The effect of neuropeptide Y on sodium, chloride and potassium transport across the rat distal colon

D. Strabel & ¹M. Diener

Institut für Veterinär-Physiologie, Universität Zürich, Winterthurerstr. 260, CH-8057 Zürich, Switzerland

- 1 Neuropeptide Y (NPY; 10^{-10} – 10^{-7} mol l⁻¹) reduced basal short-circuit current (I_{sc}) in a concentration-dependent manner in the rat distal colon but was ineffective in the proximal colon.
- 2 The action of NPY was dependent upon the presence of Cl⁻ and HCO₃⁻ anions and was blocked by prior treatment of the tissue with a Cl⁻ channel blocker. The decrease in I_{sc} was associated with an increase in mucosa-to-serosa fluxes of Na⁺, Rb⁺ (K⁺) and Cl⁻, whereas the serosa-to-mucosa flux of Cl⁻ was decreased.
- 3 The size of the inhibitory NPY effect was linearly correlated with the height of the basal I_{sc} , i.e. it inhibited 55% of basal secretory I_{sc} .
- 4 The action of NPY was unaffected by indomethacin and tetrodotoxin, when given alone, but was abolished, when the basal I_{sc} was decreased to values near zero by a combination of both inhibitors. This inhibition could be overcome by restoring basal I_{sc} with prostaglandin E₂, indicating that the effect of NPY is not mediated by nerves or prostaglandins, but that NPY is only effective, when anion secretion is stimulated by the spontaneous release of neurotransmitters and prostaglandins.
- 5 NPY inhibited the increase in I_{sc} induced by veratridine and prostaglandin E₂, but it had no effect on the I_{sc} induced by direct stimulation of the adenylate cyclase with forskolin, or on I_{sc} induced by stimulation of the Ca²⁺-pathway with carbachol. Inhibition of the response to veratridine or prostaglandin E₂ by NPY showed the same dependence on the height of the I_{sc} just prior to addition of NPY as seen in control conditions, i.e. NPY inhibited 55% of cyclic AMP-mediated secretion.
- 6 These results suggest that the effect of NPY is mediated by an inhibition of cyclic AMP-stimulated secretion, which is stimulated in the rat distal colon by a continuous release of prostaglandins and neurotransmitters.

Keywords: Cl⁻ secretion; cyclic AMP; enteric nervous system; K⁺ transport; neuropeptide Y; prostaglandins; rat colon

Introduction

Neuropeptide Y (NPY) is a 36-amino acid peptide, which was originally isolated from the brain (Tatemoto *et al.*, 1982). Like many other transmitters of the central nervous system it is also present in the gut (Furness *et al.*, 1983), where it is known to coexist with noradrenaline in sympathetic nerves and with vasoactive intestinal polypeptide (VIP) in nerves of the small intestine (Ekblad *et al.*, 1987; Cox *et al.*, 1994). At least two receptor subtypes for NPY have been identified pharmacologically, the Y₁-receptor, which is stimulated only by the intact peptide, and the Y₂-receptor, which is also stimulated by C-terminal fragments of NPY (Wahlestedt *et al.*, 1990).

NPY has been shown to exert proabsorptive and anti-secretory actions in the small intestine of different species. It inhibits basal and stimulated Cl⁻ secretion in the rat small intestine *in vivo* (Saria & Beubler, 1985) and *in vitro* (Cox & Cuthbert, 1988; Cox *et al.*, 1988) as well as in the porcine jejunum *in vitro* (Brown *et al.*, 1990), whereas in the rabbit ileum, NPY selectively stimulates Cl⁻ absorption (Hubel & Renquist, 1986). Only a few studies have been performed with the colonic mucosa. In the rabbit distal colon, NPY inhibited ion secretion stimulated by VIP (Flint *et al.*, 1990), and when comparing the effect of NPY in the rat small and large intestine, only small effects of NPY on basal short-circuit current were observed in the rat descending colon (Cox *et al.*, 1988). Different mechanisms have been proposed for the action of NPY on anion transport. In rabbit (Hubel & Renquist, 1986) and pig (Brown *et al.*, 1990) small intestine, NPY was found to stimulate exclusively the absorptive, mucosa-to-serosa flux of Cl⁻ (J_{ms}^{Cl}), whereas in the rat small intestine both an increase

in J_{ms}^{Cl} as well as a decrease in the serosa-to-mucosa flux (J_{ms}^{Cl}), i.e. a proabsorptive and an antisecretory effect of NPY have been observed (Cox *et al.*, 1988). In rabbit (Hubel & Renquist, 1986) and rat (Cox *et al.*, 1988) small intestine, the effect of NPY was unaffected by the neurotoxin, tetrodotoxin (TTX), whereas in the pig small intestine TTX abolished the response to NPY (Brown *et al.*, 1990). In contrast, in the rat small intestine, the effect of NPY was reduced by prior treatment of the tissue with the cyclo-oxygenase inhibitor, indomethacin (Cox *et al.*, 1988).

Recently, we studied the effect of another proabsorptive and antisecretory hormone, somatostatin, on ion transport in the rat distal colon. Somatostatin inhibits adenosine 3':5'-cyclic monophosphate (cyclic AMP)- and Ca²⁺-mediated secretion (Knobloch *et al.*, 1989) and stimulates basal Cl⁻ absorption by stimulating J_{ms}^{Cl} (Diener & Gartmann, 1994). This latter action has been shown to be mediated by the opening of a volume-activated basolateral Cl⁻ channel, the activation of which can be prevented by prior addition of lipoxigenase blocker or a leukotriene D₄ receptor blocker. One aim of the present study was to investigate whether the action of NPY in the rat colon involves a similar mechanism to that of somatostatin. In the light of the published literature (see above) concerning the action of NPY in other intestinal segments, special interest was placed on a possible role of the eicosanoid and the neural system. Because no data are available about an action of proabsorptive hormones on K⁺ transport, the effect of NPY on K⁺ fluxes (using ⁸⁶Rb⁺ as a marker for K⁺) was investigated and compared with two other proabsorptive hormones, somatostatin and vasopressin. The colon is a differentiated organ with well known segmental differences (see e.g. Nobles *et al.*, 1991), therefore the effect of NPY was tested in the distal and the proximal part of this organ.

