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Neuropeptide Y: a powerful modulator of epithelial ion transport

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1 Neuropeptide Y (NPY) is a major gut peptide localized in the intestinal mucosa of several mammalian species. Ileal mucosa from rabbit and guinea-pig was mounted in Ussing chambers in order to study the effect of NPY on short circuit current.

2 Neuropeptide Y inhibited the short circuit current when applied to the serosal side of the tissue. The maximum change in short circuit current was $-50 \pm 6\,\mu A\,cm^{-2}$ in the rabbit ileum and $-49 \pm 14\,\mu A\,cm^{-2}$ in the guinea-pig ileum. The EC_{50} was 3×10^{-8} M in both species.

3 Pretreatment of rabbit ileum with the α_2-adrenoceptor antagonist, yohimbine (1×10^{-6} M) for 10 min did not reduce the response of the tissue to neuropeptide Y (1×10^{-7} M).

4 When applied serosally to rabbit ileal mucosa, the related peptide YY caused a maximum change in short circuit current of $-60 \pm 13\,\mu A\,cm^{-2}$; the EC_{50} was 2×10^{-9} M.

5 Isotopic flux studies in rabbit ileum showed that 1×10^{-7} M neuropeptide Y enhanced mucosal-to-serosal Na^+ and Cl^- fluxes and reduced serosal-to-mucosal Cl^- flux.

6 Replacement of chloride with gluconate on both sides of the tissue significantly reduced the change in short circuit current produced by neuropeptide Y (1×10^{-7} M), as did a similar replacement of bicarbonate.

7 It is concluded that neuropeptide Y and peptide YY are the most potent neurotransmitters or hormones so far described in their ability to attenuate electrogenic transport in the small intestine.

Introduction

Neuropeptide Y (NPY) is a 36 amino acid member of the pancreatic polypeptide family. It shares sequence homologies with pancreatic polypeptide (PP) and peptide YY (PYY) (Tatemoto, 1982a; Tatemoto *et al.*, 1982). Whereas both PP and PYY are localized in distinct populations of endocrine cells in pancreas and gut (Larsson *et al.*, 1976; Lundberg *et al.*, 1982; 1984b), NPY is widely distributed throughout the nervous system (Lundberg *et al.*, 1984). In the guinea-pig gut, for example, NPY immunoreactivity is found in 25% of all submucosal neurones, many of which innervate the intestinal mucosa and have been traced to epithelial cells (Furness *et al.*, 1983; Keast *et al.*, 1984). Subpopulations of these NPY-immunoreactive neurones have been shown to contain noradrenaline, choline acetyltransferase, and vasoactive intestinal peptide (VIP) (Ekblad *et al.*, 1984; Furness *et al.*, 1983; 1984). Owing to its mucosal localization, we wished to determine whether NPY could act as a regulator of intestinal epithelial ion transport. We therefore studied the effect of NPY on short circuit current in the guinea-pig and rabbit ileal mucosa. Our results show that NPY and PYY are the most powerful inhibitory modulators of intestinal ion transport so far described.

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Methods

Preparation of ileal mucosa

Female Hartley guinea-pigs weighing 400 g were killed by decapitation. A 15 cm portion of distal ileum directly adjacent to the ileo-cecal valve was quickly excised and rinsed in ice cold Ringer solution of the following composition (mM): Na^+ 141, K^+ 10, Ca^{2+} 1.25, Mg^{2+} 1.1, Cl^- 127, HCO_3^- 25, $H_2PO_4^-$ 0.3, HPO_4^{2-} 1.65, pH = 7.4 when gassed with 95% O_2 and 5% CO_2. Tissue viability was promoted by the presence of 10 mM glucose. Individual pieces, 2 cm in length, were cut off and opened along the

mesenteric border. The tissue was then stretched serosal side up across the pins of a Lucite half chamber and curved forceps were used to strip off the outer longitudinal muscle. A second half chamber was clamped on top. The entire assembly was mounted with 10.0 ml Ringer solution in reservoirs on either side. The reservoirs were bubbled with a 95% O_2/5% CO_2 gas phase and maintained at 37.5°C by means of an external water-jacketed circulatory system (Field *et al.*, 1971). Glucose 10 mM was present on the serosal side; osmotic balance was achieved with 10 mM mannitol on the mucosal side. Salt-agar bridges connected the chamber to a pair of calomel electrodes for measuring the potential difference (p.d.) and a pair of Ag/AgCl electrodes for delivering current.

