Neurokinin A-Induced Vasoconstriction and Muscular Contraction in the Rat Isolated Stomach: Mediation by Distinct and Unusual Neurokinin\textsubscript{2} Receptors\textsuperscript{1}

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Accepted for publication February 4, 1997

ABSTRACT
This study examined the pharmacological identity of the tachykinin receptors which in the rat stomach mediate vasoconstriction and muscular contraction. The vasculature of the rat isolated stomach was perfused with oxygenated Krebs buffer containing 3\% dextran. Vasoconstrictor responses were recorded as increases in the vascular perfusion pressure and gastric contractions were measured as increases in the intraluminal pressure. By examining the effects of selective agonists and antagonists for tachykinin neurokinin (NK\textsubscript{1}), NK\textsubscript{2} and NK\textsubscript{3} receptors it was found that the vasculature contained only NK\textsubscript{2} receptors that were activated by the NK\textsubscript{2} receptor agonist [\textit{b}\textit{Ala}\textsuperscript{8}]-NKA-(4–10) and inhibited by the NK\textsubscript{2} receptor antagonists MEN-10,627 and GR-94,800. However, the vasoconstrictor action of NKA was blocked only when the preparations were exposed to a combination of NK\textsubscript{1}, NK\textsubscript{2} and NK\textsubscript{3} receptor antagonists (SR-140,333, MEN-10,627, PD-161,182). In contrast, the NKA-evoked contraction of the gastric musculature was suppressed by NK\textsubscript{2} receptor antagonists but little affected by NK\textsubscript{1} or NK\textsubscript{3} receptor antagonists. This observation was consistent with the predominance of NK\textsubscript{2} receptors on the muscle as revealed by the effects of receptor-selective NK\textsubscript{1}, NK\textsubscript{2} and NK\textsubscript{3} agonists and antagonists. These results demonstrate that the major tachykinin receptor type present on the gastric vasculature and musculature is a NK\textsubscript{2} receptor that is sensitive to receptor-selective agonists and antagonists. The NKA-evoked gastric contraction is also primarily due to NK\textsubscript{2} receptor activation, whereas the NKA-induced vasoconstriction is mediated by a distinct and unusual type of NK\textsubscript{2}-like receptor that is blocked by a combination of NK\textsubscript{1}, NK\textsubscript{2} and NK\textsubscript{3} receptor antagonists only.

Received for publication September 18, 1996.
\textsuperscript{1}This work was supported by the Austrian Science Foundation (FWF Grants P9473-MED and P11834-MED).

ABBREVIATIONS: BANKA, [\textit{b}\textit{Ala}\textsuperscript{8}]-NKA-(4–10); NK, neurokinin; NKA, neurokinin A; SP, substance P; SPOME, substance P methyl ester.

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Methods

Tissue preparation. All experiments of this study were approved by the Federal Ministry of Science and Research of the Republic of Austria. Sprague-Dawley rats (Institut für Versuchstierkunde, Himberg, Austria) of either sex weighing 250 to 400 g were used. Before the experiments the rats were fasted overnight but allowed free access to water. After induction of anesthesia with pentobarbital (50 mg kg⁻¹ i.p.) the rats were laparatomized to expose the stomach and the abdominal aorta. The junction of the aorta with the celiac artery was cleared of surrounding tissue, the hepatic and splenic artery ligated and the celiac artery cannulated from the aorta with a blunted 23-gauge needle. Immediately after cannulation, the vasculature of the stomach was flushed free of blood with 10 ml Krebs buffer (for composition see below) containing heparin (20 IU ml⁻¹). Care was taken to rinse the preparation as gently as possible so as to preserve the integrity of the vasculature.

Thereafter, the stomach was excised, transferred to a perfusion apparatus which consisted of an inclined plexiglass pad maintained at 37°C and covered with parafilm to avoid dehydration (Holzer et al., 1993). Oxygenated Krebs buffer of pH 7.4 enriched with dextran (20 IU ml⁻¹) and kept at 37°C was perfused through the preparation with a peristaltic pump (Gilson, Villiers le Bel, France) at a rate of 1.8 ml min⁻¹. The perfusate left the preparation via the gastric veins that were cut when the stomach was excised. The perfusion pressure was measured with a pressure transducer (ISOTEC, HSE, March-Hugstetten, Germany) connected to the inflow cannula, and the amplified signals were fed into a personal computer via an analog-digital converter. All test substances were infused via a side arm close to the stomach at a rate calculated to give the final concentrations indicated in the text.

