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Neuropeptide Y-induced contraction is mediated by neuropeptide Y Y_2 and Y_4 receptors in the rat colon

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Abstract

Ascending and descending segments of the rat colon were studied to analyze their contractile responses to neuropeptide Y and related peptides. These responses are (a) completely eliminated by tetrodotoxin (1 μM), (b) reduced to a variable extent (20 to 60%) by atropine (1 μM) and (c) not modified by indomethacin, diphenhydramine or methysergide. The order of potency of agonists for peptides related to neuropeptide Y was as follows: human pancreatic polypeptide = rat pancreatic polypeptide > peptide YY = peptide YY-(3–36) = [Leu^{31},Pro^{34}]neuropeptide Y > neuropeptide Y-(2–36) > C2-neuropeptide Y = neuropeptide Y > neuropeptide Y-(13–36), with minor differences observed between the two parts of the colon. This selectivity pattern does not correspond to the profile of any known cloned neuropeptide Y receptors. BIBP3226, a selective antagonist for the neuropeptide Y Y_1 receptor sub-type, was found to be inactive, while a neuropeptide Y Y_2 receptor antagonist, T_4-[NPY-(33–36)]$_4$, reduced the effects of neuropeptide Y, peptide YY, peptide YY-(3–36) and C2-neuropeptide Y without affecting those of human pancreatic polypeptide, rat pancreatic polypeptide and [Leu^{31},Pro^{34}]neuropeptide Y. JCF 104 (compound 28), a putative neuropeptide Y Y_5 receptor antagonist, showed no effect or a weak inhibition of human pancreatic polypeptide or [Leu^{31},Pro^{34}]neuropeptide Y-induced contraction. Taken together, these data suggest that: (1) at least two neuropeptide Y receptor types are present in the rat colon autonomic nerve terminals and modulate the release of acetylcholine and possibly other transmitters; (2) a proportion of the receptors mediating the contractile response of the rat colon (especially descending part) to neuropeptide Y and related peptides appears to be of the Y_2 type and (3) the significant portion of the response is mediated by a receptor which is insensitive to neuropeptide Y Y_1, Y_2 and to neuropeptide Y Y_5 receptor antagonists. This receptor behaves as a neuropeptide Y Y_4 receptor sub-type and appears to be located on enteric nerves.

Keywords: Neuropeptide Y; Neuropeptide Y receptor type; Gastrointestinal tract

1. Introduction

The pancreatic polypeptides, peptide YY and neuropeptide Y are homologues of 36 amino acids that were originally identified in pancreatic (Kimmel et al., 1975), and intestinal and brain extracts (Tatemoto and Mutt, 1980; Tatemoto, 1982). Peptide YY and the pancreatic polypeptides are mainly present in endocrine cells of the intestine and the pancreas while neuropeptide Y is expressed almost exclusively in the central and peripheral nervous systems. These peptides exert multiple biological effects by acting on different receptor sub-types (for reviews see Hazelwood, 1993; Gehlert, 1998). It was originally suggested that peptide YY and neuropeptide Y act on the same neuropeptide Y receptor sub-types, namely Y_1 and Y_2 (Wahlestedt et al., 1986). The receptors for the pancreatic polypeptides were not clearly identified until recently when new classes of neuropeptide Y G-protein-coupled receptors were cloned (Bard et al., 1995; Gerald et al., 1996) and found to be different from the better known neuropeptide Y_1 and Y_2 receptor sub-types. Their pharmacological characterization led to their classification as neuropeptide Y Y_4 and Y_5 receptors on the basis of the high potencies of the pancreatic polypeptides, especially

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human pancreatic polypeptide and lack of blocking activity of the neuropeptide Y Y_1 receptor antagonist BIBP 3226 (Rudolf et al., 1994; Gerald et al., 1996). Interestingly, while the neuropeptide Y Y_4 receptor sub-type cannot distinguish between rat and human versions of pancreatic polypeptides, the neuropeptide Y Y_5 receptor can with rat pancreatic polypeptide being significantly less potent than the human homologue. The pharmacological characteristics of neuropeptide Y Y_1 and Y_2 receptors have been extensively studied (Wahlestedt et al., 1986; Holst et al., 1996) while those of neuropeptide Y Y_4 and Y_5 receptors are still largely unknown due to the lack of specific antagonists and simple in vitro tissue preparations.