Male New Zealand White rabbits weighing 2–3 kg were killed by cervical dislocation. A 40 cm portion of the distal ileum directly adjacent to the ileo-caecal valve was excised, opened along the mesenteric border, and rinsed as described above. A 12 cm portion was laid serosal side up on an ice-cooled Lucite board and stripped of the circular and longitudinal muscle. Individual pieces 3 cm in length were then clamped between two Lucite half chambers and mounted as described above.

Concentration-response curves

The fluid resistance (R_f) was measured before the tissue was mounted. A 50 μA current was passed between the Ag/AgCl electrodes and R_f was calculated from the resultant p.d. according to Ohm's law: Δp.d. = 50 μA × R_f. A typical value for R_f was 20 Ω. This procedure was then repeated with the tissue mounted. A typical value for total resistance was 55 Ω. Subtraction of R_f from the total resistance provided a measure of the tissue resistance, R_t. A typical value for R_t was 35 Ω. Since R_f and R_t were of equal magnitude, R_f could be subtracted from all subsequent manipulations involving ($R_f + R_t$) without significantly detracting from the accuracy of R_t.

The short circuit current (Isc) was calculated from the p.d. as follows. The p.d. was recorded at a frequency of 1 Hz. A 50 μA current of 4 s duration was pulsed through the tissue with inter-pulse intervals of 7 s. R_t was calculated from the resultant Δp.d. according to Ohm's law: Δp.d. = 50 μA × ($R_f + R_t$). R_t and the p.d. immediately preceding each pulse were then combined to calculate the Isc according to Ohm's law: p.d. = R_t × Isc. A record of Isc was thus obtained with a frequency of 0.09 Hz.

The continuous curves generated by the concentration-response experiments represent equations of the form:

$$\Delta I = (C)\ (\Delta I_{max})/(C + K_D)$$

where ΔI = calculated maximum change in Isc, C = peptide concentration, and ΔI_{max} and K_D are parameters chosen to achieve the best fit to the data using an iterative non-linear least squares optimization routine (Yamaoka *et al.*, 1981).

Ion flux studies

The tissue was short-circuited manually as follows. The open circuit potential, (V_o), is a product of the tissue-generated current and tissue resistance, ($I_t \times R_t$). Assuming the tissue to be a flat sheet, the potential measured under short circuit conditions, (V_m), is described as:

$$V_m = I_e(R_f + R_t) + (I_t \times R_t)$$

where I_e = externally applied current (Tai & Tai, 1981). The voltage across the tissue is zero when $I_e = I_t$, or $V_m = (-I_t \times R_f) = (-V_o/R_t) \times R_f$. Thus, V_o was determined every 5 min and I_e was varied to attain the desired V_m.

In ion flux experiments, unidirectional mucosal-to-serosal (J^{ion}_{ms}) and serosal-to-mucosal (J^{ion}_{sm}) isotope fluxes were measured before and after the addition of NPY (1×10^{-7} M). Trace quantities of $^{36}Cl^-$ (0.074 μCi ml^{-1}) or $^{22}Na^+$ (0.1 μCi ml^{-1}) were added to either the mucosal or serosal side of a short circuited tissue (Field *et al.*, 1971). After the isotope had attained a steady state rate of transfer across the mucosa (20 min), two aliquots were taken from the unlabelled chamber separated by a 5 min interval (period I). Thirty minutes later, NPY was added to the serosal side of the tissue. Fifteen minutes later, two more aliquots were taken from the unlabelled side, separated by a 5 min interval (period II). The net flux, defined as ($J^{ion}_{ms} - J^{ion}_{sm}$), was obtained from paired tissues, whose resistance differed by no more than 25%. J^R_{net} was estimated from the Isc, which is defined as ($J^{Na+}_{net} J^{Cl-}_{net} + J^R_{net}$). The average Isc was determined for each tissue pair in the sodium and chloride flux studies and designated $Isc_{(Na+)}$ or $Isc_{(Cl)}$, respectively. These values were then averaged, as were the measurements of $J^{Na^+}_{net}$ and $J^{Cl^-}_{net}$, in order to obtain the average J^R_{net}:

$$(Isc_{(Na+)}/2 + Isc_{(Cl-)}/2 - J^{Na^+}{}_{net} + J^{Cl}{}_{net}) = J^R{}_{net}.$$

Ion substitution studies

The ion substitution experiments were performed by replacing Cl^- and HCO_3^- in the Ringer solution with gluconate. In all HCO_3^- free conditions, the Ringer solution was bubbled with 100% O_2 and buffered with 25 mM HEPES, pH = 7.4.

