Substance P Stimulates and Inhibits Intestinal Peristalsis *via* Distinct Receptors[1]

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Accepted for publication March 1, 1995

ABSTRACT

The tachykinins substance P (SP) and neurokinin A participate in the neural control of intestinal peristalsis. This study aimed at elucidating the types of tachykinin receptors involved in SP's ability first to stimulate and then to inhibit propulsive activity. Peristalsis in the guinea pig isolated ileum was triggered by fluid-induced distension of the intestinal wall. Unlike SP, the neurokinin (NK)-1 receptor-selective agonist SP methyl ester (1–100 nM) failed to facilitate peristalsis but caused a delayed inhibition of peristaltic activity. In contrast, the NK-2 receptor-selective agonist [β-Ala⁸]-NKA-(4–10) (BANKA, 1–100 nM) stimulated, but did not inhibit, peristalsis. The NK-3 receptor-selective agonist succinyl-[Asp⁶,N-MePhe⁸]-substance P-(6–11) (SENKTIDE, 0.1–10 nM) was most potent in facilitating propulsive activity, and only with 10 nM SENKTIDE was a delayed inhibition of peristalsis seen. The receptors responsible for the tachykinin-evoked stimulation and inhibition of peristal-

tic activity were further characterized by use of the NK-1 receptor-selective antagonist (+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994, 300 nM) and the NK-2 selective antagonist (−)-N-methyl-N[4-acetylamino-4-phenyl-piperidino-2(3,4 dichlorophenyl)butyl]-benzamide (SR-48,968, 100 nM). CP-99,994 antagonized the inhibitory effects of SP (100 nM) and SP methyl ester (100 nM) on peristalsis but did not alter the facilitation of propulsive motility brought about by SP or BANKA (100 nM). Conversely, SR-48,968 (100 nM) suppressed the ability of SP and BANKA to stimulate peristaltic activity but did not attenuate the inhibitory motor effects of SP and SP methyl ester. These data indicate that tachykinins influence intestinal peristalsis by interaction with NK-1, NK-2 and NK-3 receptors. Activation of NK-1 receptors results in inhibition, whereas activation of NK-2 and NK-3 receptors leads to facilitation, of peristaltic motor activity.

The peristaltic movements of the intestine are coordinated by excitatory and inhibitory pathways of the enteric nervous system (Furness and Costa, 1987). Acetylcholine is the major excitatory neuroeffector transmitter involved in propulsive motility, but blockade of muscarinic acetylcholine receptors does not necessarily abolish peristaltic motor activity (Kottegoda, 1970; Tonini et al., 1981), which under these conditions seems to be mediated by the tachykinins SP and NKA (Barthó et al., 1982a, 1982b; Costa et al., 1985a; Holzer and Maggi, 1994). It appears that under physiological conditions, tachykininergic transmission *via* NK-2, but not NK-1, receptors synergizes with cholinergic transmission through muscarinic receptors in the relay of excitatory pathways to the circular muscle (Holzer and Maggi, 1994). This cooperative interaction is in keeping with the co-existence of SP/NKA and

acetylcholine in enteric motor neurons in the guinea pig intestine (Brookes et al., 1991) and with their co-release from these neurons (Maggi et al., 1994a).

Tachykinins have long been known to play a role in noncholinergic excitatory neurotransmission in the propulsive motility of the intestine (Barthó et al., 1982a, 1982b, 1989; Barthó and Holzer, 1985; Costa et al., 1985a, 1985b; Furness and Costa, 1987; Grider, 1989; Holzer, 1989; Maggi et al., 1993b). The peptides' overall effect on the circular muscle, the major effector of peristalsis, is excitation and contraction that is mediated predominantly by NK-2 receptors (Barthó et al., 1992; Holzer et al., 1993; Holzer and Maggi, 1994; Maggi et al., 1994b) although NK-1 receptors are also involved (Maggi et al., 1994b). Furthermore, tachykinins can stimulate NK-3 receptors on enteric neurons, which, depending on the experimental conditions, gives rise to cholinergic (Laufer et al., 1986; Maggi et al., 1994c) and tachykininergic (Guard and Watson, 1987; Maggi et al., 1994c) contraction or to nitrergic (Maggi et al., 1993a) relaxation of intestinal smooth

ABBREVIATIONS: BANKA, [β-Ala⁸]-neurokinin A-(4–10); CP-99,994, (+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine; NK, neurokinin; NKA, neurokinin A; SENKTIDE, succinyl-[Asp⁶,N-MePhe⁸]-substance P-(6–11); SP, substance P; SPOME, substance P methyl ester; SR-48,968, (−)-N-methyl-N[4-acetylamino-4-phenyl-piperidino-2-(3,4-dichlorophenyl)butyl]-benzamide; SR-140,333, (S)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenyl-acetyl)piperidin-3-yl]-ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]-octane chloride.

muscle. In addition, SP-induced activation of NK-1 receptors can inhibit the motility of the canine intestine by a pathway involving muscarinic acetylcholine receptors (Fox *et al.*, 1986), an action that may be related to the NK-1 receptor-mediated inhibition of the stimulus-evoked release of acetylcholine from myenteric neurons in the guinea pig ileum (Kilbinger *et al.*, 1986; Löffler *et al.*, 1994).