¹ Author for correspondence.

Methods

Solutions

Most of the experiments were carried out in a Parsons solution containing (mmol l⁻¹): NaCl 107, KCl 4.5, NaHCO₃ 25, Na₂HPO₄ 1.8, NaH₂PO₄ 0.2, CaCl₂ 1.25, MgSO₄ 1 and glucose 12. The solution was gassed with carbogen (5% CO₂ in 95% O₂) and kept at a temperature of 37°C; pH was 7.4. When fluxes of ⁸⁶Rb⁺ were measured, KCl was substituted by RbCl. For the Cl⁻-free buffer, NaCl was replaced with Na gluconate, the Ca²⁺ concentration in this solution was elevated to 5.8 mmol l⁻¹ in order to compensate the Ca²⁺-buffering properties of gluconate (Kenyon & Gibbons, 1977). In the Na⁺-free solution, NaCl was replaced by N-methyl-D-glucamine chloride. The HCO₃⁻-free Tyrode solution consisted of (mmol l⁻¹): NaCl 140, KCl 5.4, CaCl₂ 1.25, MgSO₄ 1, HEPES (N-(2-hydroxyethyl)piperazine-N'-2-ethansulphonic acid) 10, glucose 12; it was gassed with O₂. If in addition Cl⁻ was omitted from this solution, NaCl was substituted by Na gluconate and the CaCl₂ concentration was elevated to 10 mmol l⁻¹.

Tissue preparation

Female SIVZ-50 rats (Institut für Labortierkunde, Universität Zürich, Switzerland) with a weight of 180–220 g were used. The animals had free access to water and food until the day of the experiment. Animals were stunned by a blow on the head and killed by exsanguination (approved by the Swiss Federal Administration Bureau for Veterinary Medicine). The serosa and muscularis propria were stripped away by hand to obtain the mucosa-submucosa preparation of the distal part of the colon descendens. For comparison, some experiments were also performed with the muscle-stripped proximal colon.

Short-circuit current measurement

The tissue was fixed in a modified Ussing chamber (Andres *et al.*, 1985), bathed with a volume of 4 ml on each side of the mucosa and short-circuited by a voltage clamp (Aachen Microclamp, AC Copy Datentechnik, Aachen, Germany) with correction for fluid resistance. The exposed surface of the tissue was 1 cm². Short-circuit current (*I*_{sc}) was continuously recorded on a chart-recorder. In addition, *I*_{sc} and tissue conductance (Gt) were printed every min by a computer printer.

Unidirectional flux measurements

Ion flux studies were performed as previously described (Andres *et al.*, 1985). After an equilibration period of 60 min, either ²²Na (59 kBq) and ³⁶Cl (29 kBq), or ⁸⁶Rb (74 kBq) were added to one side (= labelled side) of the epithelium. After an additional 20 min (to allow isotope fluxes to reach a steady state), unidirectional ion fluxes were determined in two sequential 20 min periods, i.e. a control period and a period starting 10 min after administration of the proabsorptive hormone. From the measured unidirectional fluxes net ion fluxes were calculated. Residual ion flux, i.e. the sum of the movement of all ions other than Na⁺ and Cl⁻, was calculated according to: $J_{\text{net}}^{\text{R}} = I_{\text{sc}} - J_{\text{net}}^{\text{Na}} - J_{\text{net}}^{\text{Cl}}$. A positive $J_{\text{net}}^{\text{R}}$ indicates either the absorption of a cation or the secretion of an anion.

In other experiments, Cl⁻ or Rb⁺ accumulation on the unlabelled side was measured under control conditions in five sequential 3 min periods followed by five sequential 3 min periods starting 3 min after administration of 10⁻⁷ mol l⁻¹ NPY to the serosal side. For these experiments 104 kBq ³⁶Cl⁻ or 120 kBq ⁸⁶Rb⁺, respectively, were added to the labelled side 20 min before the beginning of the flux experiment. Transport rates for Cl⁻ or Rb⁺ were calculated separately by linear regression of isotope accumulation on the unlabelled side during

the control and the NPY period. All aliquots from the labelled side were replaced by unlabelled buffer solution and appropriate correction for this replacement solution was performed.

Data evaluation

Usually, the experiments were started after an equilibration period of 1–2 h, after which the initial high *I*_{sc} had reached a stable plateau. The response to NPY was tested in the absence and presence of different inhibitors, which were administered to the compartment indicated in the text, stabilization of a new *I*_{sc} baseline was usually within 15–20 min. The baseline in *I*_{sc} was determined as the mean of three immediately preceding *I*_{sc} values, i.e. the last 3 min just before administration of NPY. In the tables, the maximal decrease in *I*_{sc} induced by NPY is expressed as the difference from the starting baseline (ΔI_{sc}).

Drugs

If not indicated otherwise, drugs were from Sigma, Buchs, Switzerland. Anthracene-9-carboxylic acid (Aldrich, Buchs, Switzerland), methazolamide, 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB; gift from R. Greger, Physiologisches Institut, Freiburg, Germany), and veratridine were dissolved in dimethylsulphoxide (DMSO, final maximal concentration 0.25%, v/v). Forskolin (gift from Hoechst, Frankfurt, Germany), indomethacin, nordihydroguaiaretic acid, and prostaglandin E₂ were dissolved in ethanol (final maximal concentration 0.25%, v/v). Neuropeptide Y (NPY; Peninsula, Belmont, CA, U.S.A.) was dissolved in 10 mmol l⁻¹ acetic acid containing 0.1% (w/v) bovine serum albumin. Neuropeptide Y_{(13–36)}}, somatostatin (generous gift from Curamed, Karlsruhe, Germany), vasoactive intestinal polypeptide (VIP), and vasopressin were dissolved in aqueous stock solutions containing 0.1% (w/v) bovine serum albumin. Tetrodotoxin (TTX) was dissolved in citrate buffer (5 mg mg⁻¹ TTX; pH 4.3). All other drugs including SK&F 104353 (2-hydroxy-3-carboxyethylthio-3-[2-(8-phenyloctyl)phenyl] propanoic acid) (generous gift from Smith Kline & French, King of Prussia, PA, U.S.A.) were in aqueous stock solutions. Radioisotopes were obtained from NEN, Dreieich, Germany, and specific activities were 3.9 TBq g⁻¹ ²²Na and 550 MBq g⁻¹ ³⁶Cl; the initial activity of ⁸⁶Rb amounted to 411 GBq g⁻¹.

