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# Differential mechanism of peptide YY and neuropeptide Y in inhibiting motility of guinea-pig colon

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**Abstract**

The effect of peptide YY on contractility, acetylcholine release and noradrenaline release was examined in the isolated guinea-pig colon, and findings were compared with those for neuropeptide Y. Peptide YY and neuropeptide Y inhibited the twitch contractions mediated by the stimulation of cholinergic neurons. Peptide YY, neuropeptide Y, [Leu$^{31}$,Pro$^{34}$]neuropeptide Y and neuropeptide Y-(13–36) inhibited the electrically stimulated release of acetylcholine. Neuropeptide Y, but not peptide YY, inhibited the high $K^+$-stimulated tetrodotoxin-resistant release of acetylcholine, while the inhibitory effect of neuropeptide Y disappeared after treatment with yohimbine. Neuropeptide Y, but not peptide YY or neuropeptide Y analogues, evoked the release of noradrenaline. After desensitization to the effects of neuropeptide Y, peptide YY inhibited electrically stimulated acetylcholine release. Thus, peptide YY inhibits acetylcholine release through stimulation of a receptor, distinct from the site of action of neuropeptide Y, located on cholinergic neurons as well as the neuropeptide Y $Y_1$ and $Y_2$ receptors in the guinea-pig colon. Neuropeptide Y inhibits acetylcholine release due to the noradrenaline release mediated by stimulation of a receptor distinct from neuropeptide Y $Y_1$ and $Y_2$ receptors, located on adrenergic neurons.

*Keywords:* Acetylcholine release; Noradrenaline release; Neuropeptide Y $Y_1$ receptor; Neuropeptide Y $Y_2$ receptor

## 1. Introduction

Neuropeptide Y, a 36-amino acid peptide of the pancreatic polypeptide family, is an abundant and widely distributed neuropeptide in central and peripheral neurons (Sundler et al., 1983; O'Donohue et al., 1985; Allen and Bloom, 1986; DeQuidt and Emson, 1986; Gray and Morley, 1986; Dumont et al., 1992), whereas the structurally related peptide, peptide YY, is a gut hormone present in endocrine cells in the lower small intestine and colon (Tatemoto, 1982; Adrian et al., 1985; O'Donohue et al., 1985; Taylor, 1985; Greeley et al., 1987; Roddy et al., 1987; Dumont et al., 1992). Both neuropeptide Y and peptide YY released locally may serve paracrine, autocrine or neurotransmitter functions. These peptides inhibit the intestinal motility of cat, dog and guinea-pig (Lundberg et al., 1982; Hellström et al., 1985; Wiley and Owyang, 1985,1987; Baba et al., 1990; Krantis and Harding, 1991; Dumont et al., 1992; Takahashi et al., 1992); however, in rat intestine the response to neuropeptide Y or peptide YY varies dependent on the concentrations of the peptide and the part of intestinine. Peptide YY produces contraction of duodenum, relaxation of ileum, and contraction and relaxation of jejunum (Krantis et al., 1988), and both neuropeptide Y and peptide YY produce contraction of colon (Cadieux et al., 1990). These effects appear to be related to a modification of neurotransmitter release from myenteric neurons rather than by a direct effect on intestinal smooth muscle (Lundberg et al., 1982; Hellström et al., 1985; Wiley and Owyang, 1987; Krantis et al., 1988; Cadieux et al., 1990; Krantis and Harding, 1991; Takahashi et al., 1992). The mechanism underlying the inhibitory effects of neuropeptide Y on colonic motility may be stimulation of noradrenaline release from sympathetic nerves which, in turn, inhibits the release of acetylcholine from the cholinergic nerve in the guinea-

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pig (Wiley and Owyang, 1987). Peptide YY does not appear to inhibit acetylcholine release through adrenergic neurons in the guinea-pig stomach (Wiley et al., 1991). To elucidate the physiological role of peptide YY in colonic motility, we examined the sites and types of receptor for peptide YY in the guinea-pig colon by measuring colonic motility and acetylcholine release, and findings were compared with those for neuropeptide Y.

## 2. Materials and methods

Adult guinea-pigs of either sex (300–350 g) were killed by cervical dislocation. Strips of colon approximately 2-cm long were removed from a region 10–18 cm distal from the ileo-colonic junction.