After the lumen of the stomach had been flushed with saline to remove any solid contents, the esophagus was cannulated and connected to a syringe. Then the stomach was filled with 5 ml saline via another cannula inserted through the pylorus. This catheter was connected to a pressure transducer (ISOTEC) and gastric luminal pressure displayed on a chart recorder (ABB Goerz, Vienna, Austria).

Experimental protocol. Each preparation was equilibrated for 30 min and then standardized by bolus injections of 20 nmol norepinephrine that caused maximal constriction of the vasculature. As soon as the responses to norepinephrine were stable, cumulative or intermittent at 5-min intervals (n = 47). Before and after any exposure to agonists or antagonists, the viability of the preparations was checked by the consistency of the constrictor responses to norepinephrine.

Drugs and solutions. SP, SPOME (Bachem, Bubendorf, Switzerland) and NKA (Peptide Institute, Osaka, Japan) were dissolved in 0.1 M acetic acid. BANKA, senktide (succinyl-[Asp⁶,N-MePhe⁸]-SP-6–11); Neosystem, Strasbourg, France) and the tachykinin antagonists [1,4]-diazepine; Boehringer-Ingelheim KG, Ingelheim, Germany) and WEB 2170 (5-(2-chlorophenyl)-3,4-dihydro-10-methyl-3-(4-morpholinyl)-1-carboxylic acid 2HCl, 7H-cyclopenta[4,5]thiophene[3,2-f][1,2,4]triazolo-[4,3-a][1,4]diazepine; Boehringer-Ingelheim KG, Ingelheim, Germany) were dissolved in saline.

For administration to the vascularly perfused stomach all stock solutions were diluted with Krebs buffer. The composition of the Krebs buffer solution in mM was: NaCl 118.8, KCl 4.7, MgSO₄ 1.18, CaCl₂ 2.5, KH₂PO₄ 1.18, NaHCO₃ 25.0, glucose 1.1. The pH of the buffer was kept at 7.4 by gassing it with a mixture of 5% CO₂ and 95% O₂. For the vascular perfusion, dextran F70 (Serva, Heidelberg, Germany) was added to the Krebs buffer at a concentration of 3%.

Statistical analysis and evaluation of results. Agonist-induced vasoconstriction was expressed as increment of the vascular perfusion pressure above the base-line pressure (Δ mm Hg). Because the base-line intraluminal pressure was close to 0, muscle contraction was expressed as the intraluminal pressure (mm H₂O) attained in the presence of the agonist concentrations under study. All figures for vasoconstriction and muscle contraction refer to the peak effects produced by the respective agonist concentrations. To compare agonist potencies, EC₅₀ values were roughly estimated by extrapolation from the average concentration-response curves shown in the graphs. The extrapolation was carried out with the curve fitting software ORIGIN (version 2.24, MicroCal, Northampton, MA) (Meddings et al., 1989).

Statistical evaluation of the results was performed with the Wilcoxon signed rank test or Friedman test as appropriate. Probability values of P < .05 were regarded as significant. Although nonparametric statistical tests were used, the data are presented as means ± S.E.M. for ease of understanding and simplicity of presentation.

Results

Vascular constriction. First, the gastric vasconstrictor action of selective tachykinin agonists and their antagonism by selective tachykinin antagonists were investigated. Control experiments showed that perfusion of the gastric vasculature with NKA (1 nM–10 μM) given in a cumulative manner caused a concentration-dependent increase in the perfusion pressure indicative of vasoconstriction. The EC₅₀ of NKA was extrapolated to be 0.29 μM and the maximal effect amounted to 35 to 40% of the constriction produced by a bolus of 20 nmol norepinephrine (fig. 1, A and B) which was a maximally effective dose. Concentration-response curves for NKA remained stable during a period of 4 hr (fig. 1A). Perfusion of dimethyl sulfoxide at the highest concentration (1.5 U) that was ever used in the agonist/antagonist experiments failed to cause any significant change in the response of the gastric vasculature to NKA (fig. 1B) and norepinephrine over a period of 4 hr.