Several studies have shown that pancreatic polypeptides, neuropeptide Y and peptide YY are distributed along the gastrointestinal tract (Holst et al., 1996) and one of their physiological role is considered to be the inhibition of gastrointestinal functions (Hazelwood, 1993; Larhammar, 1993). In the present study, we examined the biological activities of pancreatic polypeptides, peptide YY, neuropeptide Y and related peptides in vitro in the rat colon in an attempt to identify and characterize the neuropeptide Y receptor sub-type(s) mediating the contractile responses. The rat colon has already been described (Cadieux et al., 1990) as a tissue enriched in neuropeptide Y Y_3 'neuropeptide Y' preferential receptor (Dumont et al., 1993; Jacques et al., 1995) before data became available on the cloning of the neuropeptide Y Y_4 and Y_5 receptors (Michel et al., 1998). Our results suggest that the rat colon is enriched with both the neuropeptide Y Y_2 and Y_4 receptor sub-types.

2. Methods

2.1. Animals

Sprague–Dawley rats of either sex (250–300 g) were killed according to the guidelines of the Canadian Council for Animal Care. Segments (1–3 cm) of the colon (ascending, descending) were immediately removed and placed in a Tyrode solution of the following composition (mM): NaCl (136.8); KCl (2.6); $MgSO_4 \cdot 7H_2O$ (1.05); NaH_2PO_4 (0.47); $NaHCO_3$ (11.9); $CaCl_2$ (2.0) and glucose (5.5). The tissues were cleared of intestinal content, attached with thread so as to leave the lumen open to the bathing solution and suspended in organ baths (10 ml) containing oxygenated (95% O_2 and 5% CO_2) Tyrode solution maintained at 37°C. The segments were stretched to a resting tension of 1 g and allowed to equilibrate in the organ bath for 60 min before starting the experiment. The changes of tension were isometrically recorded with Grass (FT 03C) transducers and displayed on a Grass (Model 7D) polygraph.

Under these experimental conditions, the preparations show marked spontaneous activity which however is rather stable along the 6–8 h of the experiment and interferes very little with the measurement of the contractile effect. However, tetrodotoxin increases spontaneous activity to such an extent as to make difficult the quantitative evaluation of contractile responses.

2.2. Bioassays

Concentration–response curves to neuropeptide Y related peptides were obtained by adding increasing concentrations of each peptide to the organ bath at intervals of 20–30 min. In some experiments, to characterize the response of the colon to neuropeptide Y and related peptides, the tissues were incubated with one of the following agents: atropine, tetrodotoxin, diphenhydramine, indomethacin or methysergide (all at 10^{-6} M) for 15 min before injecting neuropeptide Y related peptides. Neuropeptide Y receptor antagonists (BIBP 3226, neuropeptide Y Y_1 receptor antagonist; T_4-[NPY-(33–36)]$_4$, neuropeptide Y Y_2 receptor antagonist; or JCF 104, a neuropeptide Y Y_5 receptor antagonist) were also added to the organ baths 10–15 min before repeating, in their presence, the recording of the concentration–response curves of the preferential agonists (peptide YY-(3–36) for neuropeptide Y Y_2 receptor and [Leu^{31},Pro^{34}]neuropeptide Y for neuropeptide Y Y_1, Y_4 and Y_5 receptor sub-types). The contraction induced by agonists is defined as the increase of the tone (after the injection of the agonist) but not the amplitude of the basal spontaneous activities.

