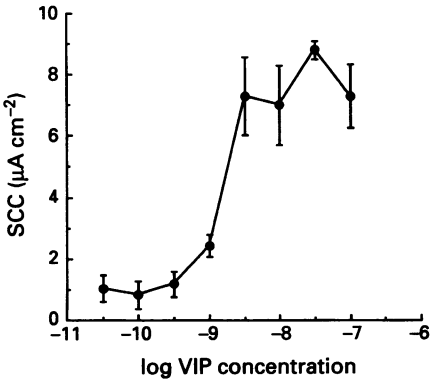

Figure 2 The concentration-response curve for VIP induced increases in SCC in Col-6 epithelia. Each point represents the mean±one s.e.mean of 5-8 observations, a single concentration being given to each filter. Data were pooled and resultant curves fitted to the iterative curve-fitting programme, Graphpad Inplot to yield an EC_{50} value of 1.6 nM.

The observation that basolateral PP (like PYY) could reduce VIP-elevated SCC was unexpected since we had found it to be inactive in Y_2-like receptor-expressing gastrointestinal epithelia (Cox *et al.*, 1988). A study of the concentration-response relationships for agonists known to activate the different Y-receptor subtypes, namely; PYY, NPY, [Leu^{31},Pro^{34}]-NPY, NPY(2–36), NPY(13–36) as well as PP and the inactive peptide desamidoNPY, was undertaken and the results are shown in Figure 4. All curves were constructed from responses stimulated by single basolateral peptide applications and the subsequent reductions in the SCC response to 10 nM VIP recorded within 15 min were pooled to provide data that yielded EC_{50} values listed in Table 1. The maxima obtained from each agonist concentration-response curve were similar (Figure 4, there was no significant difference between the size of re-

sponses to 30 nM PYY, 100 nM NPY or 1 μM PP) and the agonist order of potency was PYY > NPY > [Leu^{31},Pro^{34}]NPY > PP > NPY(2–36), while NPY(13–36) and desamidoNPY were inactive up to concentrations of 1 μM and 600 nM respectively. The Hill slopes of agonist-response curves were −0.9 for PYY, −0.7 for [Leu^{31},Pro^{34}]NPY, −1.7 for NPY, −1.2 for PP and −1.1 for NPY(2–36).

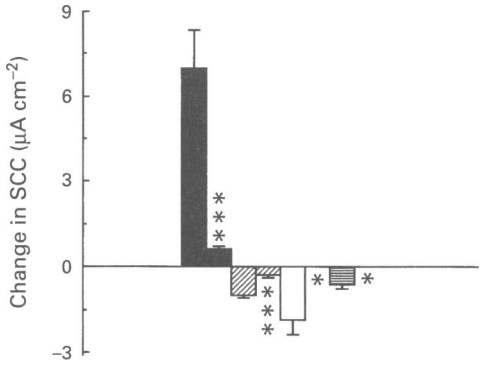

Figure 3 Sidedness of responses to VIP alone (10 nM, solid columns) and to PYY (100 nM, hatched column) Som (100 nM, open column) or PP (1 μM, horizontally lined column) added to either the basolateral (the first of each pair) or apical bathing fluid following VIP pretreatment. Each column is the mean with one s.e.mean from 3–11 observations. Significant differences between responses following apical addition compared with the respective basolateral peptide control are: $*P<0.05$, $***P<0.001$.

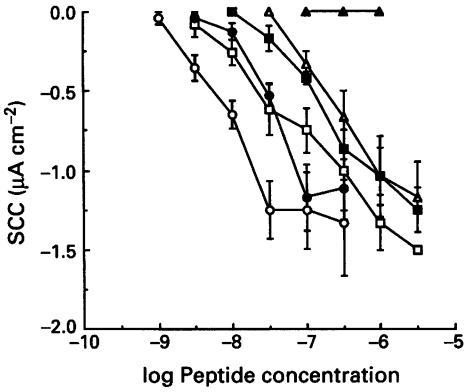

Figure 4 Concentration-response relationships in VIP-pretreated (10 nM) Col-6 epithelia for PYY (○, $n=3-10$), NPY (●, $n=4-7$), [Leu^{31},Pro^{34}]NPY (□, $n=2-5$), PP (△, $n=3$), NPY(2–36) (■, $n=3$) and NPY(13–36) (▲, $n=2$). Each point is the mean ± one s.e.mean where more than 2 observations were made. All peptide additions were made to the basolateral surface.