In view of the presence of NK-1, NK-2 and NK-3 receptors on muscle cells and enteric neurons and their ability to bring about stimulation or inhibition of intestinal motility, it is not surprising to find that SP influences peristalsis in a complex manner, first stimulating and then inhibiting propulsive motor activity in the guinea pig small intestine (Barthó *et al.*, 1982b). It is not clear what receptors and mechanisms are responsible for these intricate motor actions of SP. Because SP does not differentiate among the known tachykinin receptors (Guard and Watson, 1991; Maggi *et al.*, 1993b) our first aim was to examine which effects NK-1, NK-2 and NK-3 receptor-selective agonists have on peristalsis, and thus to explore which tachykinin receptors play a role in the stimulant and inhibitory effects of tachykinins on peristaltic motor activity. Our second aim was to exploit the availability of NK-1 and NK-2 receptor-selective antagonists and identify the receptors that are involved in the complex motor effects of tachykinins.

Materials and Methods

Recording of peristalsis. Adult guinea pigs of either sex weighing between 350 and 450 g were stunned and bled *via* the carotid arteries. The distal jejunum and ileum was excised, flushed of luminal contents and placed in Tyrode's solution at room temperature, gassed with 95% O_2 and 5% CO_2, until required. The composition of the Tyrode's solution was (in mM) NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, and glucose 5.6. For the study of peristalsis, a segment approximately 8 to 10 cm in length was dissected from the ileum (at least 10 cm proximal to the ileocecal valve) and secured horizontally in a silanized glass organ bath that contained 30 ml of Tyrode's solution maintained at 37°C (Holzer and Maggi, 1994). The oral end of the intestinal segment was tied to an inflow cannula, which permitted the continuous infusion of pre-warmed Tyrode's solution at a flow rate of 0.5 ml/min. The aboral end of the segment was attached to an intermediate tubing (I.D. 0.3 mm) fixed with a T piece (Holzer and Maggi, 1994). One arm of the T piece was connected to a pressure transducer (ISOTEC™, HSE, March, Germany) for recording the intraluminal pressure (Holzer and Maggi, 1994), and the other arm of the T piece was fitted with a vertical outlet tubing (I.D. 0.2 mm) that ended 4 cm above the fluid level in the organ bath. This arrangement (Bülbring *et al.*, 1958; Costall *et al.*, 1993) made emptying of the intestinal segment possible when peristaltic contractions raised the intraluminal pressure above 4 mbar. The records showed two distinct phases of intraluminal pressure changes (fig. 1). During the preparatory phase, the intestine is gradually filled with fluid, and the intraluminal pressure rises slowly because the outlet tubing prevents the escape of fluid from the system (Holzer and Maggi, 1994). When the intraluminal pressure reaches a threshold (about 1 mbar), an aborally moving wave of peristaltic contraction is triggered and the emptying phase of peristalsis is initiated (fig. 1). The wave of circular muscle contraction, measured as a spike-like increase in intraluminal pressure, propels the intraluminal fluid to leave the system *via* the outlet tubing and thus causes partial emptying of the segment.