2.3. Drugs

Human neuropeptide Y and related peptides, porcine peptide YY and its fragments were provided by A. Fournier (INRS-Santé, Montréal, Québec, Canada). Human and rat pancreatic polypeptide were purchased from Bachem (Torrance, CA, USA). BIBP3226 was supplied by Karl Thomae (Biberach, Germany). JCF 104 (compound 28, Gerald et al., 1996) was provided by Dr. J.L Fauchere, (Servier, Suresnes, France). T_4-[NPY-(33–36)]$_4$ was purchased from E. Grouzmann (Switzerland). Atropine, carbamylcholine, diphenhydramine, indomethacin or methysergide were obtained from Sigma (St. Louis, MO, USA). Tetrodotoxin was purchased from ICN (Costa-Mesa, CA, USA). All compounds, except indomethacin, were dissolved in bidistilled water and kept in concentrated solutions (1 mg/ml) at −20°C until use. Fresh solutions of indomethacin were prepared before each experiment in Trisma base.

2.4. Data analysis and terminology

All data are expressed as means ± standard error of the mean. Data have been statistically analyzed using the

Student two-tailed *t*-test via a software package (Tallarida and Murray, 1987). p values less than 0.05 were considered to be significant.

The pharmacological terminology adopted in this paper is consistent with the recent IUPHAR recommendation (Jenkinson et al., 1995; Vanhoutte et al., 1996). The agonist apparent affinities are given as EC_{50} which is the molar concentration of an agonist that produces 50% of the maximal response. Antagonist affinities have been evaluated according to Arunlakshana and Schild (1959) and are given in terms of pA_2, which is the $-\log$ of molar concentration of an antagonist that is necessary to double the concentration of agonist needed to elicit the original submaximal response (Schild, 1947; Jenkinson et al., 1995). α^E is intrinsic activities expressed as a fraction of the maximal responses induced by human pancreatic polypeptide or by peptides YY.

3. Results

Neuropeptide Y and its homologues evoked strong contractions of the rat ascending and descending colon. Fig. 1 shows tracings illustrating these effects. In the transverse colon, the contractions induced by these peptides were weak and due to strong interference by the spontaneous activity (not shown), this tissue was not used further. Treatment with atropine (10^{-6} M), by itself, decreased the basal tone of the tissues and exerted a variable inhibition (20–60%) of the effect of neuropeptide Y and peptide YY (Fig. 1) suggesting that acetylcholine is involved in mediating the contractile effect of neuropeptide Y and related peptides. Carbamylcholine induced a strong contraction which was abolished, as expected, by atropine (10^{-6} M) (not shown). These data suggest that non-adrenergic–non-cholinergic mechanisms are partially responsible for the effect of neuropeptide Y and predominantly mediated the effect of human pancreatic polypeptide. The actions of neuropeptide Y and related peptides ($n = 3$) were not influenced by indomethacin, diphenhydramine and methysergide (results not shown). Tetrodotoxin (10^{-6} M) by itself, increased the spontaneous activity of the tissues (Fig. 1). In its presence, however, peptide YY and human pancreatic polypeptide did not induce further contraction while carbamylcholine was still active (Fig. 1). The neuropeptide Y Y_2 receptor agonists, C2-neuropeptide Y and peptide YY-(3–36), as well as [Leu^{31},Pro^{34}]neuropeptide Y (a neuropeptide Y Y_1, Y_4 and Y_5 receptor agonist) induced concentration-dependent contractions of the rat colon. The effects of peptide YY-(3–36) and C2-neuropeptide Y (not shown) were partially blocked by a neu-