### 2.1. Measurements of mechanical activity

The strips of colon were placed in a 20-ml organ bath in the presence of Krebs-Ringer solution of the following composition (in mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.19, $NaHCO_3$ 25.0, $KH_2PO_4$ 1.18 and glucose 11, which was continuously gassed with 95% $O_2$ and 5% $CO_2$ and maintained at 34–36° C and pH 7.4. Mechanical activity was recorded by means of an isometric transducer (Nihon Kohden, Japan, SD-1T). Approximately 500 mg of resting tension was applied and was kept constant by re-adjustment during the equilibration period. Two parallel platinum electrodes were used to stimulate intramural nerves of the strip positioned between these two electrodes. The strips produced contractile responses to electrical transmural stimulation (1 ms pulse duration, 15 V intensity, at a frequency of 0.1 Hz). After stabilization of the stimulated-evoked contraction, peptide YY or neuropeptide Y was added cumulatively to the organ bath during stimulation. Each concentration was added to the organ bath when the maximum effect of the previous concentration had been reached. The increase and decrease by agents of the stimulation-evoked contraction were represented as a percent increase over and percent decrease under the stimulation-evoked contraction in the absence of agents, respectively.

### 2.2. Measurement of the release of [$^3$H]acetylcholine and [$^3$H]noradrenaline

The methods used have been described previously (Nakayama et al., 1990). Briefly the colonic strips were incubated with $2 \times 10^{-7}$ M [$^3$H]choline or $5 \times 10^{-8}$ M [$^3$H]noradrenaline for 60 min in Krebs-Ringer solution. After being washed in fresh Krebs-Ringer solution for 30 min, the preparations were mounted in the apparatus and superfused at 1.2 ml/min with the same solution gassed with 95% $O_2$ and 5% $CO_2$, maintained at 34–36° C and pH 7.4. Experiments were started 60 min after the spontaneous release of tritium had approached a plateau. The superfusates were collected every 1 min, and the radioactivity was counted in a liquid scintillation counter. The $Ca^{2+}$-free medium was Krebs-Ringer solution from which $CaCl_2$ was omitted and which contained 1 mM EGTA. For experiments on [$^3$H]acetylcholine release, Krebs-Ringer solution containing hemicholinium-3, to prevent the uptake of choline formed from acetylcholine, was used for superfusion. For [$^3$H]noradrenaline release, the incubation and perfusion media were Krebs-Ringer solution containing $10^{-5}$ M ascorbate and $10^{-4}$ M pargyline.

The proportion of unchanged [$^3$H]acetylcholine and [$^3$H]noradrenaline to the total tritium content in the superfusates was estimated as follows. Superfusates were collected immediately before and during various stimulations at 60 min after the start of the superfusion. Extraction and separation of [$^3$H]choline and [$^3$H]acetylcholine were carried out according to the method of Potter and Murphy (1967). Aliquots (1 ml) of the superfusates were collected in 1 ml of 3-heptanone-tetraphenylboron (10 mg/ml) on ice. [$^3$H]Choline and [$^3$H]acetylcholine were extracted with 1 N HCl, dried and dissolved in 1 N formic acid/acetone (15:85, v/v). The samples were then subjected to electrophoresis at a constant voltage (200 V) for 1 h on Whatman-3 chromatography paper. Separated substances were stained by iodine vapor, the recovered radioactive compounds were extracted with 0.5 ml of ethanol, and the radioactivity was measured in a liquid scintillation spectrometer. When the recovery of added [$^3$H]acetylcholine was measured after electrophoresis, at least 96% of applied [$^3$H]acetylcholine was recovered. Over 81.8% of the total radioactivity released from electrically stimulated preparations proved to be [$^3$H]acetylcholine and the total radioactivity in the superfusates from the stimulated preparation was considered to approximate the amount of [$^3$H]acetylcholine, and therefore was denoted as [$^3$H]acetylcholine release.

When electrical stimulation was applied successively 4 times to the preparation at 30 min intervals, the stimulation-evoked release of [$^3$H]acetylcholine markedly decreased or increased from the first to second stimulation period, whereas there were no significant differences between the release evoked by the second to the fourth stimulations. Therefore, the release of [$^3$H]acetylcholine evoked by the first stimulation was disregarded and the release evoked by the second stimulation served as control.