To characterize the tachykinin receptors causing constriction of the rat gastric vasculature the following receptor-selective agonists were used: SPOME (0.01 - 100 μM) as NK₁ receptor agonist, BANKA (0.01–10 μM) as NK₂ receptor agonist and senktide (0.01–100 μM) as NK₃ receptor agonist. The receptors were further characterized by the use of receptor-selective antagonists: SR-140,333 (0.1 μM, contact time 60 min) as NK₁ agonists.
antagonist, MEN-10,627 (1 μM, contact time 30 min) or GR-94,800 (0.1 μM, contact time 60 min) as NK2 antagonists and PD-161,182 (1 μM, contact time 60 min) as NK3 antagonist.

The NK1 agonist SPOME caused a concentration-dependent constriction of the rat gastric vasculature (fig. 1C). With an extrapolated EC$_{50}$ of 5.84 μM SPOME was considerably less potent than NKA (extrapolated EC$_{50}$ 0.29 μM) whereas the efficacy of SPOME closely resembled that of NKA (fig. 1, A-C). The action of SPOME to enhance the vascular perfusion pressure remained unaltered in the presence of the NK1 antagonist SR-140,333 (fig. 1C). Senktide, a NK3 agonist, was only weakly active in constricting the gastric vasculature. Because the responses were variable and of very low amplitude (fig. 1D) it was not possible to estimate the EC$_{50}$. Exposure of the preparations to the NK3 antagonist PD-161,182 failed to alter the constrictor effect of senktide (fig. 1D).

Among the receptor-selective agonists, the NK2 agonist BANKA was the most active compound to raise the vascular perfusion pressure (fig. 1, E and F). With an extrapolated EC$_{50}$ of 0.13 μM BANKA was more potent than NKA (extrapolated EC$_{50}$ 0.29 μM) whereas the efficacy of the two agonists was very similar (figs. 1, A, B, E and F). The vasoconstrictor action of BANKA was significantly inhibited in the presence of the NK2 receptor antagonists MEN-10,627 (fig. 1E) and GR-94,800 (fig. 1F).

Having found that selective NK2 receptor activation is an efficacious way to constrict the gastric vasculature, we went on to analyze the tachykinin receptors that are responsible for the constrictor responses to the naturally occurring tachykinin receptor ligands SP and NKA. Both tachykinins caused a concentration-dependent increase in vascular perfusion pressure, but SP (extrapolated EC$_{50}$ 1.64 μM) was less potent than NKA (extrapolated EC$_{50}$ 0.29 μM). The concentration-response curve for SP remained unaltered in the presence of the NK1 receptor antagonists MEN-10,627 and the NK3 antagonist PD-161,182 (fig. 2A).

The concentration-response curve for NKA remained unaltered by the NK1 antagonist SR-140,333, which was also true.
when the preparations treated with SR-140,333 were in addition exposed to the NK\(_3\) antagonist MEN-10,627 (fig. 3A). Another antagonist of NK\(_1\) receptors, RP-67,580 (1 \(\mu\)M, contact time 30 min), was similarly ineffective in antagonizing the vasoconstrictor action of NKA (data not shown, \(n = 6\)). Unlike the vasoconstrictor effect of BANKA (fig. 1, E and F), the NKA-induced rise of vascular perfusion pressure was not antagonized by MEN-10,627 (fig. 3B). When the preparations exposed to MEN-10,627 were later treated with a combination of MEN-10,627 and SR-140,333, there was a slight yet significant shift of the NKA concentration-response curve to the right (fig. 3B). Similarly, a small change in the activity of NKA to constrict the gastric vasculature was seen when the NKA concentration-response curve was recorded in the presence of the NK\(_3\) antagonist PD-161,182, alone or in combination with SR-140,333 (fig. 3C). In contrast, a combination of PD-161,182 with the NK\(_2\) antagonist MEN-10,627 attenuated the vasoconstrictor response to high NKA concentrations (1–10 \(\mu\)M) only, whereas simultaneous administration of SR-140,333, MEN-10,627 and PD-161,182 resulted in complete inhibition of the NKA-induced vasoconstriction (fig. 3D).