Experimental protocol and evaluation of results. The tissue was equilibrated in the organ bath for 20 min before the experiments were started. Thereafter, the bath fluid was renewed and basal
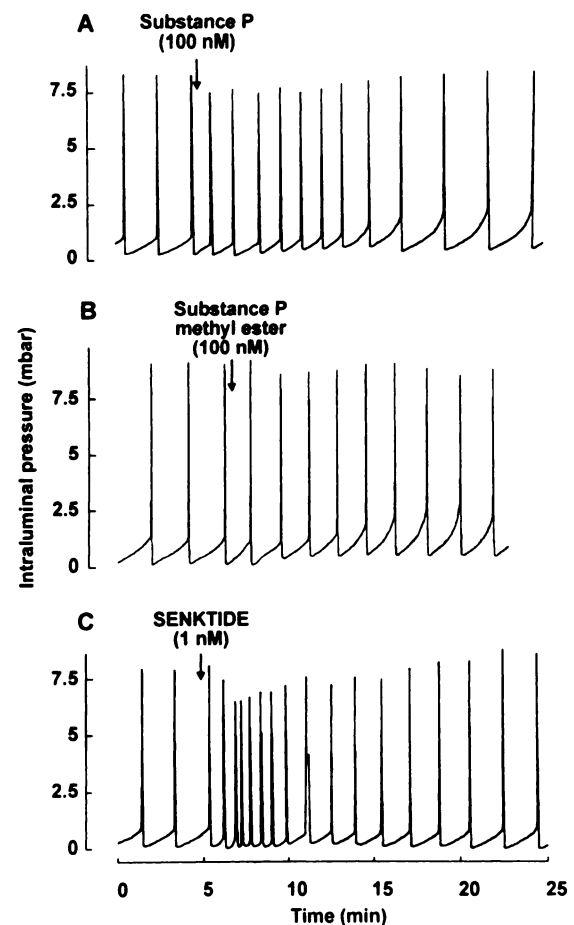
Fig. 1. Recording of the effects of SP (A), the NK-1 receptor-selective agonist SPOME (B) and the NK-3 receptor-selective agonist SENKTIDE (C) on peristaltic motor activity in the guinea pig isolated ileum. The concentrations of the drugs in the bath fluid are given in parentheses.

peristaltic activity recorded for a period of 20 min. The drugs to be tested were administered into the bath, *i.e.*, to the serosal surface of the intestinal segment, at volumes not exceeding 1% of the bath volume. Each drug was added only once to each segment and was tested on at least six segments from six different guinea pigs. All vehicle solutions used in this study were tested separately to ensure that they were devoid of any influence on peristaltic activity. The data recorded and the parameters calculated from the tracings (Holzer and Maggi, 1994) are presented as means ± S.E.M. of n experiments, n being the number of guinea pigs used in the test. The results were evaluated statistically with the Mann-Whitney U test or the Wilcoxon test for pair differences or with the Kruskal-Wallis H test if multiple comparisons were made. A probability value of less than .05 was regarded as significant.

Two parameters of peristalsis were evaluated: the frequency of peristaltic waves (min^{-1}) and the pressure threshold (mbar), which is the intraluminal pressure level at which a peristaltic wave is elicited (Holzer and Maggi, 1994). The amplitude of the peristaltic movements was not assessed because this parameter was least sensitive to the manipulations under study.

Drugs and solutions. The tachykinin receptor agonists and antagonists used here, their names, codes, potencies and stock solutions are given in table 1. The stock solutions were diluted with Tyrode's solution as required. The sources of the drugs were as follows: BANKA (Menarini, Firenze, Italy); CP-99,994 (Pfizer, Groton, CT); SINKTIDE and SPOME (Bachem, Basel, Switzerland); SR-48,968 and SR-140,333 (Sanofi, Montpellier, France) and SP (Serva, Heidelberg, Germany).

TABLE 1

Tachykinin receptor-selective agonists and antagonists used in this study

Receptor Selectivity	pD₂/pKB	Name	Code	Stock Solution
Agonists				
Nonselective	10.00 (CM)[a]	SP	SP	1 mM in 0.02 M HAc
NK-1	8.47 (LM)[b]	SPOME	SPOME	1 mM in 0.02 M HAc
NK-2	8.00 (CM)[a]	[β-Ala⁸]-neurokinin A-(4–10)	BANKA	1 mM in DMSO
NK-3	8.59 (CM)[c]	Succinyl-[Asp⁶,N-MePhe⁸]-substance P-(6–11)	SENKTIDE	1 mM in DMSO
Antagonists				
NK-1	N.D.	(+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine	CP-99,994	1 mM in Tyrode
NK-1	9.65 (LM)[d]	(S)1-{2-[3-(3,4-dichlorophenyl)-1-(3-iso-propoxyphenyl-acetyl)piperidin-3-yl]-ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]-octane chloride	SR-140,333	1 mM in DMSO
NK-2	7.83 (CM)[a]	(−)-N-methyl-N[4-acetylamino-4-phenyl-piper-idino-2-(3,4-dichlorophenyl)butyl]-benzamide	SR-48,968	1 mM in DMSO

CM = circular muscle of the guinea pig ileum; LM = longitudinal muscle of the guinea pig ileum. [a] Maggi *et al.*, 1994d; [b] Laufer *et al.*, 1986; [c] Maggi *et al.*, 1993a; [d] Emonds-Alt *et al.*, 1993. The pKB (equilibrium dissociation constant) values were evaluated by the use of receptor-selective NK-1 and NK-2 agonists. HAc = acetic acid; DMSO = dimethylsulfoxide; N.D. = not determined. The pKB value for CP-99,994 in the guinea pig ileum has not yet been determined, but this compound is at least as potent as the related NK-1 receptor antagonist CP-96,345 (Snider *et al.*, 1991; McLean *et al.*, 1993), whose pKB value for the NK-1 receptor in the circular muscle of the guinea pig ileum is 8.17 (Maggi *et al.*, 1994d).