NPY, anthracene-9-carboxylic acid, carbachol, NPPB, prostaglandin E₂, SK&F 104353, somatostatin, tetrodotoxin, vasopressin, veratridine and VIP were added to the serosal side only, while forskolin, indomethacin, methazolamide and nordihydroguaiaretic acid were applied on both sides of the tissue.

Statistics

Results are given as means ± one standard error of the mean (s.e.mean). Standard errors for calculated values, i.e. net ion transport, were calculated by the law of error propagation. The significance of differences was tested by analysis of variances and, if indicated, by Student's paired or unpaired two-tailed *t* test or a U test, respectively. An *F* test was applied to decide which test method was to be used. Regression lines were compared by analysis of co-variances (Kenakin, 1987). The quality of regressions was checked by the squared non-linear regression coefficient (*r*²).

Results

Effect of NPY on basal electrical parameters

The rat distal colon showed a baseline short-circuit current (*I*_{sc}) of 1.8 ± 0.1 µEq h⁻¹ cm⁻² and a baseline tissue conductance (Gt) of 10.9 ± 0.9 mS cm⁻² (*n* = 40). NPY (administered at the serosal side) caused a concentration-dependent decrease in *I*_{sc} in the rat distal colon (Figure 1a) with a threshold concentration of 10⁻¹⁰ mol l⁻¹ and a half-maximal effect at a

concentration of $5 \times 10^{-9} \text{ mol l}^{-1}$. The response to NPY saturated at concentrations $\geq 5 \times 10^{-8} \text{ mol l}^{-1}$ and a maximal concentration of $10^{-7} \text{ mol l}^{-1}$ was used for all further experiments.

When all responses to NPY ($10^{-7} \text{ mol l}^{-1}$) in the distal colon under control conditions were pooled, NPY evoked a decrease of I_{sc} of $-0.9 \pm 0.1 \mu\text{Eq h}^{-1} \text{ cm}^{-2}$ ($n=40$; $P<0.05$), which was associated with a small, insignificant decrease in Gt ($-0.4 \pm 0.2 \text{ mS cm}^{-2}$; $n=40$). In contrast, NPY did not evoke

any significant change in I_{sc} in the proximal colon (Figure 1a). The response to native NPY was mimicked by the C-terminal NPY-fragment, NPY₍₁₃₋₃₆₎. Neuropeptide Y₍₁₃₋₃₆₎ ($10^{-7} \text{ mol l}^{-1}$) caused a decrease in I_{sc} of $-0.6 \pm 0.1 \mu\text{Eq h}^{-1} \text{ cm}^{-2}$ ($n=8$; $P<0.05$), which was not significantly smaller than the response induced by native NPY.

The response to NPY was relatively transient. In general, NPY caused a maximal decrease in I_{sc} within 5–6 min, which

Figure 1 (a) Concentration-dependent effect of neuropeptide Y (NPY) on I_{sc} in distal (○) and proximal (●) rat colon. Values are means $\pm$ 1 s.e. mean, $n=8$. The decrease in I_{sc} induced by NPY in the distal colon was significant for all concentrations tested, whereas in the proximal colon no significant change of the I_{sc} could be evoked by the peptide. Basal I_{sc} levels were $2.6 \pm 0.3 \mu\text{Eq h}^{-1} \text{ cm}^{-2}$ ($n=8$) in the distal and $2.1 \pm 0.2 \mu\text{Eq h}^{-1} \text{ cm}^{-2}$ ($n=8$) in the proximal colon. (b) Time-course of the response to NPY ($10^{-7} \text{ mol l}^{-1}$, administered three times without intermittent washing) in the distal colon. The experiment is representative for 6 similar experiments. The inset shows the decay in the I_{sc} response to NPY (■ = measured data points starting 8 min after NPY administration), which could be fitted ($r^2=0.992$) by a mono-exponential function (dashed line) with a half-time of 13.5 min. The data were averaged from 7 experiments. (c) Transfer of serosal fluid from a first tissue (lower tracing) 15 min after NPY administration ($10^{-7} \text{ mol l}^{-1}$) to the serosal side of a second native tissue (upper tracing). The line interruption in the upper tracing is caused by omission of an artifact due to exchange of the serosal buffer. This trace is representative of 5 similar experiments.

returned to baseline within about 30 min. This decay could be adequately described ($r^2=0.992$) using a mono-exponential function with a half-time of 13.5 min ($n=7$; inset of Figure 1) suggesting that only one mechanism is responsible for the termination of the response. When NPY (10^{-7} mol l $^{-1}$) was administered a second time during the decay phase of a first NPY administration, the peptide induced a prompt decrease in I_{sc} (Figure 1b), which amounted to $89.6 \pm 3.0\%$ of the first response. Even a third application of NPY, administered when the response to the first two doses had faded without an intermediate washout, induced a decrease in I_{sc} , which amounted to $63.7 \pm 6.7\%$ of the response evoked by the first NPY application ($n=6$). In a further set of experiments, NPY (10^{-7} mol l $^{-1}$) was added to one group of tissues. The serosal fluid of these tissues was sucked away 15 min after peptide administration, i.e. when the I_{sc} response had started to fade. When this serosal fluid replaced the serosal fluid of a second, untreated group of tissues, no decrease in I_{sc} could be evoked (Figure 1c; $n=5$), although subsequent administration of fresh NPY showed that the preparations were sensitive to the peptide. This suggests that peptide degradation rather than receptor desensitization is responsible for the transient nature of the NPY response.

Figure 2 Dependence of neuropeptide Y (NPY) responses (10^{-7} mol l $^{-1}$) upon the height of the baseline I_{sc} . The effect of NPY is shown under control conditions (●), and after elevation of baseline I_{sc} with prostaglandin E_2 (10^{-6} mol l $^{-1}$; ○) or with veratridine (5×10^{-5} mol l $^{-1}$; □). The solid line gives the linear correlation pooled for all data points according to: $\Delta I_{sc} = 0.07 - 0.56 \times \text{baseline}$ ($r = 0.84$; $n = 56$; $P < 0.05$).