The possible involvement of a variety of other vasoactive mediators in the vasoconstrictor response to NKA was exam-
ined with the following drugs (final concentration, contact time and appropriate reference given in brackets): N\(^6\)-nitro-L-arginine methyl ester (nitric oxide synthase inhibitor; 40 \(\mu\)M, 30 min) (Zagorodnyuk et al., 1995), indomethacin (cyclooxygenase inhibitor; 1 \(\mu\)M, 30 min) (Zagorodnyuk et al., 1995), ketotifen (mast cell stabilizer; 1 \(\mu\)M, 30 min) (Grant et al., 1990), bosentan (endothelin receptor antagonist; 1 \(\mu\)M, 45 min) (Clozel et al., 1994), guanethidine (noradrenergic neuron blocking drug; 5 \(\mu\)M, 30 min) (Zagorodnyuk et al., 1995), WEB 2086 and WEB 2170 (platelet-activating factor receptor antagonists; 1 \(\mu\)M, 30 min) (Weber and Heuer, 1989), granisetron (5-hydroxytryptamine 5-HT\(_3\) receptor antagonist; 1 \(\mu\)M, 15 min) (Sanger and Nelson, 1989) and methysergide (5-hydroxytryptamine 5-HT\(_2\) receptor antagonist; 1 \(\mu\)M, 15 min) (Kilbinger and Pfeuffer-Friederich, 1985). None of these substances had any significant influence on the NKA-induced vasoconstriction or gastric muscle contraction (data not shown, \(n = 4–7\)).

None of the tachykinin antagonists, alone or in combination and none of the other drugs we examined had any significant effect on the constrictor responses to norepinephrine (20 nmol) as tested in every experiment.

**Muscle contraction.** Concomitantly with the vascular perfusion pressure the intraluminal pressure in the stomach, an index of gastric muscular tone, was measured in all experiments. As found in the initial control experiments, NKA caused vigorous contractions of the gastric musculature, which were concentration dependent and long lasting (fig. 4A). However, bolus administration of 20 nmol norepinephrine, which was performed after each recording of a tachykinin concentration-response curve, stopped any contraction and relaxed the gastric muscle to baseline levels. The concentration-response curve for NKA was reproducible for up to 4 hr, the longest period that was tested (fig. 4A). Vascular perfusion of dimethyl sulfoxide, at a concentration equivalent to that used in the antagonist experiments (1.5 U), for a period of 4 hr did not alter the contractile activity of NKA (fig. 4B).

As in the vasoconstriction experiments, the tachykinin receptors causing gastric muscle contraction were characterized by the use of receptor-selective agonists and antagonists. Both the NK\(_1\) agonist SPOME (fig. 4C) and the NK\(_3\) agonist senktide (fig. 4D) were virtually inactive in contracting the gastric musculature. Because of the minute efficacy of these agonists it was not possible to extrapolate the EC\(_{50}\) of

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**Fig. 4.** Concentration-response curves for the effect of tachykinins to induce muscle contraction in the isolated vascularly perfused rat stomach in the absence and presence of various tachykinin antagonists. Muscle contraction is expressed as the peak intraluminal pressure (mm H\(_2\)O) attained in the presence of the agonist concentrations under study. The data are presented as means \(\pm\) S.E.M. of 6 to 10 experiments. *\(P < .05\) vs. control (open circles). A, Effect of NKA at time 0 and after an interval of 4 hr. B, Effect of NKA in the absence and presence of the antagonist vehicle (1.5 U dimethyl sulfoxide). C, Effect of SPOME in the presence of vehicle or SR-140,333 (0.1 \(\mu\)M). D, Effect of senktide in the presence of vehicle or PD-161,182 (1 \(\mu\)M). E, Effect of BANKA in the presence of vehicle or MEN-10,627 (1 \(\mu\)M). F, Effect of BANKA in the presence of vehicle or GR-94,800 (1 \(\mu\)M).
SPOME and senktide. However, it is obvious from figure 4, C and D that the very small contractions elicited by SPOME and senktide were blocked by the NK₁ antagonist SR-140,333 and the NK₂ antagonist PD-161,182, respectively. Conversely, the NK₃ agonist BANKA (extrapolated EC₅₀ 0.036 μM) was more potent than NKA (extrapolated EC₅₀ 0.11 μM) in causing gastric muscular contraction whereas the efficacies of the two agonists were similar (fig. 4, A, B, E and F). The contractile action of BANKA was blocked by the NK₂ antagonists MEN-10,627 (fig. 4E) and GR-94,800 (fig. 4F).