The proportion of unchanged [$^3$H]noradrenaline to total tritium in the superfusate was determined by the method of Fujiwara et al. (1984). As carrier, 10 ng of unlabeled noradrenaline was added to each sample, and noradrenaline in the sample was absorbed with

activated alumina and identified by high-performance liquid chromatography (HPLC) with electrochemical detection (Yanaco). Fractions containing noradrenaline were collected from the outflow of the HPLC, and the radioactivity in these fractions was determined by liquid scintillation spectrometry. The proportion of [$^3$H]noradrenaline to the total $^3$H efflux in the superfusates collected during application of peptide YY or neuropeptide Y exceeded 85.8% ($n = 6$), respectively. The total radioactivity in the superfusates from the stimulated preparation was considered to approximate the amount of [$^3$H]noradrenaline, and therefore was denoted as [$^3$H]noradrenaline release.

### 2.3. Calculations of the release of [$^3$H]acetylcholine and [$^3$H]noradrenaline

At the end of the release experiments, the radioactivity of the tissue dissolved in Soluene was counted in a liquid scintillation spectrometer. The release of tritium was represented as the fractional rate, obtained by dividing the amount of tritium in the superfusate by the respective amount of tritium in the tissue. The tritium content of the tissue at each period was calculated by adding cumulatively each fractional tritium efflux to the tritium content of the tissue at the end of the experiment. From each of the release curves obtained by plotting the fractional release of tritium against time, the peak release of tritium evoked by stimulation in each condition was calculated as the percentage increase over the basal release. Data were analyzed by Wilcoxon's signed rank test, and a $P$ value $< 0.05$ was considered statistically significant.

### 2.4. Drugs and chemicals

The substances used were as follows: [$^3$H]noradrenaline (40.8 Ci/mmol) and [$^3$H]choline (60 Ci/mmol) (New England Nuclear, Boston, MA, USA), neuropeptide Y, peptide YY, [Leu$^{31}$,Pro$^{34}$]neuropeptide Y, neuropeptide Y-(13–36) and acetyl-[3-(2-6-dichlorobenzyl)-Try$^{27,36}$,D-Thr$^{32}$]neuropeptide Y-(27–36) (PYX-2) (Peninsula, Belmont, CF, USA), yohimbine, pargyline, hemicholinium-3 and ethylene glycol bis($\beta$-aminoethyl ether) $N,N,N',N'$-tetraacetic acid (EGTA) (Sigma Chemical Co., St. Louis, MO, USA), tetrodotoxin (Wako Pure Chemicals, Osaka, Japan), Soluene (Packard, Downers Groves, IL, USA). Other chemicals used were of reagent grade.

## 3. Results

### 3.1. Effects of peptide YY and neuropeptide Y on nerve-mediated twitch contractions

Electrical stimulation (1 ms, 30 V) at 0.1 Hz produced twitch contractions of the colonic preparations, and these contractions were prevented by treatment with $10^{-7}$ M atropine or $3 \times 10^{-7}$ M tetrodotoxin (data not shown), thereby indicating that the contractions were mediated by the stimulation of cholinergic neurons. Peptide YY and neuropeptide Y at $3 \times 10^{-9}$ to $10^{-7}$ M inhibited the twitch contractions induced by electrical stimulation, in a concentration-dependent manner (Fig. 1).

### 3.2. Effects of peptide YY and neuropeptide Y on the [$^3$H]acetylcholine release evoked by electrical stimulation and high $K^+$

The spontaneous release of [$^3$H]acetylcholine reached a steady state, and a single exponential curve was obtained with a fractional rate of $0.00660 \pm 0.00062$/min ($n = 6$) 60 min after superfusion. Both electrical stimulation (1 ms, 15 V, 180 pulses) at 3 Hz and high $K^+$ (40 mM) evoked the release of [$^3$H]acetylcholine from the colonic strips. The electrical stimulation-evoked release of [$^3$H]acetylcholine was prevented either by removal of external $Ca^{2+}$ or by treatment with $3 \times 10^{-7}$ M tetrodotoxin (Fig. 2A). The high $K^+$-evoked release of [$^3$H]acetylcholine was prevented by the removal of external $Ca^{2+}$, but was not changed by treatment with $3 \times 10^{-7}$ M tetrodotoxin (Fig. 2B).