The decrease in I_{sc} induced by NPY exhibited a certain degree of variability even under control conditions ranging from 0.0 $\mu\text{Eq h}^{-1} \text{cm}^{-2}$ to $-3.7 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ ($n=40$; ● in Figure 2). However, the decrease in I_{sc} showed a linear correlation with the baseline I_{sc} prior to addition to NPY; i.e. NPY inhibited about 55% of the spontaneous basal secretory I_{sc} .

Ionic dependence of the NPY effect

The decrease in I_{sc} caused by NPY (10^{-7} mol l $^{-1}$) was not dependent on the presence of Na^+ (Table 1). Omission of either Cl^- or HCO_3^- ions alone from the buffer did not diminish the response to NPY. If, however, both anions were omitted from the buffer, the effect of NPY was significantly attenuated (Table 1). The response to NPY was completely suppressed, when in addition the carbonic anhydrase blocker, methazolamide (10^{-4} mol l $^{-1}$) was present. Pretreatment of the mucosa with the Cl^- channel blockers, anthracene-9-carboxylic acid (10^{-3} mol l $^{-1}$) or NPPB (10^{-4} mol l $^{-1}$), also significantly reduced the response to NPY, indicating that a change in anion transport is predominantly responsible for the decrease in I_{sc} .

Measurements of unidirectional ion fluxes

The effect of NPY (10^{-7} mol l $^{-1}$) on unidirectional fluxes of $^{22}\text{Na}^+$ and $^{36}\text{Cl}^-$ was tested for a long time period, i.e. 20 min. Despite the results of the ion substitution experiments, which suggested a change in anion transport to be responsible for the decrease in I_{sc} induced by the peptide, no significant effect of NPY on Cl^- transport could be observed (Table 2). The only action of NPY under these conditions consisted of a significant increase in $J_{\text{Na}}^{\text{Na}}$ from $10.9 \pm 1.3 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ to $12.1 \pm 1.2 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ ($n=9$; $P < 0.05$).

In order to investigate the transient effect of NPY on anion transport in a more appropriate way, the effect of NPY on Cl^- transport was tested using a protocol developed by Cox *et al.* (1988) to investigate the effect of this peptide upon Cl^- transport in the rat jejunum. Therefore, tracer amounts of $^{36}\text{Cl}^-$ were added to one side of the epithelium and the accumulation of $^{36}\text{Cl}^-$ on the unlabelled side was measured under control conditions and in the presence of NPY (10^{-7} mol l $^{-1}$). Transport rates under control conditions and in the presence of NPY were calculated by linear regression, linear correlation coefficients (r) for all individual experiments were ≥ 0.96 .

Under control conditions, the ms-transport rate of $^{36}\text{Cl}^-$ amounted to $267 \pm 24 \text{ nmol min}^{-1} \text{cm}^{-2}$ (Figure 3). This rate

Table 1 Baseline electrical parameters during ion replacement experiments and in the presence of inhibitors

	Baseline I_{sc} ($\mu\text{Eq h}^{-1} \text{cm}^{-2}$)	ΔI_{sc}	n
Control	1.8 ± 0.1	-0.9 ± 0.1	40
Na^+ -free	2.4 ± 0.2	-1.0 ± 0.2	8
Cl^- -free	1.8 ± 0.2	-0.7 ± 0.1	8
HCO_3^- -free	1.8 ± 0.4	-0.5 ± 0.2	8
$\text{HCO}_3^- + \text{Cl}^-$ -free	0.9 ± 0.2	$-0.2 \pm 0.1^*$	11
$\text{HCO}_3^- / \text{Cl}^-$ -free + methazolamide	0.6 ± 0.2	$-0.1 \pm 0.1^*$	9
Anthracene-9-carboxylic acid	1.4 ± 0.2	$-0.3 \pm 0.1^*$	8
NPPB	1.0 ± 0.2	$-0.2 \pm 0.1^*$	8
Nordihydroguaiaretic acid	1.6 ± 0.3	-0.6 ± 0.1	8
SK&F 104353	1.9 ± 0.4	-0.6 ± 0.2	7
Indomethacin	1.3 ± 0.2	-0.7 ± 0.1	6
TTX	1.3 ± 0.5	-0.6 ± 0.1	6
Indomethacin/TTX	0.3 ± 0.2	$-0.1 \pm 0.0^*$	6
Indomethacin/TTX/prostaglandin E_2	3.0 ± 0.7	$-0.9 \pm 0.1^*$	6

Short-circuit current levels prior to the administration of neuropeptide (NPY)(baseline) and the effect of NPY (10^{-7} mol l $^{-1}$; ΔI_{sc}) during control conditions, during ion replacement experiments and in the presence of anthracene-9-carboxylic acid (10^{-3} mol l $^{-1}$), NPPB (10^{-4} mol l $^{-1}$), nordihydroguaiaretic acid (10^{-5} mol l $^{-1}$), SK&F 104353 (5×10^{-5} mol l $^{-1}$), indomethacin (10^{-6} mol l $^{-1}$), or tetrodotoxin (10^{-6} mol l $^{-1}$) and the combination of indomethacin, tetrodotoxin and prostaglandin E_2 (10^{-6} mol l $^{-1}$). Ion replacements were applied to both sides of the tissue. The effect of NPY is given as the difference from the preceding baseline. Values are means ± 1 s.e.mean. * $P < 0.05$ versus effect of NPY under control conditions.

increased to $321 \pm 28 \text{ nmol min}^{-1} \text{ cm}^{-2}$ in the presence of NPY ($n=9$; $P<0.05$). In parallel, the sm-transport of Cl^- was decreased by NPY ($215 \pm 23 \text{ nmol min}^{-1} \text{ cm}^{-2}$ in the absence and $155 \pm 12 \text{ nmol min}^{-1} \text{ cm}^{-2}$ in the presence of NPY; $n=8$; $P<0.05$). This led to an increase in net Cl^- transport rate from $53 \pm 6 \text{ nmol min}^{-1} \text{ cm}^{-2}$ to $166 \pm 6 \text{ nmol min}^{-1} \text{ cm}^{-2}$ ($P<0.05$; analysis of co-variances).