Materials and drugs

NPY, PYY and PP were purchased from Peninsula Laboratories (San Carlos, Calif., U.S.A.). Yohimbine was purchased from Sigma (St. Louis, Mo., U.S.A.). $^{36}Cl^-$ and $^{22}Na^+$ were purchased from Amersham (Arlington Heights, Il., U.S.A.)

Results

Concentration-response studies

Addition of NPY to the serosal side of guinea-pig and rabbit ileal mucosa caused a slow and prolonged depression in transmural potential difference (p.d.) (Figure 1). Since NPY caused no significant change in R_t (Table 1), the time-course of ΔIsc was identical to that of Δp.d. NPY was extremely potent, with an EC_{50} of 3×10^{-8} M in both species. The maximum decrease in Isc was $-49 \pm 14\ \mu A\ cm^{-2}$ in the guinea-pig ileum (Figure 2) and $-50 \pm 6\ \mu A\ cm^{-2}$ in the rabbit ileum (Figure 3). Following addition of NPY, the Isc reached a minimum value within 5 min. No effect was seen when 1×10^{-7} M NPY was added to the mucosal side.

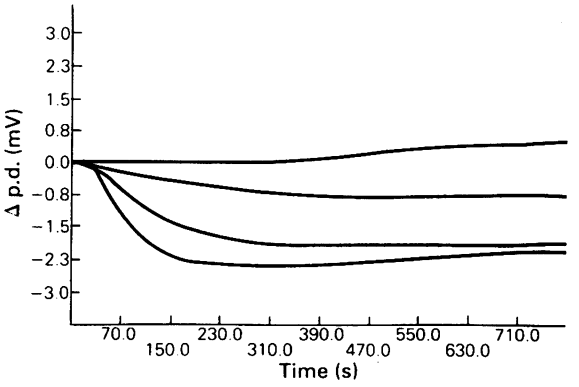

Figure 1 Neuropeptide Y (NPY)-induced depression of transmural potential difference (p.d.) and short circuit current (Isc) in rabbit ileal mucosa. Shown are typical tracings from a single rabbit mucosal preparation. Three similar experiments gave similar results. From top to bottom, curves represent responses to 1×10^{-9}M, 1×10^{-8}M, 5×10^{-8}M and 5×10^{-7}M NPY, respectively.

Table 1 Effect of neuropeptide Y (NPY) on the short circuit current (Isc) and tissue resistance (R_t) in rabbit ileum

	Isc (μEq h^{-1} cm^{-2})	R_t (Ω cm^{-2})
I	2.1 ± 0.3	33.4 ± 2.2
II	0.5 ± 0.3	32.9 ± 2.0
Δ	−1.6 ± 0.2*	−0.4 ± 0.8†

Data show means ± s.e.mean.
Period I represents baseline values 20 min after isotope addition. Period II represents values 20 min after the addition of 1×10^{-7}M NPY. Measurements were made in 24 tissues. Significance levels are based on 2-tailed Student's *t* test. *$P < 0.001$, †$P > 0.05$.

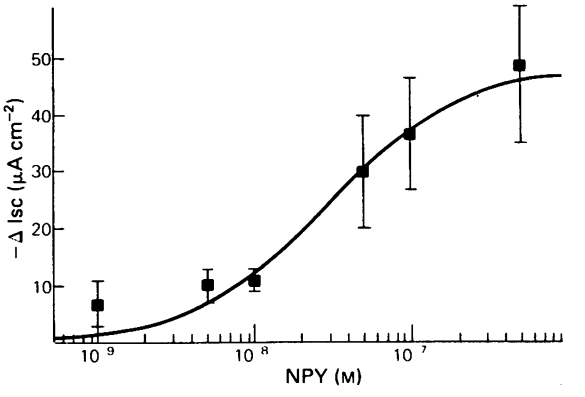

Figure 2 Concentration-response curve for neuropeptide Y (NPY) in guinea-pig ileum. The maximum change in the short circuit current (ΔIsc) was $-49 \pm 14\ \mu A\ cm^{-2}$ and the EC_{50} was 3×10^{-8}M. The number of observations (*n*) was distributed as follows: 1×10^{-9}M, (5); 5×10^{-9}M, (4); 1×10^{-8}M, (6); 5×10^{-8}M, (7); 1×10^{-7}M, (6); 5×10^{-7}M, (6). Vertical lines represent s.e.mean.