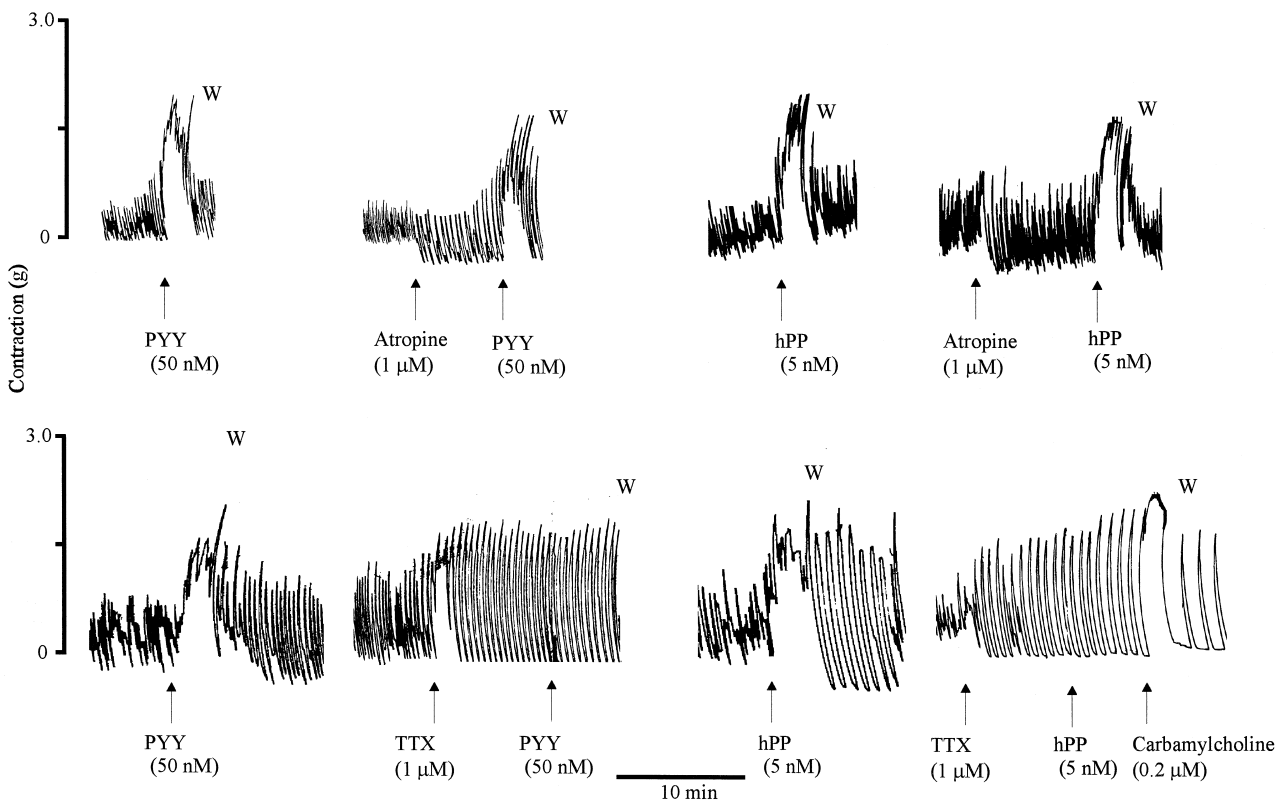

Fig. 1. Contractile effects of human pancreatic polypeptide (hPP) and peptide YY on the rat ascending colon in the absence and presence of atropine and tetrodotoxin (TTX). W: washout. Ordinate: contraction in g. Abscissa: time in min.