Sensitivity to inhibitors

The response to NPY ($10^{-7} \text{ mol l}^{-1}$) was neither affected by prior treatment of the mucosa with the cyclo-oxygenase inhibitor, indomethacin ($10^{-6} \text{ mol l}^{-1}$; Table 1), nor by the neuronal toxin, tetrodotoxin ($10^{-6} \text{ mol l}^{-1}$; Table 1), suggesting that the effect of the peptide is not mediated by prostaglandins or enteric neurones. However, prior addition of a combination of both blockers suppressed the effect of NPY; i.e. a combination of indomethacin and TTX attenuated NPY responses (Table 1). Both blockers together caused a decrease in I_{sc} from $4.0 \pm 0.4 \text{ } \mu\text{Eq h}^{-1} \text{ cm}^{-2}$ to $0.3 \pm 0.2 \text{ } \mu\text{Eq h}^{-1} \text{ cm}^{-2}$ ($n=6$; $P<0.05$; Table 1). In contrast, the lipoxygenase inhibitor, nordihydroguaiaretic acid ($10^{-5} \text{ mol l}^{-1}$), and the leukotriene D_4 receptor blocker, SK&F 104353 ($5 \times 10^{-5} \text{ mol l}^{-1}$), which both inhibit the effect of somatostatin on basal I_{sc} (Diener &

Gartmann, 1994), were without effect on the I_{sc} response induced by NPY (Table 1).

The inhibition of NPY responses by the combination of indomethacin and tetrodotoxin suggests either that the peptide effects are mediated by inhibitory prostaglandins and enteric nerves or that the spontaneous production of prostaglandins and neurotransmitters elevate the secretory state of the tissue, allowing subsequent attenuation by NPY. In order to distinguish between these possibilities, I_{sc} , which had been decreased by prior addition of indomethacin and tetrodotoxin, was elevated by prostaglandin E_2 ($10^{-6} \text{ mol l}^{-1}$; Table 1), and subsequently NPY was applied. Under these conditions, i.e. despite the inhibition of prostaglandin production by indomethacin and the inhibition of neurotransmission by tetrodotoxin, NPY caused a decrease in I_{sc} of $-0.9 \pm 0.1 \text{ } \mu\text{Eq h}^{-1} \text{ cm}^{-2}$ ($n=6$; $P<0.05$), which was not significantly different from control responses to NPY (Table 1).

Effect of NPY on the response to secretagogues

Pretreatment of preparations with NPY ($10^{-7} \text{ mol l}^{-1}$) did not affect the response to carbachol ($5 \times 10^{-5} \text{ mol l}^{-1}$), a secretagogue acting on the Ca^{2+} -pathway, nor to VIP ($10^{-7} \text{ mol l}^{-1}$), a secretagogue acting on the cyclic AMP-pathway, nor to vera-

Table 2 Effect of neuropeptide Y (NPY) on $^{22}\text{Na}^+$ and $^{36}\text{Cl}^-$ fluxes

	$J_{\text{ms}}^{\text{Na}}$	$J_{\text{sm}}^{\text{Na}}$	$J_{\text{net}}^{\text{Na}}$	$J_{\text{ms}}^{\text{Cl}}$	$J_{\text{sm}}^{\text{Cl}}$	$J_{\text{net}}^{\text{Cl}}$	I_{sc}	$J_{\text{net}}^{\text{R}}$	Gt
				($\mu\text{Eq h}^{-1} \text{ cm}^{-2}$)					(mS cm^{-2})
Control	10.9 ± 1.3	4.8 ± 0.4	6.1 ± 1.4	16.2 ± 1.7	9.9 ± 0.7	6.3 ± 1.8	1.7 ± 0.1	1.9 ± 2.3	11.3 ± 0.1
NPY	$12.1 \pm 1.2^*$	5.5 ± 0.4	6.6 ± 1.2	15.2 ± 1.8	9.3 ± 0.9	5.9 ± 2.0	$1.3 \pm 0.1^*$	0.6 ± 2.3	11.5 ± 0.9

The effect of NPY ($10^{-7} \text{ mol l}^{-1}$ at the serosal side) on mucosa-to-serosa (ms), serosa-to-mucosa (sm) and net fluxes during a 20 min flux measurement. The I_{sc} and Gt values are the means $\pm$ s.e.mean of the complete control or NPY period, respectively, where $n=9-10$. * $P<0.05$ versus the preceding period.

Figure 3 Effect of neuropeptide Y (NPY, $10^{-7} \text{ mol l}^{-1}$) upon $^{36}\text{Cl}^-$ unidirectional fluxes. Mucosa-to-serosa (ms; $\square$), serosa-to-mucosa (sm; $\blacksquare$), and net ($\bullet$) fluxes. The dashed lines represent the best fit line for Cl^- transport under control conditions. The inset shows the change in I_{sc} ($\circ$) induced by NPY in this series of experiments. Values are means $\pm$ s.e.mean, $n=8-9$.

Table 3 Effect of pretreatment with neuropeptide Y (NPY) on the response to secretagogues

	Distal		Proximal	
	Control	+ NPY ($\mu\text{Eq h}^{-1} \text{cm}^{-2}$)	Control	+ NPY
Carbachol peak	9.5 ± 1.2	8.7 ± 1.7	9.0 ± 1.2	6.3 ± 1.2
10 min	1.9 ± 0.5	2.5 ± 0.7	1.9 ± 1.1	2.3 ± 0.5
Veratridine peak	8.2 ± 0.7	10.5 ± 1.2	7.0 ± 0.9	4.9 ± 1.4
15 min	3.6 ± 0.5	5.1 ± 0.6	4.1 ± 0.6	3.2 ± 0.6
VIP	2.8 ± 0.4	2.9 ± 0.3	4.4 ± 0.3	5.0 ± 0.7

Increase in I_{sc} induced by carbachol ($5 \times 10^{-5} \text{ mol l}^{-1}$), veratridine ($5 \times 10^{-5} \text{ mol l}^{-1}$) and vasoactive intestinal polypeptide (VIP) ($10^{-7} \text{ mol l}^{-1}$) in the absence (control) and presence of NPY ($10^{-7} \text{ mol l}^{-1}$) in the distal (left) and the proximal (right) rat colon. Carbachol and veratridine induced a biphasic increase in I_{sc} , which are referred to as peak and then the response 10 or 15 min after administration of the drug, respectively. For VIP, which induced a monophasic increase in I_{sc} , only the peak response is presented. Values are means $\pm$ 1 s.e. mean, $n = 5-7$. There were no significant differences between the responses to secretagogues under control conditions and those in the presence of NPY in either colonic segment.