Table 1 EC_{50} values for peptides in Colony-6 epithelial monolayers

Peptide	*EC_{50}* (nM)
VIP	1.6 (1.52–1.64, 6)
PYY	9.9 (9.0–10.9, 4)
NPY	32.3 (24.4–47.9, 3)
[Leu^{31},Pro^{34}]NPY	78.7 (49.0–128.7, 5)
PP	177.2 (138.9–227.7, 4)
NPY(2–36)	323.4 (219.8–476.4, 3)
NPY(13–36)	No effect
DesamidoNPY	No effect

The concentration-response relationships shown in Figures 2 and 4 were subjected to the iterative curve fitting programme Graphpad Inplot, yielding EC_{50} values with 95% confidence limits and degrees of freedom in parentheses. For abbreviations see text.

A comparison of the response profiles generated by near maximally effective concentrations of PYY (30 nM) NPY (100 nM) and [Leu^{31},Pro^{34}]NPY (1 μM) and PP (1 μM) revealed some differences (Figure 5). [Leu^{31},Pro^{34}]NPY responses appeared to be monophasic returning to the original baseline within 15 min while NPY responses were prolonged reaching baseline around 30 min (data not included in Figure 5). PYY and PP responses were initially rapid and transient reaching a stable inhibitory level 8–20 min after peptide addition. The latencies between peptide additions to the basolateral reservoir and the start of each decline in current increased from 50 ± 7 s ($n=4$) for 1 μM PP, 80 ± 13 s ($n=6$) for 30 nM PYY, compared with 200 ± 36 s ($n=6$) for 100 nM NPY and 200 ± 20 s ($n=3$) with 1 μM [Leu^{31},Pro^{34}]NPY. At higher concentrations of NPY and [Leu^{31},Pro^{34}]NPY (300 nM and 3 μM respectively) there was a reduction in response latency to 135 ± 23 s ($n=7$) and 172 ± 38 s ($n=3$) respectively. The latter two peptides achieved peak responses 12 and 6 min after basolateral addition respectively, while PP effects were maximal at 2 min and PYY responses reached a peak at 3 min.

Cross-desensitization studies were performed where cells were pretreated with either PYY (100 nM), [Leu^{31},Pro^{34}]NPY (1 μM) initially, and subsequently a second addition of one of these three peptides, without intermediate washing. In controls when two applications of PYY or [Leu^{31},Pro^{34}]NPY were made (25 min apart) there was no further reduction in SCC following the second peptide addition (Figure 6) even though the original SCC levels had been reached. [Leu^{31},Pro^{34}]NPY responses were significantly inhibited following 1 μM PP ($P<0.05$) and after 100 nM PYY ($P<0.001$). However, responses to PYY (100 nM) were not significantly affected by pretreatment with 1 μM PP ($P=0.13$, Figure 6). PP responses were not significantly attenuated after PYY pretreatment ($P=0.17$) but the reductions in SCC to [Leu^{31},Pro^{34}]NPY were reduced significantly ($P<0.05$). Thus homologous desensitization was complete for PYY and [Leu^{31},Pro^{34}]NPY, and there was heterologous desensitization between the Y_1-agonist, [Leu^{31},Pro^{34}]NPY and both PP and PYY, but not between PP and PYY. In a separate study, responses to PYY (30 nM, which reduced the SCC by −1.3 ± 0.2 μA cm^{-2}, $n=9$) in untreated Col-6 epithelia were compared with those following exposure to increasing concentrations of either NPY(2–36), NPY(13–36) or desamidoNPY. There was no significant change in the size of PYY responses after NPY(13–36) (100 nM–1 μM) or desamidoNPY (600 nM only) addition compared with controls. However, significant reductions were observed following 100 nM, 300 nM and 1 μM NPY(2–36) (where responses, in μA cm^{-2}, were −0.5 ± 0, $P<0.05$; −0.2 ± 0.04, $P<0.01$; and −0.04 ± 0.04, $P<0.01$, respectively, $n=3$ throughout).