Pretreatment with peptide YY at $3 \times 10^{-8}$ M for 1 min significantly inhibited the electrical stimulation-evoked release of [$^3$H]acetylcholine by $75.2 \pm 9.2\%$ ($n = 7$), but did not change the high $K^+$-evoked release of [$^3$H]acetylcholine in the presence of $3 \times 10^{-7}$ M tetrodotoxin (Fig. 2). Neuropeptide Y at $3 \times 10^{-8}$ M

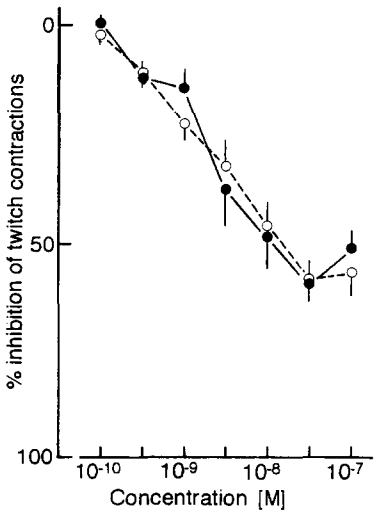


Fig. 1. Inhibitory effects of peptide YY and neuropeptide Y on twitch contractions induced by electrical stimulation of guinea-pig colon. Electrical stimulation was of 1 ms pulse duration, 30 V intensity, and 0.1 Hz. Peptide YY (●) and neuropeptide Y (○) were added cumulatively to the bath medium at 3 min intervals. Each point represents the mean ± S.E.M. for 5 animals.

significantly inhibited both the electrical stimulation-evoked release of [$^3$H]acetylcholine by 60.8 ± 14.3% ($n$ = 7) and the high $K^+$-evoked release of [$^3$H]acetylcholine in the presence of $3 \times 10^{-7}$ M tetrodotoxin by 43.9 ± 12.9% ($n$ = 8) (Fig. 2). The neuropeptide Y ($3 \times 10^{-8}$ M)-induced inhibition of the high $K^+$-evoked release of [$^3$H]acetylcholine was antagonized by acetyl-[3-(2-6-dichlorobenzyl)-Try$^{27,36}$,D-Thr$^{32}$]neuropeptide Y-(27–36) (PYX-2), an antagonist of the neuropeptide Y receptor (Tatemoto et al., 1992), at $10^{-7}$ M for 10 min pretreatment (Fig. 2B).

### 3.3. Effect of yohimbine on the neuropeptide Y-induced inhibition of high $K^+$- evoked release of [$^3$H]acetylcholine

The inhibitory effect of neuropeptide Y was examined on the high $K^+$-evoked tetrodotoxin-resistant release of [$^3$H]acetylcholine from the yohimbine ($5 \times 10^{-6}$ M)-treated preparations. Neuropeptide Y ($3 \times 10^{-8}$ M) did not alter the high $K^+$-evoked release of [$^3$H]acetylcholine.

### 3.4. Effects of peptide YY, neuropeptide Y and neuropeptide Y analogues on [$^3$H]noradrenaline release

The fractional rate of spontaneous release of [$^3$H]noradrenaline from the colonic strips preloaded with [$^3$H]noradrenaline was 0.00221 ± 0.00028/min ($n$ = 6) 60 min after superfusion, a time when the spontaneous release had approached a plateau level. Neuropeptide Y ($3 \times 10^{-8}$ M), but not peptide YY ($3 \times 10^{-8}$ M), [Leu$^{31}$,Pro$^{34}$]neuropeptide Y ($10^{-7}$ M), a selective neuropeptide Y $Y_1$ receptor agonist (Fuhlendorff et al., 1990) or neuropeptide Y-(13–36) ($10^{-7}$ M), a selective neuropeptide Y $Y_2$ receptor agonist (Wahlestedt et al., 1986), produced an increase greater than the spontaneous release of [$^3$H]noradrenaline (Fig. 3). The neuropeptide Y ($3 \times 10^{-8}$ M)-evoked release of [$^3$H]noradrenaline was abolished in the $Ca^{2+}$-free medium.