Figure 4 Effect of neuropeptide Y (NPY, $10^{-7} \text{ mol l}^{-1}$) after pretreatment of the mucosa with (a) prostaglandin E_2 ($10^{-6} \text{ mol l}^{-1}$), (b) veratridine ($5 \times 10^{-5} \text{ mol l}^{-1}$), or (c) forskolin ($5 \times 10^{-6} \text{ mol l}^{-1}$). In the case of the prostaglandin E_2 experiments, tissues were pretreated with indomethacin ($10^{-6} \text{ mol l}^{-1}$). Values are representative of between 6-9 experiments.

tridine ($5 \times 10^{-5} \text{ mol l}^{-1}$; Table 3). In order to establish whether NPY, which does not affect basal I_{sc} in the proximal colon (Figure 1a), may interfere with secretagogue-stimulated I_{sc} in the proximal colon, the experiments were repeated with this colonic segment. However, NPY proved to be completely ineffective (Table 3).

Because the missing effect of NPY pretreatment on these secretory responses might be due to the short duration of the

action of the peptide, the sequence of the protocol was reversed; i.e. the respective secretagogue was administered first and NPY was added, when the I_{sc} had risen to a stable elevated plateau. Instead of VIP, which induces only a transient increase in I_{sc} in the rat colon (Knobloch *et al.*, 1989), prostaglandin E_2 was used to induce a prolonged receptor-mediated stimulation of the cyclic AMP-pathway (Diener *et al.*, 1988). Tissues were pretreated with indomethacin ($10^{-6} \text{ mol l}^{-1}$) to enhance the sensitivity of the mucosa to exogenously applied prostaglandins (Bukhave & Rask-Madsen, 1980). Prostaglandin E_2 ($10^{-6} \text{ mol l}^{-1}$) caused an increase in I_{sc} by $2.9 \pm 0.3 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ in the distal colon ($n = 9$; $P < 0.05$). After pretreatment with prostaglandin E_2 , the response to a subsequent administration of NPY ($10^{-7} \text{ mol l}^{-1}$) was greatly enhanced. In the presence of prostaglandin E_2 , NPY induced a decrease in I_{sc} of $-2.0 \pm 0.3 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ ($n = 6$; $P < 0.05$) compared with NPY response under control conditions, Figure 4a). Also pretreatment of the mucosa with the stimulating neurotoxin, veratridine, enhanced the response to NPY. Veratridine ($5 \times 10^{-5} \text{ mol l}^{-1}$) caused an increase in I_{sc} in the distal colon to a stable plateau of $6.4 \pm 0.7 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ (Figure 4b; $n = 7$). Subsequent addition of NPY induced a decrease in I_{sc} of $-3.8 \pm 0.5 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ ($n = 7$; $P < 0.05$), a response, which was about 4 times greater than the response to NPY under control conditions ($P < 0.05$). Interestingly, the decrease in I_{sc} induced by NPY in the presence of these two secretagogues showed the same dependence on the height of the I_{sc} (○ and □ in Figure 2) as was observed for the control response to NPY (● in Figure 2) and all data could be fitted by a common regression line.

In contrast, when the cyclic AMP-pathway was stimulated by forskolin, the activator of the adenylate cyclase (Seamon & Daly, 1981), the response to NPY was unaffected. Forskolin ($5 \times 10^{-6} \text{ mol l}^{-1}$) induced an increase of I_{sc} of $3.2 \pm 1.0 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ (Figure 4c; $n = 5$; $P < 0.05$), and NPY administered during this plateau caused only a decrease in I_{sc} of $-0.8 \pm 0.7 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ ($n = 5$; not significantly different from the response to NPY under control conditions). Also pretreatment with the agonist of the Ca^{2+} -pathway, carbachol, did not enhance the response to NPY. Carbachol ($5 \times 10^{-5} \text{ mol l}^{-1}$; in the presence of TTX, $10^{-6} \text{ mol l}^{-1}$) caused an increase of I_{sc} to a stable plateau of $0.7 \pm 0.2 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ above baseline ($P < 0.05$; $n = 6$). Neuropeptide Y ($10^{-7} \text{ mol l}^{-1}$), administered during this plateau (about 25-35 min after administration of carbachol), induced a decrease of I_{sc} of $-0.7 \pm 0.2 \mu\text{Eq h}^{-1} \text{cm}^{-2}$ ($n = 6$), a response which was not significantly different from controls.

When the effect of NPY was tested in the proximal colon with the same protocol, i.e. administration of NPY ($10^{-7} \text{ mol l}^{-1}$) during the plateau phase of the secretagogue-induced rise in I_{sc} , the peptide had no effect on the responses to veratridine, prostaglandin E_2 or carbachol ($n = 5-6$ for each substance; data not shown).

Effect of NPY on K^+ transport

$^{86}\text{Rb}^+$ was used as a marker for K^+ (Foster *et al.*, 1984) in order to test, whether NPY (10^{-7} mol l^{-1}) had an effect on K^+ transport. The protocol (two consecutive periods of 3×5 min) was the same as that used for the determination of NPY effects on $^{36}\text{Cl}^-$ transport, i.e. transport rates under control conditions and in the presence of NPY were calculated by linear regression. Linear correlation coefficients (r) for all individual experiments were ≥ 0.93 . The ms-transport rate of $^{86}\text{Rb}^+$ amounted to 7.8 ± 1.9 nmol $\text{min}^{-1} \text{cm}^{-2}$ under control conditions (Figure 5). It increased to 9.5 ± 2.2 nmol $\text{min}^{-1} \text{cm}^{-2}$ in the presence of NPY ($n=10$; $P<0.05$). In parallel, the sm-transport of $^{86}\text{Rb}^+$ decreased from 7.3 ± 0.9 nmol $\text{min}^{-1} \text{cm}^{-2}$ to 5.8 ± 0.5 nmol $\text{min}^{-1} \text{cm}^{-2}$ ($n=9$); an effect which did not however reach statistical significance. The resultant was an increase in the calculated net transport rate for Rb^+ transport from 0.6 ± 0.2 nmol $\text{min}^{-1} \text{cm}^{-2}$ to 3.8 ± 0.4 nmol $\text{min}^{-1} \text{cm}^{-2}$ ($P<0.05$; analysis of co-variances).