Of the two endogenous tachykinins tested, SP (fig. 2B) was evidently less potent (extrapolated EC₅₀ 1.87 μM) and efficacious in contracting the gastric muscle than NKA (fig. 5). The SP-evoked contractions were left unaltered by SR-140,333 and PD-161,182, but abolished by MEN-10,627 (fig. 2B).

The receptor pharmacology of the contractile effect of NKA (fig. 5) was similar to that of the SP response (fig. 2B). SR-140,333 caused only a slight but significant shift of the NKA concentration-response curve to the right (fig. 5A) whereas MEN-10,627 suppressed the NKA-induced contraction (fig. 5B). When SR-140,333 was administered to preparations treated with MEN-10,627, the contractile responses to NKA were virtually abolished (fig. 5B). The same result was obtained when, vice versa, MEN-10,627 was administered to preparations that had been exposed to SR-140,333 beforehand (fig. 5A). The ability of NKA to contract the gastric musculature remained unchanged by PD-161,182 (fig. 5C) or a combination of PD-161,182 and SR-140,333 (fig. 5C). When a mixture of MEN-10,627 and PD-161,182 was perfused through the gastric vasculature, a nearly complete inhibition of the NKA-evoked muscular contractions was observed (fig. 5D). Perfusion of SR-140,333, MEN-10,627 and PD-161,182 in combination abolished the contractile response to NKA (fig. 5D).

**Discussion**

The current data demonstrate that the tachykinins SP and NKA contract the musculature and constrict the vasculature of the rat isolated stomach. Pharmacological analysis has shown that the contractile and constrictor actions are not related to each other, because they are differentially inhibited by tachykinin receptor-selective antagonists or combinations thereof.

As regards the contraction of the gastric musculature, our data indicate that this response to tachykinins is predominantly mediated by NK₂ receptors. Accordingly, the order of potency in inducing gastric muscular contractions is BANKA > NKA > SP, BANKA and NKA being most efficacious. In contrast, NK₁ receptor activation with SPOME and NK₃ receptor activation with senktide (Wormser et al., 1986) has been proved by the antagonism of their contractile actions with receptor-selective antagonists. For this purpose, the NK₁ receptor antagonists SR-140,333 (Emonds-Alt et al., 1993) and RP-67,580, a combi-
pound selective for the rat type NK₁ receptor (Garret et al., 1991), the NK₂ receptor antagonist MEN-10,627 (Maggi et al., 1994) and the NK₃ receptor antagonist PD-161,182 (Boden et al., 1996) were chosen and used at concentrations that were both effective and receptor-selective.

The predominance of NK₂ receptors on the gastric musculature is further reflected by the observation that the contractile responses to the naturally occurring tachykinins SP and NKA are blocked by a NK₂ antagonist. In addition, a small but distinct component in the gastric contractions evoked by NKA, but not SP, is due to NK₁ receptor activation whereas NK₃ receptors do not seem to contribute. The prevalence of NK₂ receptors in the rat gastric musculature is in keeping with other in vitro and in vivo experiments that have analyzed the potency rank order with which various tachykinin receptor ligands contract the rat gastric fundus (Burcher et al., 1993; Mussap and Burcher, 1993; Smits and Lefebvre, 1994) and corpus (Holzer-Petsche et al., 1987; Holzer-Petsche, 1991). These data are extended by the use of receptor-selective agonists and unequivocally proved by the inhibitory action of receptor-selective antagonists. Experiments with the rat gastric fundus in vitro have also shown that the contractile response to tachykinins is blocked by NK₂ receptor antagonists but left unaltered by blockade of NK₁ receptors (Mussap and Burcher, 1993; Smits and Lefebvre, 1994).

