

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/13807080>

Characterization of receptors mediating contraction induced by tachykinins in the guinea-pig isolated common bile duct

ARTICLE *in* BRITISH JOURNAL OF PHARMACOLOGY · DECEMBER 1997

Impact Factor: 4.84 · DOI: 10.1038/sj.bjp.0701560 · Source: PubMed

CITATIONS

19

READS

26

3 AUTHORS:



Riccardo Patacchini

Chiesi Farmaceutici S.p.A.

267 PUBLICATIONS 7,642 CITATIONS

SEE PROFILE



Lorand Bartho

University of Pécs

121 PUBLICATIONS 3,021 CITATIONS

SEE PROFILE



Carlo Alberto Maggi

819 PUBLICATIONS 23,857 CITATIONS

SEE PROFILE



Characterization of receptors mediating contraction induced by tachykinins in the guinea-pig isolated common bile duct

¹Riccardo Patacchini, *Lorand Barthó & Carlo Alberto Maggi

Pharmacology Department, Menarini Ricerche SpA, via Rismondo 12/A, 50131, Florence, Italy and *Pharmacology Department, University Medical School of Pécs, Pécs, Hungary

1 We studied the effect of the natural tachykinins and of synthetic agonists selective for the tachykinin NK₁, NK₂ and NK₃ receptors, on the motility of guinea-pig isolated common bile duct longitudinally-oriented smooth muscle.

2 All the tachykinins tested (both natural and synthetic) produced a concentration-dependent contractile response of the guinea-pig isolated common bile duct: these effects underwent a marked tachyphylaxis, especially the responses elicited by NK₁ and NK₃ receptor-selective agonists.

3 Among the natural tachykinins neurokinin B (EC₅₀ = 3.2 nM; 95% c.l. = 2.0–5.1; *n* = 4) was the most potent, being about 40 and 25 fold more potent than substance P (EC₅₀ = 121.6 nM; 95% c.l. = 94–157; *P* < 0.01; *n* = 4) and neurokinin A (EC₅₀ = 83.4 nM; 95% c.l. = 62–112; *P* < 0.01; *n* = 4), respectively. Among the synthetic analogues the NK₃ receptor-selective agonist senktide (EC₅₀ = 1.1 nM; 95% c.l. = 0.7–1.8; *n* = 8) was the most potent, being about 120, 110 and 20 fold more potent than [Sar⁹]substance P sulfone (NK₁ receptor-selective) (EC₅₀ = 130.4 nM; 95% c.l. = 99–172; *P* < 0.01; *n* = 8), [βAla⁸]NKA (4–10) (NK₂ receptor-selective) (EC₅₀ = 120.1 nM; 95% c.l. = 95–151; *P* < 0.01; *n* = 8) and septide (NK₁ receptor-selective) (EC₅₀ = 22.6 nM; 95% c.l. = 18–28; *P* < 0.01; *n* = 8), respectively. All tachykinins (natural or synthetic receptor agonists) produced a similar E_{max}, averaging about 50% of that produced by KCl (80 mM).

4 Atropine (1 μM) did not affect the responses to either NK₁ or NK₂ receptor-selective agonists, whereas it reduced the E_{max} of senktide by about 50%, without affecting its potency (EC₅₀). Tetrodotoxin (1 μM) totally blocked senktide-induced contractions, as did the combined pretreatment with atropine plus the tachykinin NK₁ and NK₂ receptor-selective antagonists GR 82334 and MEN 11420 (1 μM each), respectively.

5 GR 82334 (1 μM) blocked with apparent competitive kinetics septide- (apparent pK_B = 7.46 ± 0.10; *n* = 5) and [Sar⁹]substance P sulfone- (apparent pK_B = 6.80 ± 0.04; *n* = 4) induced contractions. MEN 11420 (30–300 nM), a novel potent NK₂ receptor antagonist, potently antagonized [βAla⁸]NKA (4–10), with competitive kinetics (pK_B = 8.25 ± 0.08; *n* = 12; Schild plot slope = –0.90; 95% c.l. = –1.4; –0.35). The NK₃ receptor-selective antagonist SR 142801 (30 nM) produced insurmountable antagonism of the senktide-induced contractions (E_{max} inhibited by 64%). None of the above antagonists, tested at the highest concentrations employed against tachykinins, affected the concentration–response curve to methacholine (0.1–300 μM).

6 We conclude that tachykinins produce contraction of the guinea-pig isolated common bile duct by stimulating NK₁, NK₂ and NK₃ receptors. The responses obtained by activating NK₁ and NK₂ receptors are atropine-resistant. The contraction obtained by stimulating NK₃ receptors is totally neurogenic, being mediated by the release of endogenous acetylcholine and tachykinins; the latter act, in turn, on postjunctional tachykinin NK₁/NK₂ receptors. The role of the NK₃ receptor as prejunctional mediator of the excitatory transmission operated by tachykinins is discussed.

Keywords: Guinea-pig common bile duct; tachykinins; tachykinin receptors; tachykinin receptor antagonists; MEN 11420

Introduction

The tachykinins are a family of neuropeptides distributed in the mammalian central and peripheral nervous system: they produce a wide range of biological effects through the stimulation of at least three distinct receptor types, termed NK₁, NK₂ and NK₃ (Regoli *et al.*, 1989; Guard & Watson, 1991; Maggi *et al.*, 1993b). Recently, evidence has been provided for the existence of two structurally distinct subtypes of the NK₃ receptor (Krause *et al.*, 1997). With regard to the NK₁ receptor, the existence of NK₁ receptor subtypes has also been proposed, based on functional but not structural evidence (Petitet *et al.*, 1992): more recently these data have been accounted for by a theory which postulates the existence of two different conformers of the NK₁ receptor (i.e. the 'general tachykinin' and the 'substance P preferring' conformers) (Hastrup & Schwartz, 1996).