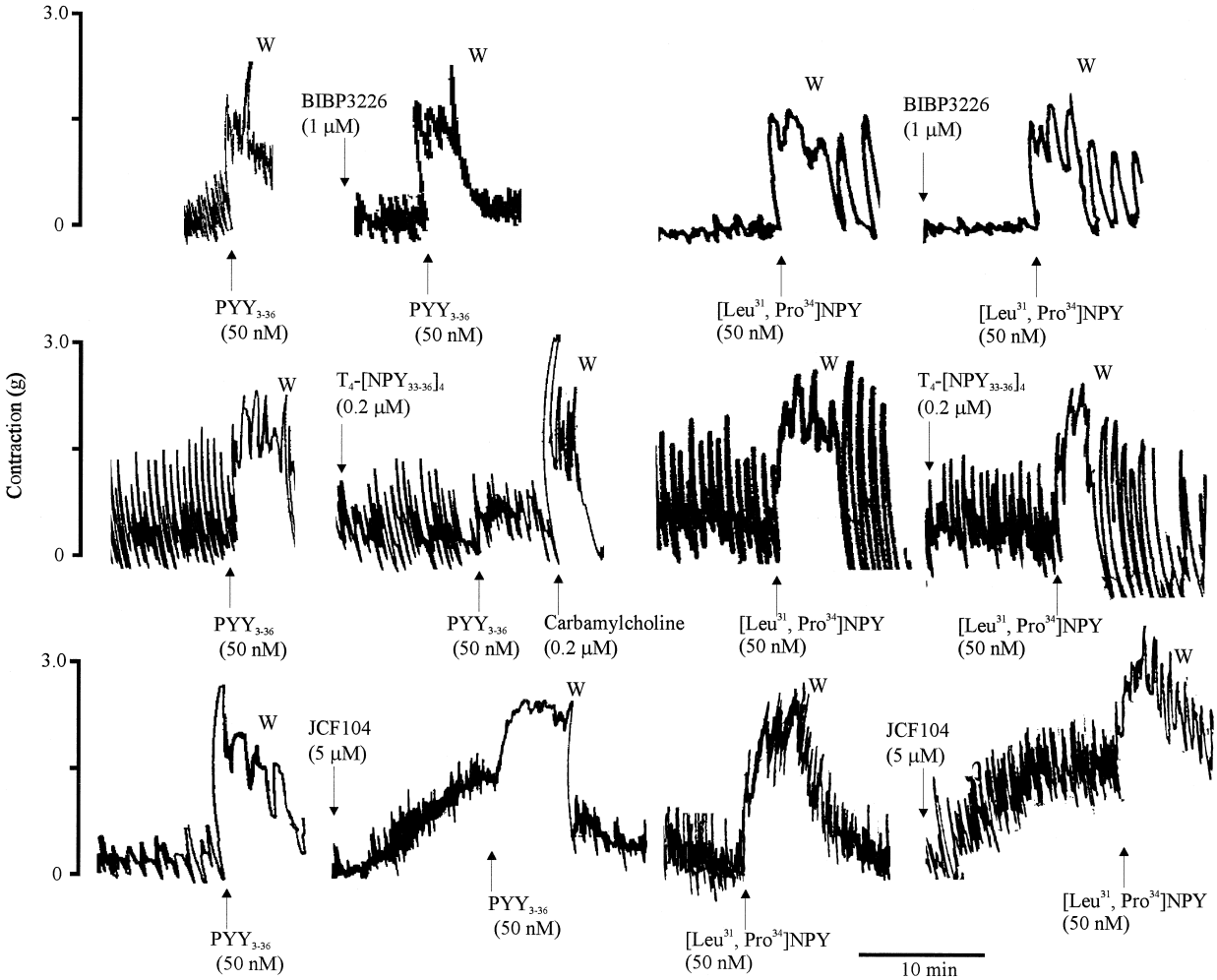

Fig. 2. Contractile effects of peptide YY-(3–36) (PYY_{3-36}) and [Leu^{31},Pro^{34}]neuropeptide Y on the rat ascending colon in the absence or the presence of neuropeptide Y antagonists, BIBP3226, T_4-[NPY-(33–36)]$_4$ and JCF104. W: washout. Ordinate: contraction in g. Abscissa: time in min.

ropeptide Y Y_2 receptor selective antagonist, T_4-[NPY-(33–36)]$_4$ (Grouzmann et al., 1997), while those of [Leu^{31},Pro^{34}]neuropeptide Y were not modified (Fig. 2, Table 2) suggesting the presence of two distinct popula-

Table 1
Pharmacological characterization of rat colon contractile responses to neuropeptide Y and related peptides