### 3.5. Effects of [Leu$^{31}$,Pro$^{34}$]neuropeptide Y and neuropeptide Y-(13–36) on the [$^3$H]acetylcholine release evoked by electrical stimulation

The effects of [Leu$^{31}$,Pro$^{34}$]neuropeptide Y and neuropeptide Y-(13–36) were examined on the release of [$^3$H]acetylcholine evoked by electrical stimulation (1 ms, 15 V, 180 pulses at 3 Hz). Pretreatment with [Leu$^{31}$,Pro$^{34}$]neuropeptide Y and neuropeptide Y-(13–36) at $10^{-7}$ M for 1 min significantly inhibited the electrical stimulation-evoked release of [$^3$H]acetylcholine by 50.2 ± 10.3% ($n$ = 8) and 38.9 ± 14.2% ($n$ = 8), respectively (Fig. 4).

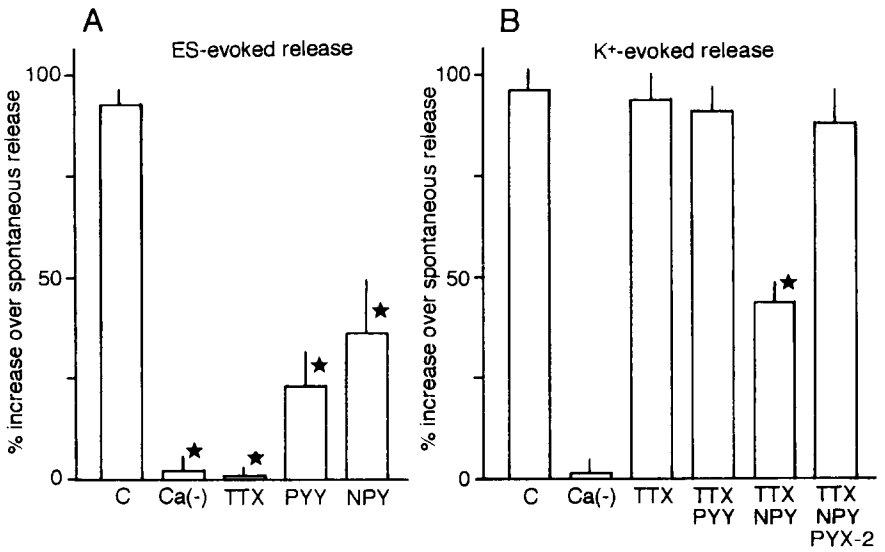


Fig. 2. Effects of external $Ca^{2+}$ removal, tetrodotoxin, peptide YY and neuropeptide Y on the electrical stimulation-evoked (A) and high $K^+$-evoked (B) release of [$^3$H]acetylcholine from the colonic strips. The electrical stimulation (ES) (1 ms pulse duration, 15 V intensity, 180 pulses) at 3 Hz or stimulation with high $K^+$ (40 mM, for 1 min) was applied to the non-treated strips (C) and to the strips pretreated with $Ca^{2+}$-free medium (Ca(−)) for 15 min, tetrodotoxin (TTX, $3 \times 10^{-7}$ M) for 15 min, peptide YY (PYY, $3 \times 10^{-8}$ M) for 1 min or neuropeptide Y (NPY, $3 \times 10^{-8}$ M) for 1 min. Acetyl-[3-(2-6-dichlorobenzyl)-Try$^{27,36}$,D-Thr$^{32}$]neuropeptide Y-(27–36) (PYX-2, $10^{-7}$ M) was added to $3 \times 10^{-7}$ M tetrodotoxin-containing medium 9 min before and during the application of NPY ($3 \times 10^{-8}$ M). Each column is presented as the mean ± S.E.M. of the percent increase in [$^3$H]acetylcholine (ACh) release over spontaneous release from 7 animals (in A) and 8 animals (in B). The percent increase over spontaneous release was calculated by dividing the fractional rate during stimulation by the spontaneous release.
* Significantly different from value in the non-treated preparations ($P < 0.05$).