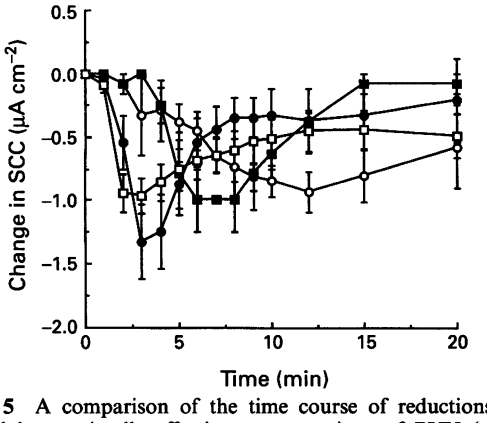

Figure 5 A comparison of the time course of reductions in SCC induced by maximally effective concentrations of PYY (●, 30 nM, $n=6$), NPY (O, 100 nM, $n=6$), [Leu^{31},Pro^{34}]NPY (■, 1 μM, $n=3$) and PP (□, 1 μM, $n=4$). Basolateral peptide was added at time zero to cells which had been pretreated with 10 nM VIP for 20 min. Each point is the mean ± one s.e.mean.

Discussion

Col-6 epithelial monolayers respond in a classical manner to the secretagogue VIP and the inhibition of this elevated secretory level by PYY, NPY and Som is reminiscent of the respective peptide responses observed in *in vitro* preparations of rat jejunum (Cox & Cuthbert, 1988; Cox *et al.*, 1988) and colon (Ferrar *et al.*, 1990). These preparations express Y_2-like receptors since functional studies have shown that the Y_1 agonist [Leu^{31},Pro^{34}]NPY was inactive (Cox & Krstenansky, 1991) and PP (at high nanomolar concentrations) did not significantly alter electrogenic ion transport (Cox *et al.*, 1988). The present study produced a rank order of agonist EC_{50} values: PYY > NPY > [Leu^{31},Pro^{34}]NPY > PP > NPY(2–36), with NPY(13–36) and desamidoNPY being inactive. This is not an order expected for either Y_1- or Y_2- NPY receptors where commonly PYY is marginally more potent than NPY, and either [Leu^{31},Pro^{34}]NPY or the C-terminal fragments of NPY are effective (but less potent than the native peptides) respectively (see Table 2). In fact the EC_{50} values calculated for PYY and NPY are higher in Col-6 cells compared with those in rat jejunum (Cox *et al.*, 1988). The metabolism of PYY and NPY by dipeptidyl peptidase-IV (Mentlein *et al.*, 1993; Dos Santos Mederois & Turner, 1994) may be responsible for a slight loss of potency since the resultant fragment PYY(3–36) is known to be a Y_2-preferring agonist.