Agonists	Ascending			Descending		
	EC_{50} (nM)	R.P. (%)	α^E	EC_{50} (nM)	R.P. (%)	α^E
Human pancreatic polypeptide	1.6 ± 0.9	100	1.0	0.7 ± 0.1	100	1.0
Rat pancreatic polypeptide	0.5 ± 0.2	320	1.1	1.9 ± 0.7	37	0.9
Human Neuropeptide Y	24.9 ± 9.7	6	0.8	76.0 ± 11.6	1	0.8
Peptide YY	1.7 ± 0.5	94	1.0	10.0 ± 3.7	7	1.0
[Leu^{31},Pro^{34}]neuropeptide Y	5.6 ± 2.1	29	1.0	8.6 ± 0.61	8	1.0
Neuropeptide Y-(2–36)	15.0 ± 4.9	11	1.0	9.0 ± 4.8	8	1.0
Neuropeptide Y-(13–36)	> 1000	> 1	0.8	47.0 ± 9.5	1	0.5
Peptide YY-(3–36)	1.8 ± 0.2	89	1.0	3.3 ± 1.8	21	1.0
C2-Neuropeptide Y	77 ± 0.7	2	1.0	23.0 ± 9.4	3	1.0
[D-Trp^{32}]neuropeptide Y	> 1000	> 1		> 1000	> 1	–

EC_{50} represents the concentration of agonists inducing 50% of the maximal response (n = 3–6). R.P., relative potency expressed as percent of EC_{50} of the reference (human pancreatic polypeptide). α^E, intrinsic activities expressed as a fraction of the maximal responses induced by human pancreatic polypeptide or by peptide YY.

Table 2
Pharmacological characterization of rat colon contractile responses to neuropeptide Y and related peptides using antagonists

Antagonist	pA_2			
	Ascending		Descending	
	[Leu^{31},Pro^{34}]Neuropeptide Y[a]	Peptide YY-(3–36)[b]	[Leu^{31},Pro^{34}]Neuropeptide Y	Peptide YY-(3–36)
BIBP3226 (Y_1)	I	I	I	I
T_4-[NPY-(33–36)]$_4$ (Y_2)	I	6.7	I	6.3
JCF104 (Y_5)	< 5.3	< 5.3	< 5.3	< 5.3

[a] Neuropeptide Y Y_1, Y_4 and Y_5 receptor agonist; [b] neuropeptide Y Y_2 receptor agonist.
pA_2: the concentration of antagonist that is needed to reduce the effect of a double concentration of agonist to that of single concentration. I: inactive at 10 μM.

tions of receptors. JCF 104 (5 μM), which has been reported as a neuropeptide Y Y_5 receptor antagonist by one group of investigators (Gerald, 1996) induced an increase of the basal tone of the colon and altered non-significantly the contractions induced by either human pancreatic polypeptide (not shown), peptide YY-(3–36) or [Leu^{31},Pro^{34}]neuropeptide Y (Fig. 2). BIBP3226 (1 μM), a neuropeptide Y Y_1 receptor selective antagonist was inactive to block the contractions induced by either human pancreatic polypeptide (not shown), peptide YY-(3–36), [Leu^{31},Pro^{34}]neuropeptide Y ($n = 3$–5) (Fig. 2, Table 2).

Table 1 summarizes the results obtained with several neuropeptide Y-related peptides in rat ascending and descending colon. The order of potency of agonists in these two segments of the rat colon shows that the most active homologues are human pancreatic polypeptide and rat pancreatic polypeptide followed by peptide YY and neuropeptide Y. All peptides, except for neuropeptide Y and neuropeptide Y-(13–36), appear to exert full agonistic activity but with different affinities. Peptide YY-(3–36) and [Leu^{31},Pro^{34}]neuropeptide Y were almost as potent as peptide YY. Neuropeptide Y-(13–36) is a weak agonist especially in the ascending colon. The truncated analogue, C2-neuropeptide Y, a selective Y_2 receptor agonist (McLean et al., 1990) was more potent than neuropeptide Y-(13–36) but significantly weaker than peptide YY-(3–36) (Table 1). [D-Trp^{32}] neuropeptide Y (1 μM), a putative neuropeptide Y Y_5 receptor agonist (Gerald, 1996) was inactive.