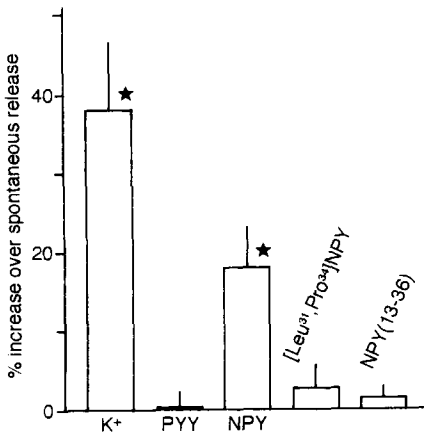


Fig. 3. Effects of peptide YY, neuropeptide Y, [Leu$^{31}$,Pro$^{34}$]neuropeptide Y and neuropeptide Y-(13–36) on the release of [$^3$H]noradrenaline from the guinea-pig colon. KCl ($K^+$, 40 mM), peptide YY (PYY, $3 \times 10^{-8}$ M), neuropeptide Y (NPY, $3 \times 10^{-8}$ M), [Leu$^{31}$,Pro$^{34}$]NPY ($10^{-7}$ M) and NPY(13–36) ($10^{-7}$ M) were added to the superfusion medium for 1 min. Each column is presented as the mean ± S.E.M. percent increase in [$^3$H]noradrenaline (NA) release over spontaneous release from 6 animals (in $K^+$, PYY and NPY) and 11 animals (in [Leu$^{31}$,Pro$^{34}$]NPY and NPY(13–36)). The percent increase in [$^3$H]NA release over spontaneous release was calculated by dividing the fractional rate during stimulation by the spontaneous release. * Significantly different from value of spontaneous release ($P < 0.05$).

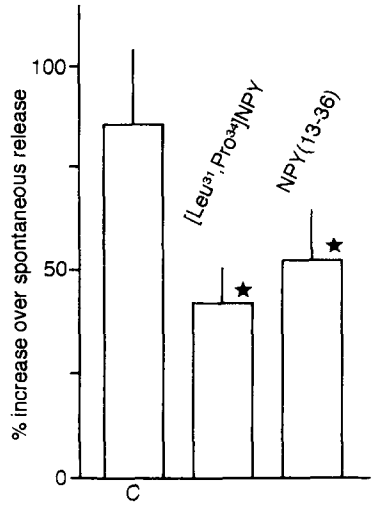


Fig. 4. Effects of [Leu$^{31}$,Pro$^{34}$]neuropeptide Y and neuropeptide Y-(13–36) on the electrical stimulation-evoked release of [$^3$H]acetylcholine from the guinea-pig colon. The electrical stimulation (1 ms pulse duration, 15 V intensity, 180 pulses) at 3 Hz was applied to the non-treated strips (C) and the strips pretreated with [Leu$^{31}$, Pro$^{34}$]neuropeptide Y ([Leu$^{31}$,Pro$^{34}$]NPY, $10^{-7}$ M) or neuropeptide Y-(13–36) (NPY(13–36), $10^{-7}$ M) for 1 min. Each column is presented as the mean ± S.E.M. percent increase in [$^3$H]ACh release over spontaneous release from 8 animals. The percent increase in [$^3$H]ACh release over spontaneous release was calculated as described in Fig. 2. * Significantly different from value in the non-treated preparations ($P < 0.05$).

### 3.6. Effects of tachyphylaxis of neuropeptide Y on the response to peptide YY

When peptide YY at $3 \times 10^{-8}$ M was present in the superfusion medium for 30 min, a second addition of the same concentration of peptide YY failed to inhibit the electrical stimulation (1 ms, 15 V, 180 pulses at 3 Hz)-evoked release of [$^3$H]acetylcholine (data not shown), thereby indicating that peptide YY may produce tachyphylaxis. Neuropeptide Y also produced tachyphylaxis (Fig. 5A). After tachyphylaxis had developed to $3 \times 10^{-8}$ M neuropeptide Y, peptide YY at $3 \times 10^{-8}$ M inhibited the electrical stimulation (1 ms, 15 V, 180 pulses at 3 Hz)-evoked release of [$^3$H]acetylcholine by 41.1 ± 4.7% (Fig. 5B), although the inhibitory effect of peptide YY was significantly reduced after tachyphylaxis had developed to neuropeptide Y.