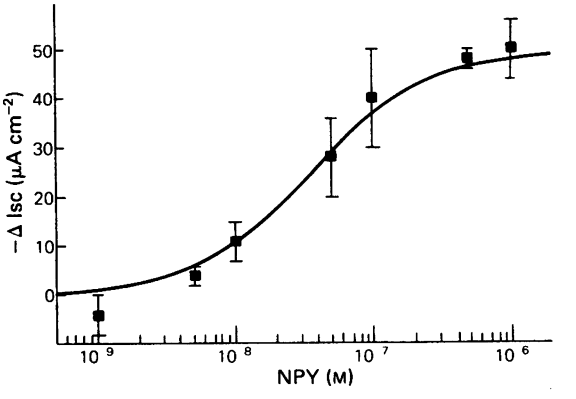

Figure 3 Concentration-response curve for neuropeptide Y (NPY) in rabbit ileum. The maximum change in the short circuit current (ΔIsc) was $-50 \pm 6\ \mu A\ cm^{-2}$ and the EC_{50} was 3×10^{-8}M. For each point, $n = 3$. Vertical lines represent s.e.mean.

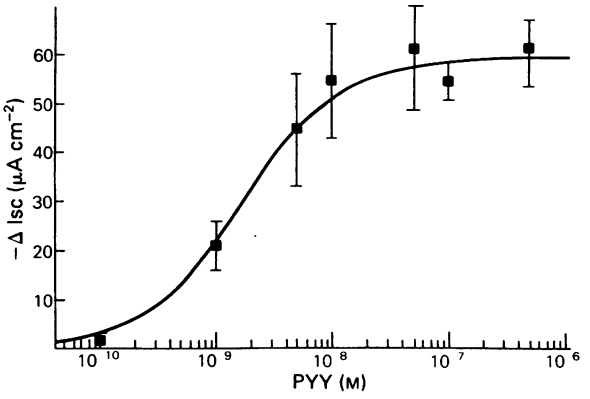

Figure 4 Concentration-response curve for peptide YY (PYY) in rabbit ileum. The maximum change in the short circuit current (ΔIsc) was $-60 \pm 13\,\mu A\,cm^{-2}$ and the EC_{50} was 2×10^{-9}M. The number of observations (n) was distributed as follows: 1×10^{-10}M, (3); 1×10^{-9}M, (4); 5×10^{-9}M, (3); 1×10^{-8}M, (4); 5×10^{-8}M, (3); 1×10^{-7}M, (5); 5×10^{-7}M, (4). Vertical lines represent s.e.mean.

Noradrenaline produced similar changes in p.d. and Isc through α_2-adrenoceptor stimulation in the small intestine (Field & McColl, 1973; Chang *et al.*, 1982; 1983). We therefore tested the possibility that NPY stimulates the release of noradrenaline from mucosal nerve terminals. Serosal addition of the α_2-adrenoceptor antagonist yohimbine (1×10^{-6} M) 10 min prior to the addition of 1×10^{-7} M NPY had, however, no effect on NPY-induced changes in p.d. or Isc ($n = 6$). Thus, neither release of noradrenaline nor stimulation of α_2-adrenoceptors can account for the transmural effects of NPY in the ileum.

As NPY shares many sequence homologies with the pancreatic polypeptides, we also examined the effects of two related peptides in the rabbit ileum. Rat pancreatic polypeptide had no effect on Isc in concentrations of up to 5×10^{-7} M. PYY, however, which differs from NPY at 11 positions, depressed the Isc in a manner similar to that of NPY. PYY was even more potent than NPY. In rabbit ileum, the EC_{50} for PYY was 2×10^{-9} M. The maximum decrease in Isc was $-60 \pm 13\,\mu A\,cm^{-2}$ (Figure 4).