Results

Effects of SP and tachykinin receptor-selective agonists. Continuous intraluminal infusion of Tyrode's solution resulted in peristaltic activity (fig. 1) that stayed constant during the experimental period of 40 to 60 min ($n = 7$). Exposure of the intestinal segment to SP (1–100 nM) caused a prompt stimulation of peristalsis, as shown by an increase in the frequency and a decrease in the pressure threshold of the peristaltic waves (figs. 1A and 2). The SP-evoked stimulation of peristaltic motor activity was transient, and after 7 to 10 min the frequency of peristalsis became slower, and the pressure threshold higher, than the respective parameters measured before administration of the peptide (fig. 1A). The delays, after which the effects of SP to first lower and then enhance the pressure threshold peaked, are given in table 2. Both the initial stimulant and the delayed inhibitory effect of SP were related to the concentration of the peptide in the bath (fig. 2). With 100 nM SP in the bath, it took over 30 min until the peptide's depressant effect wore off and peristalsis returned to the level measured before exposure of the ileal segment to SP. Administration of the vehicle (Tyrode's solution) to the bath failed, during a 40-min observation period, to alter the frequency and pressure threshold of the peristal-
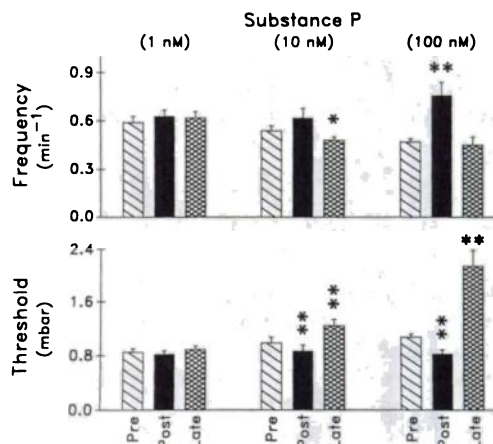

Fig. 2. Effect of SP (concentrations indicated in parentheses), added to the bath fluid, on the frequency and pressure threshold of peristaltic motor activity in the guinea pig isolated ileum. The columns show the parameters measured immediately before addition of the agonist ("Pre"), the parameters measured concurrently with the initial peak decrease in the pressure threshold after addition of the agonist ("Post") and the parameters measured concurrently with the delayed peak increase in the pressure threshold after addition of the agonist ("Late"). Means ± S.E.M., $n = 6$ to 7. * $P < .05$, ** $P < .01$ vs. "Pre."

TABLE 2

Delays in the peak effects of tachykinin receptor-selective agonists to first lower (initial effect) and then enhance (late effect) the pressure threshold of intestinal peristalsis

Agonist	Concentration (nM)	Delay of Initial Effect (min)	Delay of Late Effect (min)
SP	10	2.07 ± 0.48	12.10 ± 1.28
	100	1.78 ± 0.31	13.30 ± 1.87
SPOME	10	—	8.74 ± 1.85
	100	—	12.14 ± 1.70
BANKA	10	3.78 ± 0.55	—
	100	3.38 ± 0.66	—
SENKTIDE	1	4.03 ± 0.92	21.07 ± 3.05
	10	3.60 ± 0.61	16.43 ± 1.30

The delays indicate the times that elapsed between addition of the agonists to the bath and recording of the respective peak effects. The agonist-induced initial decrease in the pressure threshold was not seen with SPOME, whereas BANKA failed to cause a delayed increase in the pressure threshold. Means ± S.E.M., $n = 6$ to 7.

tic contractions to any appreciable extent ($n = 7$; data not shown).

After this characterization of the responses to SP, we explored the effects of tachykinin receptor-selective agonists on intestinal peristalsis. The NK-1 receptor-selective agonist SPOME, added to the bath at concentrations of 1 to 100 nM, failed to stimulate peristalsis to any significant extent, as would have been apparent from a reduction of the pressure threshold and an augmentation of the frequency of peristaltic waves (figs. 1B and 3). With some delay (table 2), however, SPOME caused a concentration-related elevation of the pressure threshold (fig. 3), whereas the frequency of peristaltic contractions did not change significantly (figs. 1B and 3). As was the case with SP, the delayed inhibitory effect of 100 nM SPOME on peristalsis lasted over 30 min.