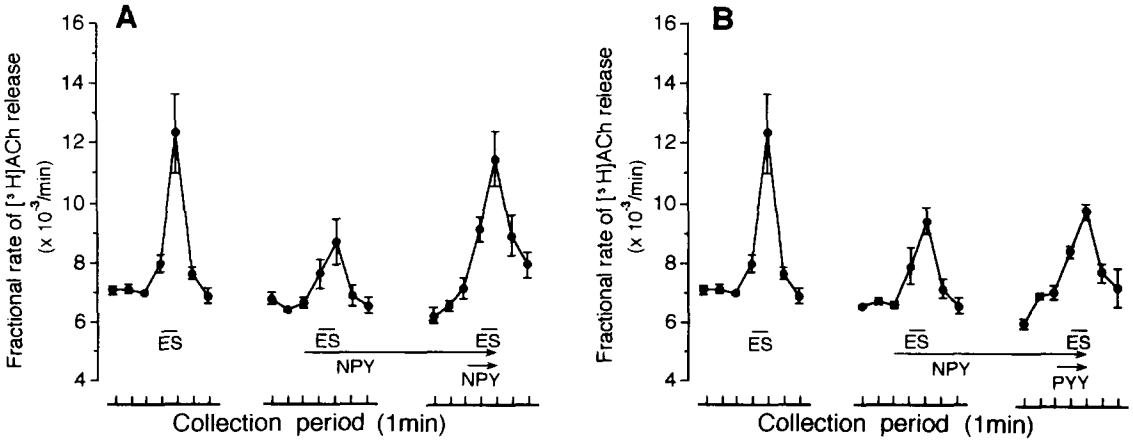


Fig. 5. Effect of desensitization to neuropeptide Y on the peptide YY-induced inhibition of electrical stimulation-evoked [$^3$H]acetylcholine release. In the presence of neuropeptide Y (NPY) at $3 \times 10^{-8}$ M for 30 min, NPY at $3 \times 10^{-8}$ M was added (A) and peptide YY (PYY) at $3 \times 10^{-8}$ M was added (B) 1 min before and during the application of electrical stimulation (1-ms pulse duration, 15 V intensity, 180 pulses) at 3 Hz. Each point represents a fractional rate, mean ± S.E.M. for 5 animals.

## 4. Discussion

Peptide YY and neuropeptide Y both inhibited the cholinergic nerve-mediated twitch contractions, in a concentration-dependent manner. The inhibitory effects of both peptides on the cholinergic transmission were confirmed in release experiments, in which both peptides reduced the electrical stimulation-evoked release of acetylcholine from the colonic preparations, as reported by Wiley and Owyang (1985). An opposite result has been shown, namely that peptide YY did not reduce the cholinergic nerve-mediated contractions to 1,1-dimethyl-4-phenylpiperazinium iodide in the guinea-pig distal colon (Krantis and Harding, 1991). The discrepancy cannot be explained, although in our experiments, the inhibitory effect of peptide YY was observed on the cholinergic nerve-mediated twitch contractions with low frequency of electrical stimulation and on the release of acetylcholine. In the guinea-pig stomach, peptide YY produces relaxation of isolated muscle strips by inhibiting the cholinergic neurotransmission (Wiley et al., 1991), and neuropeptide Y and peptide YY inhibit the nicotinic fast excitatory postsynaptic potentials of myenteric neurons (Schemann and Tamura, 1992).

Neuropeptide Y, but not peptide YY, inhibited the high $K^+$-evoked tetrodotoxin-resistant release of acetylcholine. Both electrical stimulation-evoked and high $K^+$-evoked releases of acetylcholine were $Ca^{2+}$-dependent. However, there was a difference in sensitivity to tetrodotoxin between electrical stimulation-evoked and high $K^+$-evoked release of acetylcholine. Electrical stimulation-evoked release of acetylcholine was tetrodotoxin-sensitive, while high $K^+$-evoked release was resistant to tetrodotoxin. The tetrodotoxin-sensitive release of neurotransmitter is considered to be induced by the stimulation of soma-dendritic regions of the neuron (Vizi et al., 1973), and the tetrodotoxin-resistant release of neurotransmitter is assumed to be due to direct depolarization of nerve terminals (Starke, 1981). As peptide YY inhibited the tetrodotoxin-sensitive release, but did not affect the tetrodotoxin-resistant release, the site of action of peptide YY is in soma-dendritic regions rather than in the nerve terminals of the postganglionic cholinergic neurons.