Ion flux studies

The electrical changes induced by NPY and PYY presumably reflect changes in intestinal ion transport (Field, 1974). The principal ions known to contribute to the Isc in the ileum are Na^+, Cl^-, and HCO_3^- (Field *et al.*, 1971; Dietz & Field, 1973; Sheerin & Field, 1975). To determine how NPY affects intestinal ion transport, tracer quantities of $^{22}Na^+$ or $^{36}Cl^-$ were added to either the serosal or mucosal side of a short-circuited tissue from the rabbit ileum. Unidirectional mucosal-to-serosal (J^{ion}_{ms}) and serosal-to-mucosal (J^{ion}_{sm}) isotope fluxes were measured before and after the addition of 1×10^{-7} M NPY.

The data in Table 2 show that NPY (1×10^{-7} M) enhanced net Na^+ absorption by increasing $J^{Na^+}_{ms}$. This was accompanied by an increase in $J^{Cl^-}_{ms}$ and a decrease in $J^{Cl^-}_{sm}$. No statistically significant increase was observed in the net residual flux (J^R_{net}), which is defined as that portion of the Isc not accounted for by $J^{Na^+}_{net}$ and $J^{Cl^-}_{net}$.

Ion substitution studies

Table 1 shows that NPY (1×10^{-7} M) decreased the Isc by $1.6 \pm 0.2\,\mu Eq\,h^{-1}\,cm^{-2}$. The two known components of electrogenic transport in rabbit ileum are J^R_{net} and Cl^- secretion (Field, 1971; Field *et al.*, 1971; Dietz & Field, 1973; Sheerin & Field, 1975; Bolton & Field, 1977). In order to clarify further the relationship between Isc, J^R_{net} and Cl^- secretion, we determined whether removal of either Cl^- or HCO_3^- from the bathing media reduced the effect of NPY on Isc. Table 3 shows that the ΔIsc produced by NPY was reduced

Table 2 Effect of neuropeptide Y (NPY) on ion flux in rabbit ileum

	Fluxes ($\mu Eq\,h^{-}\,cm^{-2}$)						
	Sodium			*Chloride*			J^R_{net}
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}	
I	18.1 ± 2.2	13.9 ± 1.7	4.2 ± 1.1	14.5 ± 1.2	8.9 ± 0.6	5.6 ± 1.0	3.5 ± 1.4
II	22.9 ± 2.2	14.3 ± 1.7	8.6 ± 1.0	17.2 ± 0.7	6.6 ± 1.3	10.6 ± 1.5	2.5 ± 1.4
Δ	4.8 ± 1.0	0.4 ± 1.1	4.4 ± 1.1*	2.7 ± 1.1	−2.3 ± 1.4	5.0 ± 1.0†	−1.0 ± 1.5‡

Results show mean values ± s.e.mean.
Period I represents baseline fluxes measured 20 min after the addition of isotope to the Ussing chambers. Period II represents fluxes measured 20 min after the addition of 1×10^{-7}M NPY to the Ussing chambers. Six tissue pairs were studied in each of the sodium and chloride flux groups. Significance levels are based on 2-tailed Student's t test.
*$P < 0.01$, †$P < 0.005$, ‡$P > 0.05$.

Table 3 Effect of selective ion substitution on neuropeptide Y (NPY)-induced change in the short circuit current (ΔIsc) in rabbit ileum

	Control (ΔIsc: fraction of control)	*Substituted (ΔIsc: fraction of control)*	P
(a)	1.0 ± 0.19 ($n = 6$)	0.30 ± 0.042 ($n = 6$)	<0.02
(b)	1.0 ± 0.041 ($n = 5$)	0.65 ± 0.051 ($n = 5$)	<0.001
(c)	1.0 ± 0.029 ($n = 6$)	0.22 ± 0.022 ($n = 6$)	<0.001

Results show mean values ± s.e.mean.
The concentration of NPY was 1×10^{-7}M. (a) Cl^- was replaced with gluconate on both sides of the tissue. (b) HCO_3^- was replaced with gluconate on both sides of the tissue. (c) Cl^- and HCO_3^- were replaced with gluconate on both sides of the tissue. Significance levels are based on 2-tailed Student's *t* test.

by 70% when external Cl^- was replaced with gluconate on both sides of the tissue and by 35% when external HCO_3^- was similarly replaced. When both Cl^- and HCO_3^- were replaced, the response to NPY was reduced by 78%.