The NK-2 receptor-selective agonist BANKA led to a prompt, concentration-related decrease in the pressure threshold, whereas the frequency of peristaltic waves re-

Substance P methyl ester
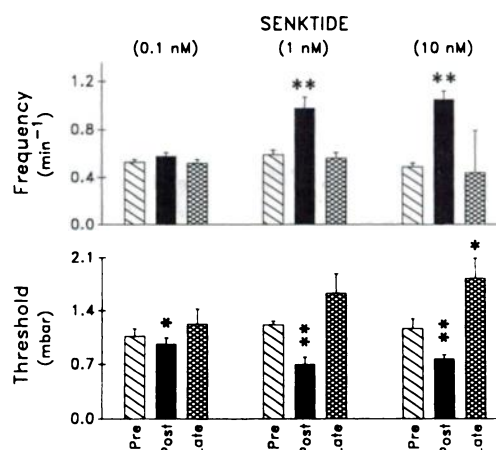(1 nM) (10 nM) (100 nM)
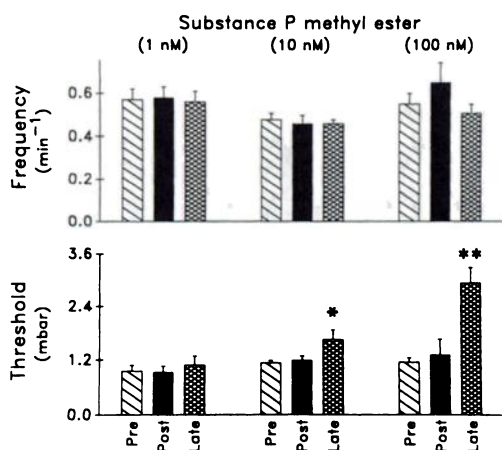
Fig. 3. Effect of SPOME (concentrations indicated in parentheses), added to the bath fluid, on the frequency and pressure threshold of peristaltic motor activity in the guinea pig isolated ileum. The columns show the parameters measured immediately before addition of the agonist ("Pre"), the parameters measured concurrently with the initial peak decrease in the pressure threshold after addition of the agonist ("Post") and the parameters measured concurrently with the delayed peak increase in the pressure threshold after addition of the agonist ("Late"). Means ± S.E.M., n = 6 to 7. * P < .05, ** P < .01 vs. "Pre."

[β–Ala^8]–NKA–(4–10)
(1 nM) (10 nM) (100 nM)
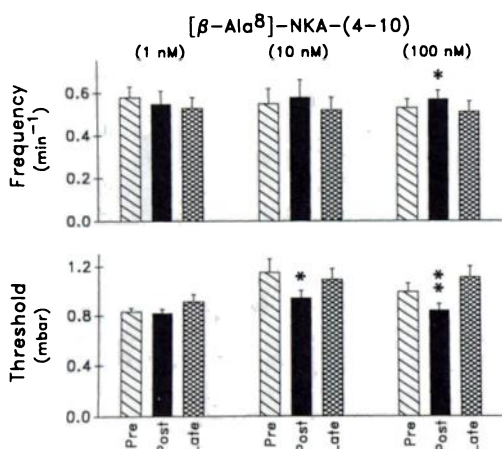
Fig. 4. Effect of BANKA (concentrations indicated in parentheses), added to the bath fluid, on the frequency and pressure threshold of peristaltic motor activity in the guinea pig isolated ileum. The columns show the parameters measured immediately before addition of the agonist ("Pre"), the parameters measured concurrently with the initial peak decrease in the pressure threshold after addition of the agonist ("Post") and the parameters measured concurrently with the delayed peak increase in the pressure threshold after addition of the agonist ("Late"). Means ± S.E.M., n = 6 to 7. * P < .05, ** P < .01 vs. "Pre."

mained unaltered (fig. 4). The stimulant effect of BANKA peaked somewhat later than the SP-evoked stimulation of peristalsis (table 2). The stimulation of peristaltic motor activity induced by BANKA usually waned within 25 min and, unlike the stimulant effects of SP and SPOME, was not followed by inhibition of peristalsis (fig. 4).