Comparison of the proabsorptive effect of NPY on K^+ transport with somatostatin and vasopressin

The effect of NPY on Rb^+ (K^+) was compared with two other proabsorptive hormones, somatostatin and vasopressin. Because both hormones exhibit a long-lasting decrease in I_{sc} , a 2×20 min-flux protocol was used. Somatostatin (10^{-7} mol l^{-1}) caused a long-lasting decrease in I_{sc} and Gt (Table 4). $J_{\text{sm}}^{\text{Rb}}$ was significantly inhibited in the presence of the hormone, whereas $J_{\text{ms}}^{\text{Rb}}$ was not altered significantly. In contrast, vasopressin (10^{-9} mol l^{-1}), which caused a decrease in I_{sc} of similar amplitude, had no significant action on $J_{\text{sm}}^{\text{Rb}}$ or $J_{\text{ms}}^{\text{Rb}}$ (Table 4).

Figure 5 Effect of neuropeptide Y (NPY, 10^{-7} mol l^{-1}) upon $^{86}\text{Rb}^+$ unidirectional fluxes. Mucosa-to-serosa (ms; $\square$), serosa-to-mucosa (sm; $\blacksquare$), and net ($\bullet$) fluxes. The dashed lines represent the best fit line for Rb^+ transport under control conditions. Values are means $\pm$ 1 s.e. mean, $n=9-10$.

Discussion

NPY exerts a segment-specific effect on I_{sc} in the rat colon, i.e. it inhibits basal and stimulated secretory I_{sc} in the distal colon, but is ineffective in the proximal colon. It is not clear, whether a reduced receptor number or a different receptor-effector coupling is responsible for the observed segment specificity of the NPY response. The effect of NPY in the distal colon declined with a half time of 13.5 min (Figure 1b) and the effectiveness of a second peptide addition (Figure 1b, c) suggests that peptide degradation rather than desensitization of the receptor may be responsible for the transient nature of this effect. The decrease in I_{sc} induced by NPY was dependent on the secretory state of the mucosa. The effect of NPY was higher, when basal I_{sc} was high (Figure 2) and the peptide-induced decrease in I_{sc} was about 55% of the spontaneous I_{sc} . When basal anion secretion was blocked by addition of Cl^- channel blockers such as NPPB or anthracene-9-carboxylic acid, the effect of NPY was inhibited (Table 1). Interestingly, in anion substitution studies the response to NPY could only be significantly reduced when both Cl^- and HCO_3^- anions were removed from the buffer. Responses were virtually abolished by the carbonic anhydrase blocker, methazolamide. This is probably due to the fact that the apical anion channels, which are thought to mediate cyclic AMP-sensitive Cl^- secretion e.g. outward-rectifying Cl^- channel (Reinhardt *et al.*, 1987) or cystic fibrosis transmembrane regulator Cl^- channel (Poulsen *et al.*, 1994), are also partially permeable to HCO_3^- anions. Consequently, only the simultaneous omission of both anions from the buffer reduced basal I_{sc} to low values and inhibited the action of NPY (Table 1). This decrease in I_{sc} was associated with an increase in the absorptive, i.e. J_{ms} fluxes of Na^+ and Cl^- (and K^+ ; see below). Whereas the effect of NPY on $J_{\text{ms}}^{\text{Na}}$ could be demonstrated using a conventional 20 min flux period protocol, the effects of NPY on unidirectional Cl^- transport were transient and could only be shown using a short-period flux protocol; an observation made initially in the rat jejunum (Cox *et al.*, 1988). Here, $J_{\text{sm}}^{\text{Cl}}$ was also decreased in the presence of NPY (Figure 3) and an increase in the net Cl^- transport rate from 53 ± 6 nmol $\text{min}^{-1} \text{cm}^{-2}$ was observed (corresponding to an increase in net Cl^- transport of 6.8 ± 0.5 $\mu\text{Eq h}^{-1} \text{cm}^{-2}$). This increase in calculated net Cl^- absorption is much larger than the observed decrease in I_{sc} , which was reduced by NPY from a mean of 1.7 ± 0.2 $\mu\text{Eq h}^{-1} \text{cm}^{-2}$ during the control period to 0.7 ± 0.2 $\mu\text{Eq h}^{-1} \text{cm}^{-2}$ and then increased slowly again (insert of Figure 3). A similar discrepancy between calculated $J_{\text{net}}^{\text{Cl}}$ and I_{sc} has been observed in the rat jejunum (Cox *et al.*, 1988). Consequently, NPY must induce the movement of other ions which partially counteract the expected decrease in I_{sc} . In short, a significant component of NPY's effects upon Cl^- transport are a consequence of modulation of electroneutral transport.

Basal secretory I_{sc} is maintained in the rat distal colon by the spontaneous activity of submucosal neurones (Andres *et al.*, 1985) and by the spontaneous production of prostaglandins (see e.g. Diener *et al.*, 1988). Whereas indomethacin and tetrodotoxin, when given alone, did not affect the response to NPY, the combination of both abolished it (Table 1). When

Table 4 Effect of somatostatin and vasopressin on $^{86}\text{Rb}^+$ fluxes

	$J_{\text{ms}}^{\text{Rb}}$	$J_{\text{sm}}^{\text{Rb}}$	$J_{\text{net}}^{\text{Rb}}$	I_{sc}	Gt
		($\mu\text{Eq h}^{-1} \text{cm}^{-2}$)			(mS cm^{-2})
Control	0.91 ± 0.18	0.32 ± 0.05	0.59 ± 0.19	2.5 ± 0.3	8.5 ± 0.4
Somatostatin	0.84 ± 0.14	$0.22 \pm 0.04^*$	0.62 ± 0.15	$0.7 \pm 0.1^*$	$7.5 \pm 0.4^*$
Control	0.88 ± 0.18	0.38 ± 0.04	0.50 ± 0.18	2.8 ± 0.2	10.2 ± 0.5
Vasopressin	1.08 ± 0.23	0.32 ± 0.03	0.76 ± 0.23	$1.5 \pm 0.1^*$	$9.4 \pm 0.5^*$