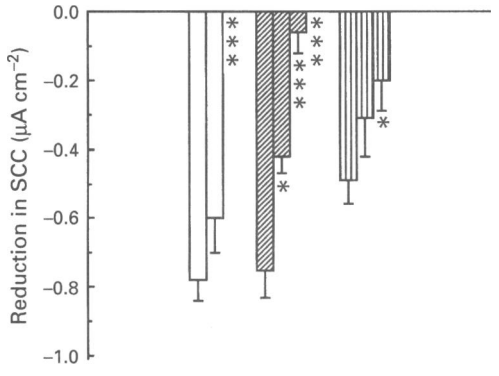

Figure 6 Desensitization between PYY (100 nM), [Leu^{31}, Pro^{34}]NPY (1 μM) and PP (1 μM) in VIP (10 nM) pretreated Col-6 cells. Each column represents the mean response to basolateral peptide with one s.e.mean and there was a 20 min interval between each peptide addition. The number of observations in each group are given in parentheses. Open columns are: first the PYY (100 nM, 7) responses in control naive epithelia; second, those following 1 μM PP (5) addition and third, those following an earlier PYY (100 nM, 3) addition. A similar sequence is used where [Leu^{31},Pro^{34}]NPY is the agonist (hatched columns): first [Leu^{31},Pro^{34}]NPY (1 μM, 11), second PP(1 μM, 5), third PYY (100 nM, 4) and fourth [Leu^{31},Pro^{34}]NPY (1 μM, 3). Finally one control and two experimental groups are shown for PP responses (vertical lined columns). Here the sequence (left to right) is: PP controls (1 μM, 12), PP responses after PYY (100 nM, 6) and PP responses after [Leu^{31},Pro^{34}]NPY (1 μM, 8). Signficance levels are: $*P<0.05$, $**P<0.001$, for data groups compared with their respective control agonist.

The attenuation of VIP-stimulated ion transport by the pancreatic polypeptides in rabbit descending colon (Ballantyne *et al.*, 1993) resembles the pharmacology of the Col-6 epithelial Y-receptor. C-terminal fragments were inactive, while the PP and [Leu^{31},Pro^{34}]NPY were full agonists with EC_{50} values of 14 and 30 nM respectively. Unlike the present study with Col-6 cells (where PP was 18 times less potent and, [Leu^{31},Pro^{34}]NPY 8 times less potent than PYY) these two agonists were roughly equipotent with NPY (EC_{50} of 24 nM) and PYY (EC_{50} of 16 nM). All four peptides reduced VIP-stimulated secretion by 70–90% in rabbit colon compared with the 25% inhibition observed in Col-6 and this 3 fold difference in efficacy may contribute to the differences in absolute potency. Table 2 summarizes the agonist potency orders observed for the Y_1, Y_2 and Y_3 NPY receptor subtypes together with the PP receptor and new phenotype(s) expressed in Col-6 epithelia and rabbit colon. It is clear that the order of EC_{50} values in Col-6 cells also bears little resemblance to that of the putative Y_3 receptor (at which NPY is significantly more potent than PYY, Wahlestedt *et al.*, 1991; Foucart *et al.*, 1993) and other potential variants described recently (Inui *et al.*, 1992).

Assuming that there are few (if any) spare Y-receptors in these cells, a potential explanation for the unique agonist order observed in the present study is that more than one receptor subtype is expressed by these cells. The cross desensitization observed between [Leu^{31},Pro^{34}]NPY and PP, [Leu^{31},Pro^{34}]NPY and PYY, and between PYY and NPY(2–36) but not between PP and PYY, argues for one subtype that is PYY-preferring (with little if any affinity for PP) and another receptor population that is Y_1-like (again PYY-preferring) with affinity for [Leu^{31},Pro^{34}]NPY and PP. The functional consequence of target cells expressing two receptor subtypes with affinity for the PP-fold peptides (as seen previously with PC-12 cells, Schwartz *et al.*, 1987, and superior cervical neurones, Foucart *et al.*, 1993) is worthy of further consideration especially since both Y_1 and Y_2-like receptors are linked to similar transduction mechanisms (Michel, 1991; Grundemar & Håkanson, 1994). In gastrointestinal epithelia, Y_2-receptors are typically involved in reducing electrolyte secretion via a cyclic AMP-dependent inhibitory mechanism (Servin *et al.*, 1989) and this is also common for Y_1 receptor activated responses in other cell lines (van Valen *et al.*, 1992; Krause *et al.*, 1992). A concomitant mobilisation of intracellular Ca^{2+} is also a common feature of activation of Y_1 receptors in certain cells (Krause *et*