Among the tachykinins tested here, the NK-3 receptor-selective agonist SENKTIDE was most potent in stimulating intestinal peristalsis (figs. 1C and 5; table 2). Concentrations as low as 0.1 nM were able to lower the pressure threshold of the peristaltic waves (fig. 5), and higher concentrations of the peptide (1–10 nM) caused a concentration-related reduction of pressure threshold and elevation of peristaltic frequency (fig. 5). Only with 10 nM SENKTIDE was a delayed inhibi-

SENKTIDE
(0.1 nM) (1 nM) (10 nM)

Fig. 5. Effect of SENKTIDE (concentrations indicated in parentheses), added to the bath fluid, on the frequency and pressure threshold of peristaltic motor activity in the guinea pig isolated ileum. The columns show the parameters measured immediately before addition of the agonist ("Pre"), the parameters measured concurrently with the initial peak decrease in the pressure threshold after addition of the agonist ("Post") and the parameters measured concurrently with the delayed peak increase in the pressure threshold after addition of the agonist ("Late"). Means ± S.E.M., n = 6. * P < .05, ** P < .01 vs. "Pre."

tion of peristalsis seen to follow the initial stimulant effect. The inhibitory effect of the NK-3 agonist became apparent from an increase in the pressure threshold, whereas the frequency of peristaltic waves had returned to levels that did not significantly differ from those seen before administration of SENKTIDE (fig. 5).

Effects of tachykinin receptor-selective antagonists. Addition of one of the NK-1 receptor-selective antagonists CP-99,994 (30–300 nM) and SR-140,333 (30 nM) to the bath caused a concentration-related decrease in the pressure threshold of the peristaltic contractions (table 3). The NK-2 receptor-selective antagonist SR-48,968 (100 nM) caused a

TABLE 3

Effects of tachykinin receptor-selective antagonists on intestinal peristalsis and on the ability of the NK-1 receptor-selective agonist SPOME to enhance the pressure threshold, and the ability of the NK-2 receptor-selective agonist BANKA to lower the pressure threshold, of intestinal peristalsis

Antagonist (nM)	Agonist (nM)	Change in Pressure Threshold (mbar)	Delay in Peak Effect (min)
CP-99,994 (30)	—	−0.13 ± 0.04*	9.60 ± 2.20
CP-99,994 (300)	—	−0.19 ± 0.05**	7.30 ± 1.20
SR-140,333 (30)	—	−0.19 ± 0.09*	9.40 ± 1.20
SR-48,968 (100)	—	+0.31 ± 0.08**	12.40 ± 2.36
Vehicle	SPOME (100)	+1.78 ± 0.29	N.D.
CP-99,994 (300)	SPOME (100)	+0.09 ± 0.03††	N.D.
SR-48,968 (100)	SPOME (100)	+1.77 ± 0.40	N.D.
Vehicle	BANKA (100)	−0.15 ± 0.02	N.D.
CP-99,994 (300)	BANKA (100)	−0.24 ± 0.10	N.D.
SR-48,968 (100)	BANKA (100)	−0.03 ± 0.02††	N.D.

The vehicle (Tyrode's solution) or SR-48,968 was added 15 min, and CP-99,994 10 min, before the administration of SPOME or BANKA. The values in the "Change in Pressure Threshold" column represent the increases (+) or decreases (−) in the pressure threshold caused by the antagonist: SPOME or BANKA. The values in the "Delay in Peak Effect" column indicate the times that elapsed between addition of the drugs to the bath and recording of the respective peak effects. Means ± S.E.M., n = 6 to 7. * P < .05, ** P < .01 vs. the pressure threshold recorded before addition of the antagonist. †† P < .01 vs. the agonist effects recorded in the presence of the vehicle. N.D. = not determined.
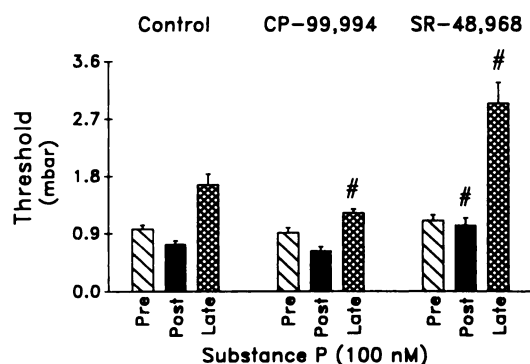
Fig. 6. Antagonism of the effects of SP (100 nM) on peristaltic motor activity in the guinea pig isolated ileum by CP-99,994 (300 nM) and SR-48,968 (100 nM). CP-99,994 was added to the bath fluid 10 min, and SR-48,968 15 min, before administration of SP. In the control experiments, equivalent volumes of the vehicle (Tyrode's solution) were added to the bath. The columns show the pressure threshold of peristaltic contractions measured immediately before addition of the agonist ("Pre"), the peak decrease in the threshold measured after addition of the agonist ("Post") and the delayed peak increase in the pressure threshold measured after addition of the agonist ("Late"). Means ± S.E.M., n = 6. # P < .05 vs. the respective values measured under control conditions.

small but significant inhibition of peristalsis as indicated by a moderate rise of the pressure threshold (table 3). None of the tachykinin antagonists tested here had any appreciable effect on the frequency of peristaltic waves.