Neuropeptide Y, but not peptide YY, evoked the release of noradrenaline from the colonic preparations, as noted by Wiley and Owyang (1987). The inhibitory effect of neuropeptide Y was not seen with the preparations in which the $\alpha_2$-adrenoceptors located on the cholinergic neuron were blocked by yohimbine, an event which shows that neuropeptide Y induces noradrenaline release from sympathetic neurons, with the noradrenaline in turn inhibiting acetylcholine release by acting on presynaptic $\alpha_2$-adrenoceptors located on the cholinergic nerves (Wiley and Owyang, 1987). Thus, the site of action of neuropeptide Y is the adrenergic nerve terminals, not the cholinergic nerve terminals, and the neuropeptide Y-induced inhibition of acetylcholine release is due to noradrenaline released from the adrenergic neurons. The fact that the neuropeptide Y receptor antagonist, PYX-2 (Tatemoto et al., 1992), antagonized the neuropeptide Y-induced inhibition of acetylcholine release indicates the localization of neuropeptide Y receptors on the adrenergic nerve terminals.

The neuropeptide Y receptors have been classified in at least 3 types, as deduced from pharmacological and molecular biological studies (Wahlestedt et al., 1990; Rimland et al., 1991). The $Y_1$ and $Y_2$ types show equal affinities for the two endogenous peptides, neuropeptide Y and peptide YY, or a slightly higher affinity for peptide YY, while the $Y_3$ type exhibits a higher affinity for neuropeptide Y than for peptide YY (Rimland et al., 1991). In the guinea-pig colon used in the present study, neuropeptide Y stimulated the adrenergic neurons, while peptide YY, a neuropeptide Y $Y_1$ receptor agonist and a neuropeptide Y $Y_2$ receptor agonist did not affect the release of noradrenaline. Thus, there is the possibility that the neuropeptide Y receptor located on the adrenergic nerve terminals is predominantly of the $Y_3$ type rather than the $Y_1$ or $Y_2$ type. The central neuropeptide Y receptors located on noradrenergic neurons are of the $Y_2$ type and mediate inhibition of the stimulated release of noradrenaline (Martire et al., 1993). In peripheral tissues, the prejunctional neuropeptide Y receptors inhibiting the noradrenaline release are of the $Y_1$ type in the rabbit vas deferens and of the $Y_2$ type in the rat vas deferens (Doods and Krause, 1991).

The release of acetylcholine induced by the stimulation of soma-dendritic regions of cholinergic neurons was inhibited by both selective neuropeptide Y $Y_1$ and $Y_2$ receptor agonists, therefore the soma-dendritic regions of postganglionic cholinergic neurons may possess both $Y_1$ and $Y_2$ types. Similar findings have been shown by receptor autoradiography, namely that both $Y_1$ and $Y_2$ types of neuropeptide Y receptor are present in myenteric and submucosal ganglia of porcine and human colons (Walsh et al., 1993). An electrophysiological study demonstrated the inhibitory effects of peptide YY and neuropeptide Y on the myenteric neurons of guinea-pig stomach (Schemann and Tamura, 1992). The responses to peptide YY and neuropeptide Y became tachyphylactic, while the response to peptide YY was observed after tachyphylaxis had developed to neuropeptide Y. Thus, peptide YY may act on receptors distinct from the site of action of neuropeptide Y, located on the soma-dendritic regions of the postganglionic cholinergic neurons.

The localization of subtypes of neuropeptide Y re-

ceptors remains to be elucidated. The $Y_1$ and $Y_2$ types are present in the postjunctional and prejunctional receptors of sympathetic neurons in rat vas deferens and blood vessels, respectively (Wahlestedt et al., 1986), while the $Y_2$ type is also present postjunctionally in vascular beds (Michel et al., 1990).

The present study demonstrated that peptide YY inhibits acetylcholine release through the $Y_1$ and $Y_2$ types of neuropeptide Y receptor and possibly through the peptide YY-specific receptor, which is mainly located on the soma-dendritic regions of postganglionic cholinergic neurons in the guinea-pig colon. Neuropeptide Y inhibits the acetylcholine release due to noradrenaline released by stimulation of the neuropeptide Y receptor, possibly the $Y_3$ type, located on adrenergic neurons. Further analyses of receptors for peptide YY and neuropeptide Y are part of ongoing studies.

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