Table 2 Comparison of agonist potency orders in isolated mucosal tissue and other epithelial preparations containing NPY and PP receptors

Receptor	*Potency order*	*Tissues/cells*
Y_1-like	PYY ≥ NPY ≥ [Leu^{31},Pro^{34}]NPY > > NPY(13–36)	Rat gastric mucosa (Penner *et al.*, 1993)
Y_2-like	PYY > NPY > NPY(13–36) > > [Leu^{31},Pro^{34}]NPY	Rat jejunum and renal epithelia (Cox *et al.*, 1988) (Sheikh *et al.*, 1989)
Y_3-like	NPY > [Leu^{31},Pro^{34}]NPY > NPY(13–36) > > PYY	(No published data to date in any epithelial preparations)
New Y	PYY > NPY > [Leu^{31},Pro^{34}]NPY > PP > > NPY(13–36)	Col-6 adenocarcinoma cell line (Cox & Tough, this study)
PP	PP > > > PYY > NPY	Canine small intestine epithelia (Gilbert *et al.*, 1988)
Nonselective	PP ≥ PYY ≥ NPY ≥ [Leu^{31},Pro^{34}]NPY > > NPY(13–36)	Rabbit distal colon (Ballantyne *et al.*, 1993)

For abbreviations, see text.

al., 1992; Michel *et al.*, 1992). Since inhibition of accumulated cyclic AMP production is the primary underlying mechanism involved in NPY/PYY antisecretory responses it is possible that functional cross-desensitization observed between particular agonists may not in fact indicate desensitization at a common receptor, but rather that down-regulation of a common transduction pathway has occurred. This may be true for the desensitization observed between [Leu^{31},Pro^{34}]NPY and PP in the present study. The time-course studies comparing equieffective concentrations of PYY, NPY, PP and [Leu^{31},Pro^{34}]NPY show similarity between PYY and PP responses, while those to NPY and [Leu^{31},Pro^{34}]NPY were also similar, being slower in onset than the former peptide pair. Further studies, preferably using a selective NPY receptor antagonist will help elucidate whether one or more Y receptors are expressed by these cells.

The predominance of peptide receptors in the basolateral membranes of Col-6 monolayers was similar to, not only the sidedness seen for other agonists (MacVinish *et al.*, 1993; Cox & Tough, 1994) in epithelial lines derived from the same parent cell line (Marsh *et al.*, 1993) but also to the sidedness of NPY and PYY responses in jejunal and colonic mucosal preparations (Cox *et al.*, 1988). Thus these cells can provide a useful model system for the study of peptide receptors in polarized epithelia in the absence of other mucosal cell types.

In conclusion we have identified responses to PYY, NPY, [Leu^{31},Pro^{34}]NPY and PP in an epithelial cell line derived from a human adenocarcinoma of the lower bowel. These peptide responses (in VIP-prestimulated epithelia) were predominantly basolateral as were those to Som and VIP itself. Cross-desensitization was observed between [Leu^{31},Pro^{34}]NPY and both PYY and PP, and between PYY and NPY(2–36), but was not significant between PYY and PP. Thus it is possible that more than one receptor phenotype is being expressed by Col-6 cells, each with high affinity for PYY though with differing affinities for NPY, [Leu^{31},Pro^{34}]NPY and PP. Future studies with a Y_1-receptor antagonist should help determine whether one or more receptors exist in Col-6 cells, or whether a single new Y-phenotype is expressed in these epithelia.

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References

BALLANTYNE, G.H., GOLDENRING, J.R., FLEMING, F.X., RUSH, S., FLINT, J.S., FIELDING, L.P., BINDER, H.J. & MODLIN, I.M. (1993). Inhibition of VIP-stimulated ion transport by a novel Y-receptor phenotype in rabbit distal colon. *Am. J. Physiol.*, **264**, G848–G854.