CP-99,994 (300 nM) and SR-48,968 (100 nM) were chosen as the NK-1 and NK-2 receptor antagonists, respectively, to be used in the further experiments. The concentrations of these two compounds were selected such that they were at least 30 times in excess of their respective equilibrium dissociation constants determined in the guinea pig ileum (table 1). Their selectivity as NK-1 and NK-2 receptor antagonists was analyzed by examining their activity in antagonizing the effects of SPOME and BANKA on the peristaltic reflex. As table 3 shows, CP-99,994 (300 nM) prevented the increase in the pressure threshold of peristaltic waves that the NK-1 receptor agonist SPOME (100 nM) caused when vehicle (Tyrode's solution) instead of CP-99,994 was added to the bath. In contrast, SR-48,968 (100 nM) did not influence the ability of SPOME to enhance the pressure threshold but prevented the decrease in the pressure threshold induced by BANKA (100 nM). The effect of this NK-2 receptor agonist was not affected by CP-99,994 (table 3). The frequency of the peristaltic waves was not evaluated in these experiments.

In order to evaluate the participation of NK-1 and NK-2 receptors in the effect of SP (100 nM) on intestinal peristalsis, we tested CP-99,994 (300 nM) and SR-48,968 (100 nM) for their antagonism of the peptide's ability first to stimulate

and then to inhibit the peristaltic reflex (fig. 6, table 4). CP-99,994 had no influence on the initial SP-induced decrease in the pressure threshold of the peristaltic waves but significantly attenuated the delayed increase in the pressure threshold, and it had no effect on the SP-induced change in the frequency of peristaltic contractions (fig. 6, table 4). SR-48,968, on the other hand, inhibited the initial SP-evoked decrease in the pressure threshold and augmented the delayed increase in the pressure threshold to a significant extent (fig. 6, table 4). In addition, the NK-2 antagonist led to a significant alteration in SP's delayed effect on the frequency of peristaltic waves (table 4).

Discussion

The current findings demonstrate that the tachykinin SP influences intestinal peristalsis in a complex manner through the activation of multiple tachykinin receptors. In particular, the initial facilitation of propulsive motility is brought about by receptors that are pharmacologically distinct from those responsible for the delayed inhibition of peristalsis. This conclusion is based on two sets of data obtained with tachykinin receptor-selective agonists and antagonists, respectively.

The first part of the study revealed that NK-1, NK-2 and NK-3 receptor-selective agonists display distinct patterns of motor activity with regard to the parameters of the peristaltic reflex measured here (peristaltic frequency and pressure threshold). The activity pattern evoked by NK-1 receptor stimulation with SPOME (no initial effect, delayed increase in pressure threshold but no change in peristaltic frequency) was completely contrary to the pattern induced by NK-2 receptor activation with BANKA (initial decrease in pressure threshold, small increase in peristaltic frequency, no delayed effect). The early phase of the activity pattern elicited by NK-3 receptor stimulation with SENKTIDE (initial decrease in pressure threshold, marked increase in peristaltic frequency) resembled that of NK-2 receptor activation, whereas the late attenuation of peristalsis (increase in pressure threshold) caused by SENKTIDE was not seen with BANKA.

A comparison of the effects of the tachykinin receptor-selective agonists (figs. 3, 4 and 5) with those of SP (fig. 2) indicates that the initial facilitation of peristalsis caused by SP combines characteristics of the stimulant effects of BANKA and SENKTIDE. The SP-evoked increase in peristaltic frequency may be largely a NK-3 receptor-mediated response, whereas the decrease in the pressure threshold includes traits of both NK-2 and NK-3 receptor activation. Because the ability of SP to inhibit peristaltic performance after some delay is fully shared by SPOME only (figs. 2 and

TABLE 4

Effects of CP-99,994 (300 nM) and SR-48,968 (100 nM) on the SP (100 nM)-induced changes in intestinal peristalsis

Treatment	SP-Evoked Initial Stimulation		SP-Evoked Delayed Inhibition	
	Frequency (min^{-1})	Threshold (mbar)	Frequency (min^{-1})	Threshold (mbar)
Vehicle	+0.22 ± 0.02	−0.24 ± 0.04	−0.06 ± 0.01	+0.69 ± 0.12
CP-99,994	+0.20 ± 0.04	−0.27 ± 0.05	−0.06 ± 0.05	+0.32 ± 0.09*
SR-48,968	+0.12 ± 0.05	−0.06 ± 0.03*	+0.01 ± 0.02*	+1.64 ± 0.33*

The vehicle (Tyrode's solution) or SR-48,968 was added 15 min, and CP-99,994 10 min, before the administration of SP. The values shown represent the SP-induced increases (+) and decreases (−) in the frequency and pressure threshold of the peristaltic waves, respectively. Means ± S.E.M., n = 6. * P < .05 vs. the respective values measured after treatment with vehicle.