The effect of somatostatin (10^{-7} mol l^{-1}) and vasopressin (10^{-9} mol l^{-1}) on mucosa-to-serosa (ms), serosa-to-mucosa (sm) and net fluxes during a 20 min-flux measurement. The I_{sc} and Gt values are means of the complete control or hormone period, respectively. Values are means $\pm$ 1 s.e. mean from 8–10 observations. * $P<0.05$ versus the preceding period.

baseline I_{sc} , which had been decreased by prior addition of indomethacin and tetrodotoxin, was elevated again by prostaglandin E_2 (10^{-6} mol l^{-1}), NPY was fully effective despite the presence of indomethacin and tetrodotoxin. Because prostaglandins (Craven & DeRubertis, 1981) and many neurotransmitters of the enteric nervous system such as VIP (Prieto *et al.*, 1979) act by stimulating adenylate cyclase in the intestinal epithelium, these results suggest that the continuous release of prostaglandins and neurotransmitters leads to a stimulation of basal cyclic AMP production in the colonic mucosa and the inhibition of basal I_{sc} by NPY is caused by an attenuation of this stimulated cyclic AMP-pathway (see below). In contrast, inhibitors of the lipooxygenase pathway were without effect on NPY responses. This differs from the effect of another proabsorptive hormone, somatostatin (Diener & Gartmann, 1994) and suggests that a swelling-induced basolateral Cl^- channel, the activation of which can be prevented by nordihydroguaiaretic acid or SK&F 104353, is not involved in the NPY effect.

In addition to the inhibition of basal anion secretion, NPY also inhibited stimulated Cl^- secretion in the distal colon. This inhibition could only be shown, when NPY was applied after the secretagogues (Figure 4), but not, when the tissue was pretreated with NPY (Table 3). This apparent absence of NPY inhibition is most probably explained by the short-lasting effect of the peptide (due to the assumed rapid degradation). The effect of NPY on I_{sc} was enhanced, when the tissue was pretreated with prostaglandin E_2 , which stimulates adenylate cyclase by a receptor-mediated mechanism (Smith *et al.*, 1987). The same was observed with veratridine and the action of this toxin on I_{sc} in the rat distal colon is suppressed by tetrodotoxin but not significantly affected by atropine (unpublished observations) and is therefore not a muscarinic-mediated effect. In contrast, NPY had no effect on the stimulation of I_{sc} by forskolin, a direct activator of the adenylate cyclase (Figure 4). These results suggest that NPY exerts its action at a step proximal to the generation of cyclic AMP. An inhibition of the adenylate cyclase mediated by a G_i -protein has indeed been shown in several tissues e.g. rat kidney (Hackenthal *et al.*, 1987) or rat cardiac myocytes (Kassis *et al.*, 1987). A similar pattern, i.e. a pertussis toxin-sensitive inhibition of the response to receptor stimulation of the cyclic AMP-pathway via VIP, but insensitivity of the response to direct stimulation of the adenylate cyclase via forskolin has been observed for somatostatin in the rat colon (Knobloch *et al.*, 1989). It is therefore tempting to speculate that the action of NPY on rat colonic anion secretion is mediated by an inhibition of adenylate cyclase as has been demonstrated in the rabbit large intestine (Flint *et al.*, 1990). In rat cardiac myocytes iso-

prealine stimulated adenylate cyclase responses were blocked by NPY but the response to forskolin was unaffected. In other tissues such as rat small intestine (Cox & Cuthbert, 1988), rabbit colon (Flint *et al.*, 1990) or porcine small intestine (Brown *et al.*, 1990) significant inhibition of the forskolin response by NPY has been observed. It is not clear, whether segment or species differences or other reasons are responsible for this discrepancy.

The effect of NPY after exogenously applied prostaglandin E_2 and following the release of endogenous neurotransmitters stimulated by veratridine, showed a dependence on the level of I_{sc} as seen with NPY effects on basal I_{sc} (Figure 2). This suggests (but does not prove) that both actions may be mediated by a common pathway, i.e. inhibition of the adenylate cyclase, e.g. mediated by a G_i -protein. In contrast, NPY had no effect upon secretion stimulated by carbachol, i.e. an agent acting via intracellular Ca^{2+} . Carbachol causes the opening of a (basolateral) K^+ conductance (Böhme *et al.*, 1991), which indirectly stimulates Cl^- secretion via apical Cl^- channels normally stimulated by cyclic AMP in this epithelium (Strabel & Diener, 1995). The absence of an effect of NPY on the carbachol response differs from that observed in the rat small intestine, where NPY also inhibits Ca^{2+} -mediated secretion (Cox & Cuthbert, 1988).

Unexpectedly, NPY also increased K^+ absorption measured using $^{86}Rb^+$ as marker for K^+ . In the presence of NPY, the ms-transport rate for Rb^+ increased significantly, whereas a parallel decrease in the sm-transport did not reach statistical significance. An increase in Rb^+ absorption was also observed in the presence of another antisecretory peptide, somatostatin, although the effect of this hormone was exclusively due to an inhibition of J^{Rb}_{sm} . In contrast, vasopressin, an antisecretory peptide, which only inhibits Ca^{2+} - but not cyclic AMP-mediated secretion (Knobloch *et al.*, 1989), was without effect on unidirectional and net Rb^+ transport (Table 4). Up to now, only the opposite, i.e. a stimulation of K^+ secretion by cyclic AMP (Foster *et al.*, 1983) or agents, which increase intracellular cyclic AMP like β -receptor agonists (Smith & McCabe, 1986) or endothelin-1, which causes a release of prostaglandin E_2 (Roden *et al.*, 1992), have been shown. This suggests that transmitters of the enteric nervous system like NPY or somatostatin may be involved in the regulation of K^+ transport across the colonic wall, possibly mediated by a decrease in the intracellular cyclic AMP-concentration.

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