Although not analyzed in detail, it seems likely that the NK₂ receptors in the rat gastric musculature are located directly on the muscle, because the motor effects of NKA in strips of the rat gastric corpus (Holzer-Petsche et al., 1987) and that of tachykinins in strips of the rat gastric fundus (Mussap and Burcher, 1993; Smits and Lefebvre, 1994) are left unaltered by atropine and tetrodotoxin. To the contrary, the ability of SP to contract the rat gastric corpus is inhibited by tetrodotoxin and atropine (Holzer-Petsche et al., 1987), which is consistent with the presence of NK₁ receptor-like immunoreactivity on neurons of the myenteric plexus in the rat stomach (Sternini et al., 1995). However, our findings indicate that these neuronal NK₁ receptors contribute little to the contractile action of SP in the rat isolated stomach. Further evidence for a direct action of NKA on the gastric muscle comes from the failure of N³-nitro-L-arginine methyl ester, indomethacin, ketotifen, boventan, guanethidine, BAY × 1005, WEB 2086, WEB 2170, granisetron and methysergide to alter the NKA-evoked contractions.

The major aim of our study was to identify the receptors that mediate the constrictor action of tachykinins on the rat gastric vasculature. This question was addressed in a modified preparation of the vascularly perfused rat isolated stomach (Holzer et al., 1993) that was drained via its cut veins. Because changes in the vascular tone were measured via changes in the inflow perfusion pressure, it is assumed that the tachykinin-induced pressor effects reflect constrictor responses of the gastric resistance vessels (small arteries and arterioles), whereas the venous side of the system is unlikely to make a significant contribution to the overall responses. The preparation was established to stay viable for a period of at least 4 hr, during which time the responses to tachykinins and norepinephrine were reproducible and insensitive to the concentrations of dimethyl sulfoxide that served as vehicle for the water-insoluble tachykinin receptor agonists and antagonists.

The data obtained with this preparation indicate that tachykinins constric the resistance vessels of the rat stomach, as demonstrated by a concentration-dependent increase in the vascular perfusion pressure, and that the receptors by which NKA causes vasoconstriction are different from those by which the peptide gives rise to gastric contraction. A further level of complexity is added by the finding that the receptor pharmacology of the NKA-evoked vasoconstriction is at variance with the tachykinin receptor distribution characterized with receptor-selective agonists. The potency rank order (BANKA > NKA > SP > SPOME/senktide) with which various tachykinin receptor agonists contract the gastric vasculature suggests that the major type of tachykinin receptor present on the vasculature is the NK₂ receptor. This conjecture has been corroborated by the high efficacy of BANKA and NKA as compared to that of SPOME and senktide and by the ability of two NK₂ antagonists, MEN-10,627 (Maggi et al., 1994) and GR-94,800 (McElroy et al., 1992), to suppress the response to BANKA. Whether the small vasoconstrictor effects of SPOME and senktide are also mediated by NK₂ receptors is not possible to deduce from the present data. However, it need be assumed that in the rat gastric vasculature SPOME and senktide are not selective NK₁ and NK₃ receptor agonists, because they were not antagonized by SR-140,333 and PD-161,182, respectively.

The similar potency and efficacy of BANKA and NKA in constricting the gastric vasculature predicts that NK₂ receptors mediate the action of either agonist. However, this surmise was not confirmed by pharmacological analysis of the vasoconstrictor responses to NKA and SP. Neither NK₂ nor NK₁ or NK₃ receptor antagonists inhibited the action of the two naturally occurring tachykinins to any substantial degree. Further experiments revealed that combinations of NK₁ and NK₃ or NK₁ and NK₃ antagonists were similarly ineffective whereas a mixture of NK₁ and NK₃ antagonists caused a partial inhibition of the NKA effect. In contrast, combined administration of NK₁, NK₂ and NK₃ receptor antagonists abolished the NKA-evoked vasoconstriction.