BILCHIK, A.J., HINES, O. J., ADRIAN, T.E., MCFADDEN, D.W., BERGER, J.J., ZINNER, M.J. & ASHLEY, S.W. (1993). Peptide YY is a physiological regulator of water and electrolyte absorption in the canine small bowel *in vivo*. *Gastroenterol.*, **105**, 1441–1448.

COX, H.M. (1993). The role of NPY and related peptides in the control of gastrointestinal function. In *The Biology of Neuropeptide Y and Related Peptides* ed Colmers, W.F. & Wahlestedt, C. 273–313. Humana Press, New Jersey.

COX, H.M. & CUTHBERT, A.W. (1988). Neuropeptide Y antagonises secretagogue evoked chloride transport in rat jejunal epithelium. *Pflügers Arch.*, **413**, 38–42.

COX, H.M. & CUTHBERT, A.W. (1990). The effects of neuropeptide Y and its fragments upon basal and electrically stimulated ion secretion in rat jejunum mucosa. *Br. J. Pharmacol.*, **101**, 247–252.

COX, H.M., CUTHBERT, A.W., HÅKANSON, R. & WAHLESTEDT, C. (1988). The effect of neuropeptide Y and peptide YY on electrogenic ion transport in rat intestinal epithelia. *J. Physiol.*, **398**, 65–80.

COX, H.M. & KRSTENANSKY, J.L. (1991). The effects of selective amino acid substitution upon neuropeptide Y antisecretory potency in rat jejunum mucosa. *Peptides*, **12**, 323–327.

COX, H.M. & TOUGH, I.R. (1994). Calcitonin gene-related peptide receptors in human gastrointestinal epithelia. *Br. J. Pharmacol.*, **113**, 1243–1248.

DOS SANTOS MEDEIROS, M. & TURNER A.J. . (1994). Processing and metabolism of peptide YY: pivotal roles of dipeptidylpeptidase-IV, aminopeptidase-P, and endopeptidase-24.11. *Endocrinol.*, **134**, 2088–2094.

EDWARDS, R.M. (1990). Neuropeptide Y inhibits cAMP accumulation in renal proximal convoluted tubules. *Regul. Pept.*, **30**, 201–206.

FERRAR, J.A., CUTHBERT, A.W. & COX, H.M. (1990). The antisecretory effects of somatostatin and analogues in rat descending colon mucosa. *Eur. J. Pharmacol.*, **184**, 295–303.

FOUCART, S., BLEAKMAN, D., BINDOKAS, V.P. & MILLER, R.J. (1993). Neuropeptide Y and pancreatic polypeptide reduce calcium in acutely dissociated neurones from adult rat superior cervical ganglia. *J. Pharmacol. Exp. Ther.*, **265**, 903–909.

GILBERT, W.R., FRANK, B.H., GAVIN, J.R. & GINGERICH, R.L. (1988). Characterisation of specific pancreatic polypeptide receptors on basolateral membranes of the canine small intestine. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 4745–4749.

GRUNDEMAR, L. & HÅKANSON, R. (1994). Neuropeptide Y effector systems: perspectives for drug development. *Trends Pharmacol. Sci.*, **15**, 153–159.

HOLZER-PETSCHE, U., PETRITSCH, W., HINTERLEITNER, T., EHERER, A. SPERK, G. & KREJS, G.J. (1991). Effect of neuropeptide Y on jejunal water and ion transport in humans. *Gastroenterol.*, **101**, 325–330.

HUBEL, K.A. & RENQUIST, K.S. (1986). Effect of neuropeptide Y on ion transport by the rabbit ileum. *J. Pharmacol. Exp. Ther.*, **238**, 167–169.

INUI, A., SANO, K., MIURA, M., HIROSUE, Y., NAKAJIMA, M., OKITA, M., BABA, S. & KASUGA, M. (1992). Evidence for further heterogeneity of the receptors for neuropeptide-Y and peptide YY in tumor cell lines derived from neural crest. *Endocrinol.*, **131**, 2090–2096.