Discussion

NPY produced a slow and prolonged inhibition of p.d. and Isc in the distal ileum of guinea-pig and rabbit. The responses were qualitatively similar to those seen upon activation of the α_2-adrenoceptors but could not be blocked by yohimbine, an α_2-adrenoceptor antagonist. Thus, NPY does not mediate its effect through α_2-adrenoceptors. More definitive data on the NPY receptor will come from binding experiments, currently in progress, and from autoradiography.

The homologous peptide PYY was more potent than NPY as an inhibitor of electrogenic transport. This is consistent with the relatively greater potency of PYY in several other physiological processes, including pancreatic secretion, vasoconstriction, and inhibition of contractions in vas deferens (Tatemoto, 1982b; Tatemoto *et al.*, 1982; Lundberg & Tatemoto, 1982; Chang *et al.*, 1985). The potent effects described here again suggest an important role for PYY as a regulator of intestinal ion transport. In addition, it would be interesting to know whether these two structurally similar peptides localized in two different cell populations play distinct roles in gastrointestinal physiology.

The isotope flux experiments enabled us to dissect the electrogenic effects of the peptide into ionic components. It is important to note that unidirectional fluxes consist of both active and passive ion transport. A comparison of unidirectional fluxes before and after the addition of a modulatory agent is valid only insofar as that agent does not significantly alter the paracellular permeability to passive ion flux. Since NPY caused no significant change in R_t (Table 1), a comparison of unidirectional fluxes before and after NPY should reflect only changes in active transport. We therefore considered the effects of NPY on unidirectional, as well as net fluxes.

The isotope flux experiments clearly showed that NPY enhanced $J^{Na^+}_{ms}$ and $J^{Cl^-}_{ms}$ and decreased $J^{Cl^-}_{sm}$. It is interesting to note that VIP, the most abundant and potent endogenous peptide stimulant of electrolyte secretion in the small intestine, produces changes in p.d., Isc, and ion transport which mirror those of NPY. VIP acts by increasing the concentration of adenosine 3′:5′-cyclic monophosphate (cyclic AMP; Schwartz *et al.*, 1974). One might speculate, therefore, that NPY acts by reducing the concentration of cyclic AMP in epithelial cells. The co-localization of NPY and noradrenaline in certain submucosal neurones raises the possibility that NPY might modulate the effects of noradrenaline in the gut; such modulation has been demonstrated in several other systems (Edvinsson *et al.*, 1984; Lundberg *et al.*, 1984a; Carter *et al.*, 1985).

The isotope flux experiments also provide an estimate of J^R_{net}, which in the ileum is generally attributed to HCO_3^- secretion (Dietz & Field, 1973). The estimated decrease in J^R_{net} provided a preliminary indication that NPY might modify bicarbonate flux. In order to probe this possibility further, we examined the relevance of HCO_3^- transport to the NPY effect by performing ion substitution experiments (Table 3). The results clearly indicate that the electrical effects of NPY depended upon the presence of both HCO_3^- and Cl^- in the external bathing media. We observed, however, that the effect of replacing both HCO_3 and Cl^- simultaneously did not quite account quantitatively for the effects of replacing them independently. This may have been due to variations in the degree of metabolically generated HCO_3^- and/or flux. Yet another possible factor may have been coupled Cl^-/HCO_3^- exchange at the luminal border (Field *et al.*, 1971), i.e., the removal of one ion may have affected the amount of the other available for electrogenic transport. In conclusion, the most parsimonious interpretation of these results is that both Cl^- and HCO_3^- contributed to basal electrogenic transport, which appeared to be inhibited by NPY.

Certain unexplained aspects of intestinal function underscore the significance of characterizing the action of NPY. For example, electrical field stimulation

of the rabbit ileum induces a neuronally-mediated change in electrolyte transport which cannot be accounted for by any of the well-characterized gut neurotransmitters (Hubel, 1978). Also, the enkephalins have an effect in the ileum similar to that of NPY and appear to act by releasing an as yet unidentified neurotransmitter (Binder *et al.*, 1984). One attractive possibility is that NPY is the endogenously released neurotransmitter.

In summary, we have shown that NPY and PYY are the most potent neurotransmitters or hormones so far described in their ability to attenuate electrolyte secretion in the small intestine. This action, together with their anatomical localization in mucosal endocrine cells and in neurones innervating the mucosa, suggests an important role for these peptides in the regulation of intestinal ion transport. Moreover, as potent antisecretory substances are important in the development of novel antidiarrhoea drugs, the pharmacological implications of these results should also be considered.

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