In an attempt to provide further evidence for neuropeptide Y receptor sub-types involved in the contraction of the rat colon, some neuropeptide Y receptor antagonists were tested. Results summarized in Table 2 indicate that BIBP 3226 (neuropeptide Y Y_1 receptor antagonist) and the neuropeptide Y Y_2 receptor antagonist were inactive against [Leu^{31},Pro^{34}]neuropeptide Y-induced contractions. They were also inactive against human pancreatic polypeptide and rat pancreatic polypeptide. In contrast, the neuropeptide Y Y_2 receptor antagonist (10^{-6} M) inhibited the contractile effects of human neuropeptide Y, peptide YY, C2-neuropeptide Y (not shown) and peptide YY-(3–36) (Fig. 2, Table 2). JCF 104, a weak neuropeptide Y Y_5 receptor antagonist (5 μM) reported by Gerald (1996) had some agonistic effect on its own (Fig. 2) and was only weakly effective against human pancreatic polypeptide (p$A_2 < 5.0$). In addition, JCF 104 (5 μM, $n = 5$) failed to block the effects of human neuropeptide Y and peptide YY (50 nM) (not shown).

4. Discussion

Our results suggest that two different functional neuropeptide Y receptor sub-types are present in the rat colon to mediate the contractions induced by neuropeptide Y and related peptides. The first sub-type is sensitive to peptide YY and neuropeptide Y, with peptide YY being more potent than neuropeptide Y in the two segments of the colon studied here. These effects are blocked by a selective neuropeptide Y Y_2 receptor antagonist. The neuropeptide Y Y_2 receptor sub-type is thus involved and is likely located on the parasympathetic nerve terminals where it mediates the release of acetylcholine, since the effects of peptide YY and neuropeptide Y are significantly reduced in the presence of atropine. Moreover, peptide YY and neuropeptide Y appear to be acting mostly via the neuropeptide Y Y_2 receptor sub-type since their effects are attenuated by the weak neuropeptide Y Y_2 receptor antagonist; a similar finding being obtained with the preferential neuropeptide Y Y_2 receptor agonist, C2-neuropeptide Y.

The second receptor is preferentially activated by human pancreatic polypeptide, rat pancreatic polypeptide and [Leu^{31},Pro^{34}]neuropeptide Y, those effects being insensitive to BIBP3226 (neuropeptide Y Y_1 receptor antagonist) and the neuropeptide Y Y_2 receptor antagonist excluding the involvement of the neuropeptide Y Y_1 and Y_2 receptors. The high potency of pancreatic polypeptides also exclude the participation of a putative 'neuropeptide Y Y_3' receptor (see Balasubramaniam et al., 1990; Wahlestedt et al., 1992, but not cloned yet) pointing toward a possible role for the neuropeptide Y Y_4 and/or Y_5 receptors (Gerald

et al., 1996; Hu et al., 1996). To discriminate between these two receptor classes, we first used the neuropeptide Y Y_5 receptor antagonist JCF104 (or compound 28) (Gerald, 1996) which has been shown to reduce significantly the effect of human pancreatic polypeptide in the rabbit ileum (Pheng et al., 1997). At high concentration (5 μM), JCF 104 exerted, by itself, a weak contractile effect in the rat colon but mostly failed to significantly reduce the action of neuropeptide Y and homologues. Moreover, rat pancreatic polypeptide and human pancreatic polypeptide demonstrated similar EC_{50}s to induce the contraction of the rat colon, different values being expected if a neuropeptide Y Y_5 receptor sub-type was involved (Gerald et al., 1996). Taken together, these data suggest that the response of the rat colon to human pancreatic polypeptide and rat pancreatic polypeptide is mediated by a neuropeptide Y Y_4 receptor sub-type.

In conclusion, the contraction of the rat colon in response to neuropeptide Y and related peptides likely results from the activation of two neuropeptide Y-related receptors, the neuropeptide Y Y_2 and Y_4 receptor sub-types. The presence of two functional receptors inducing the same effect could explain the unusual pharmacological profile observed earlier leading to the classification of the rat colon as a neuropeptide Y Y_3 receptor in vitro bioassay (Cadieux et al., 1990; Dumont et al., 1993; Jacques et al., 1995). The most recent availability of putative neuropeptide Y Y_2 and Y_5 receptor antagonists and the cloning of neuropeptide Y Y_4 and Y_5 receptors prove most useful to provide more definite evidence as to the classes of neuropeptide Y receptors that may be present in the rat colon.

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