KRAUSE, J., EVA, C., SEEBURG, P.H. & SPRENGEL, R. (1992). Neuropeptide Y_1 subtype pharmacology of a recombinantly expressed neuropeptide receptor. *Mol. Pharmacol.*, **41**, 817–821.

MACVINISH, L.J., PICKLES, R.J. & CUTHBERT, A.W. (1993). Cyclic AMP and Ca^{2+} interactions affecting epithelial chloride secretion in human cultured colonic epithelia. *Br. J. Pharmacol.*, **108**, 462–468.

MARSH, K.A., STAMP, G.W.H. & KIRKLAND, S.C. (1993). Isolation and characterisation of multiple cell types from a single human colonic carcinoma: tumorigenicity of these cells in a xenograft system. *J. Pathol.*, **170**, 441–450.

MENTLEIN, R., DAHMS, P., GRANDT, D. & KRUGER, R. (1993). Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. *Regul. Pept.*, **49**, 133–144.

MICHEL, M.C. (1991). Receptors for neuropeptide Y: multiple subtypes and multiple second messengers. *Trends Pharmacol. Sci.*, **12**, 389–394.

MICHEL, M.C., GAIDA, W., BECK-SICKINGER, A.G., WIELAND, H.A., DOODS, H., DURR, H., JUNG, G. & SCHNORRENBERG, G. (1992). Further characterisation of neuropeptide Y receptor subtypes using centrally truncated analogs of neuropeptide Y: evidence for subtype-differentiating effects on affinity and intrinsic efficacy. *Mol. Pharmacol.*, **42**, 642–648.

PENNER, S.B., SMYTH, D.D. & GLAVIN, G.B. (1993). Effects of NPY and [Leu^{31},Pro^{34}]NPY on experimental gastric lesion formation and gastric secretion in the rat. *J. Pharmacol. Exp. Ther.*, **266**, 339–343.

PLAYFORD, R.J., DOMIN, J., BEACHAM, J., PARMAR, K.B., TATEMOTO, K., BLOOM, S.R. & CALAM, J. (1990). Role of peptide YY in defence against diarrhoea. *Lancet*, **335**, 1555–1557.

SARIA, A. & BEUBLER, E. (1985). Neuropeptide Y and Peptide YY inhibit prostaglandin E_2-induced intestinal fluid and electrolyte secretion in the rat jejunum in vivo. *Eur. J. Pharmacol.*, **119**, 47–52.

SCHWARTZ, T.W., SHEIKH, S.P. & O'HARE, M.M.T. (1987). Receptors on phaeochromocytoma cells for two members of the PP-fold family–NPY and PP. *FEBS Letts.*, **225**, 209–214.

SERVIN, A.L., ROUYER-FESSARD, C., BALASUBRAMANIAN, A., SAINT PIERRE, S. & LABURTHE, M. (1989). Peptide YY and neuropeptide Y inhibit vasoactive intestinal polypeptide-stimulated adenosine 3',5'-monophosphate production in rat small intestine: structural requirements of peptides for interacting with peptide YY-preferring receptors. *Endocrinol.*, **124**, 692–700.

SHEIKH, S.P., IQBAL SHEIKH, M. & SCHWARTZ, T.W. (1989). Y_2-type receptors for peptide YY on renal proximal tubular cells in the rabbit. *Am. J. Physiol.*, **257**, F978–F984.

VAN VALEN, F., WINKELMANN, W. & JURGENS, H. (1992). Expression of functional Y_1 receptors for neuropeptide Y in human Ewing's sarcoma cell lines. *Cancer Res. Clin. Oncol.*, **118**, 529–536.

WAHLESTEDT, C., REGUANTHAN, S. & RIES, D.J. (1991). Identification of cultured cells selectively expressing Y_1-, Y_2-, or Y_3-type receptors for neuropeptide Y/PYY. *Life Sci.*, **50**, PL7–PL12.

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