3), we assume that this effect of the peptide is predominantly brought about by activation of NK-1 receptors.

The information obtained with the tachykinin receptor-selective agonists was confirmed by the use of the NK-1 receptor-selective antagonist CP-99,994 (McLean *et al.*, 1993) and the NK-2 receptor-selective antagonist SR-48,968 (Emonds-Alt *et al.*, 1992). The specificity and efficacy of these nonpeptide compounds, at the concentrations used, were proved by their selective antagonism of the motor effects of SPOME and BANKA, respectively (table 3). When tested against SP, CP-99,994 specifically attenuated the late SP-induced inhibition of peristaltic performance but did not alter the initial stimulant effect of the peptide (fig. 6, table 4). This result indicates that the delayed attenuation of propulsive motility is primarily a NK-1 receptor-mediated event, a conclusion that is consistent with the peristaltic activity profile of the NK-1 agonist SPOME.

SR-48,968 reduced the initial stimulant effect of SP on peristaltic performance, which demonstrates that NK-2 receptors play a significant part in the SP-evoked facilitation of propulsive motility, at least with regard to the lowering of the pressure threshold (fig. 6, table 4). The increase in peristaltic frequency caused by SP, however, was not significantly diminished by SR-48,968. This failure of the NK-2 antagonist reinforces the notion that the SP-induced acceleration of peristalsis is brought about by activation of NK-3 receptors. Interestingly, SR-48,968 exacerbated the delayed inhibition of peristaltic motor activity (increase in pressure threshold) caused by SP, an effect that points to a unilateral interaction between NK-1 and NK-2 receptors in the peristaltic motor action of SP. It appears that the inhibitory effect of NK-1 receptor activation is to a certain extent curtailed by the facilitatory consequences of NK-2 receptor activation.

The current findings demonstrate that different receptors account for the complex effects of the receptor-nonselective tachykinin SP first to stimulate and then to inhibit peristaltic motility. This conclusion negates the possibility that the delayed inhibition of peristalsis elicited by SP is a mere reflection of desensitization to the endogenously released peptide. In order to comprehend the motor actions of SP and the underlying mechanisms, it is necessary to consider the distribution of tachykinin receptors in the gut. There is evidence that in the guinea pig intestine, both NK-1 and NK-2 receptors are present on circular muscle cells (Maggi *et al.*, 1990, 1994b, 1994c; Barthó *et al.*, 1992; Holzer *et al.*, 1993), whereas NK-3 receptors are located exclusively on enteric neurons (Laufer *et al.*, 1986; Maggi *et al.*, 1993a, 1993b, 1994c). It would hence seem that the stimulant effect of SP on peristalsis is due to activation of muscular NK-2 and neuronal NK-3 receptors. It remains to be shown, however, whether the NK-1 receptor-mediated inhibition of peristaltic performance is related to a NK-1 receptor-mediated inhibition of acetylcholine release from myenteric neurons (Kilbinger *et al.*, 1986; Löffler *et al.*, 1994) and whether the NK-3 receptor-mediated attenuation of propulsive motility arises from the activation of inhibitory enteric pathways (Maggi *et al.*, 1993a, 1994c).

Further support for a role of NK-1 and NK-2 receptors in modulation of the peristaltic reflex comes from the motor effects that CP-99,994 and SR-48,968 themselves have (table 3). The small facilitation of peristalsis (decrease in pressure threshold) seen with CP-99,994 and with another nonpeptide

NK-1 antagonist, SR-140,333 (Emonds-Alt *et al.*, 1993), confirms a similar observation previously made with the NK-1 antagonist CP-96,345 (Holzer and Maggi, 1994). The consistent, though small, stimulation of peristaltic performance obtained with these different NK-1 antagonists suggests that endogenously released tachykinins can dampen peristaltic activity by an action on NK-1 receptors (Holzer and Maggi, 1994). Conversely, the slight inhibitory motor effect of SR-48,968 and other NK-2 receptor-selective antagonists (Holzer and Maggi, 1994) points to a facilitatory influence of NK-2 receptors activated by endogenous tachykinins.

Taken together, these findings and considerations indicate that the modulatory effect of tachykinins on intestinal peristalsis involves the activation of multiple tachykinin receptors on muscle cells and enteric neurons. Depending on their location and transduction mechanisms, these receptors give rise to either facilitation or inhibition of peristaltic performance. The current findings also emphasize the need for tachykinin receptor-selective agonists and antagonists, the availability of which is an essential prerequisite to elucidating the physiological, pharmacological and pathophysiological significance of tachykinins in the control of intestinal motility.

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