Although these results cannot be conclusively explained, they represent a novel trait in tachykinin receptor pharmacology. The differential inhibition of the vasoconstrictor effects of BANKA and NKA by various tachykinin receptor antagonists or combinations thereof is subject to more than one way of interpretation. Given that no evidence for the presence of vascular NK₁ and NK₃ receptors was found with the use of receptor-selective agonists and antagonists, the possibility of NK₁, NK₂ and NK₃ receptors synergizing in the vasoconstrictor action of NKA can be dismissed. Whether the vasoconstrictor tachykinin receptor operated by NKA is a subtype of a NK₂ receptor that is distinct from the NK₂ receptor on the gastric musculature will not be possible to find out without molecular pharmacological techniques.

It is conceivable in this context that BANKA and NKA interact with different binding and/or transduction epitopes (Maggi, 1995; Schwartz et al., 1995) on a common receptor that gives rise to vasoconstriction. If so, the existence of binding domains for NK₁, NK₂ and NK₃ receptor antagonists on the vascular tachykinin receptor operated by NKA has to be assumed. Together with the surmise that BANKA and NKA bind to different sites on the receptor molecule, a differential allosteric interaction between the antagonist binding domains and the BANKA and NKA binding epitopes,
respectively (Schwartz et al., 1995), may be anticipated. With respect to NKA, only combined administration of NK1, NK2 and NK3 receptor antagonists will alter the conformation of the receptor molecule in such a way that receptor activation by the antagonist is prevented. This assumption is in keeping with the noncompetitive nature of the antagonism that characterizes the ability of tachykinin antagonists to suppress the vasoconstrictor effects of BANKA and NKA. Whatever the molecular basis of these findings, the observation that the vasoconstrictor effects of BANKA and NKA fail to evoke gastric mucosal vasodilatation (Holzer and Guth, 1991), that NKA (Heinemann et al., 1996) and NKA-related peptides (Stroff et al., 1996) reduce gastric mucosal blood flow and that SP constricts gastric mucosal venules in a leukotriene C4-independent manner (Katori et al., 1993). Tachykinin-evoked constriction is also seen in the rat isolated mesenteric and portal venous bed in which NK3 receptors seem to play a predominant role (Mastrangelo et al., 1987; D’Orleans-Juste et al., 1991; Clai ng et al., 1992).

In summary, our study has shown that tachykinins have two distinct actions on the isolated, vascularly perfused stomach of the rat: muscle contraction and vasoconstriction. Pharmacological analysis has revealed that the muscle contraction is primarily due to activation of NK1 receptors. The gastric vasculature similarly contains NK3 receptors but the vasoconstrictor action of NK3 is suppressed only when a combination of NK1, NK2 and NK3 receptor antagonists is administered. This unprecedented complexity in tachykinin pharmacology awaits elucidation at the level of the receptor molecule.

Acknowledgments

The authors thank Dr. A. Heinemann for technical support, Mr. W. Schlueter for organizational help and Dr. C. A. Maggi for helpful discussions and suggestions on this work. The gifts of MEN-10,627 (Dr. C. A. Maggi, A. Menarini, Florence, Italy), RP-67,580 (Dr. C. Garret, Rhône-Poulenc Rorer, Vitry sur Seine, France), SR-140,333 (Dr. X. Emonds-Alt, Sanofi, Montpellier, France), PD-16,182 (Drs. D. C. Horwell and M. C. Pritchard, Parke-Davis, Cambridge, U.K.), bosentan (Dr. M. Clozel, Hoffmann-La Roche, Basel, Switzerland), griseatrin (Dr. G. J. Sanger, SmithKline Beecham, Welwyn, U.K.), WEB 2086 and WEB 2170 (Dr. H. O. Heuer, Boehringer-Ingelheim KG, Ingelheim, Germany) and BAY X1005 (Drs. E. Möller and M. Mardin, Bayer, Leverkusen, Germany) are gratefully acknowledged.

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