Both anatomical and biochemical evidence indicates the existence of tachykinin-like immunoreactivity (TK-LI) throughout the gastrointestinal tract of several species: at this level tachykinins play a role as excitatory neurotransmitters (Barthó & Holzer, 1985; Maggi, 1995, for reviews). In the smooth muscles of the mammalian gastrointestinal tract tachykinins almost invariably produce contraction, either if applied exogenously, or released from intrinsic neurones and/or from peripheral endings of capsaicin-sensitive primary afferents (Maggi, 1995, for review). Each one of the three tachykinin receptors may be involved in mediating contractions produced by tachykinins in the gastrointestinal tract, although the relative contribution of each receptor type varies greatly with both the intestinal segment and the species considered (Maggi *et al.*, 1993b, for review). Previous studies have shown that tachykinins produce excitatory motor responses in the cat (Dahlstrand *et al.*, 1988), dog (Guo *et al.*, 1989), human (Feeley *et al.*, 1987) and guinea-pig gallbladder (e.g. Shook &

¹ Author for correspondence.

Burks, 1987; Meldrum *et al.*, 1987; Maggi *et al.*, 1989): in the latter species the tachykinin-induced contraction is mediated by activation of receptors of the NK₂ type only (Patacchini & Maggi, 1992). In contrast little, if any, information is available about tachykinin effect(s) on the motility of the biliary tract.

The present work has been designed to study the effect of natural tachykinins (substance P, neurokinin A, and neurokinin B) on the guinea-pig isolated common bile duct. To characterize the receptor type(s) mediating the response to tachykinins in this preparation, we used several synthetic agonists showing improved selectivity for the NK₁, NK₂ and NK₃ receptors, and the following tachykinin receptor-selective antagonists: GR 82334 (NK₁ receptor-selective; Hagan *et al.*, 1991), MEN 11420 (NK₂ receptor-selective; Santicoli & Maggi, 1997) and SR 142801 (NK₃ receptor-selective; Emonds-Alt *et al.*, 1995; Patacchini *et al.*, 1995a).

Methods

General

Male albino guinea-pigs (300–350 g) were stunned and bled. The common bile duct, including the terminal enlarged segment (or *ampulla*: see Figure 1), was carefully dissected from surrounding tissue and excised from the outer surface of the duodenum to the junction with the hepatic duct. The duct was placed in a Petri dish filled with warmed and oxygenated (96% O₂ and 4% CO₂) Krebs solution of the following composition (mM): NaCl 119, NaHCO₃ 25, KH₂PO₄ 1.2, MgSO₄ 1.5, CaCl₂ 2.5, KCl 4.7 and glucose 11. The preparation was then tied at each end with cotton threads, placed in a 5 ml organ bath and connected to an isotonic transducer (load 2 mN) for recording mechanical activity along its longitudinal axis. For some experiments in which an internal control was required, the duct was opened along its longitudinal axis and cut in two parallel strips. The strips were then suspended against a load of 1.0–1.5 mN. All the experiments commenced after an equilibration period of 90–120 min.

Contractile responses to the natural tachykinins and to the tachykinin receptor-selective agonists septide and [Sar⁹]substance P sulfone (NK₁ receptor), [β Ala⁸]NKA (4–10) (NK₂ receptor) and senktide (NK₃ receptor), were studied either in

the absence or in the presence of atropine (1 μ M). Cumulative concentration–response curves to these agonists were constructed, by adding the next concentration of peptide when the effect of the preceding one had reached a steady state. The contractile responses to tachykinins (both natural and synthetic) underwent a marked tachyphylaxis, particularly the responses elicited by NK₁ and NK₃ receptor-selective agonists. To prevent this phenomenon, cumulative curves to NK₁ and NK₃ receptor-selective agonists were constructed by increasing the concentration of peptide by 10 fold at each administration, and leaving at least 90 min to elapse between two curves. Moreover, to evaluate the effects of atropine, tetrodotoxin, SR 142801 and GR 82334 plus MEN 11420 on NK₃ receptor-mediated responses, matched strips from the same tissue were used in parallel: one served as control, and the other was pretreated with the test compound, before a concentration–response curve to senktide was constructed. All the experiments were performed in the presence of a mixture of peptidase inhibitors: thiorphan, captopril and bestatin (1 μ M each; 15 min before), to prevent peptide degradation. The antagonist activity of GR 82334 (15 min incubation), MEN 11420 (15 min incubation) and SR 142801 (60 min incubation) was tested against the corresponding receptor-selective agonist(s). In order to obtain a correct estimate of the potency of MEN 11420 at tachykinin NK₂ receptors, the experiments with this compound were conducted in the presence of the NK₁ receptor-selective antagonist SR 140333 (Emonds-Alt *et al.*, 1993) (0.1 μ M, 30 min before) to prevent stimulation of NK₁ receptors by the agonist used ([β Ala⁸]NKA (4–10)), at high concentrations (see Patacchini *et al.*, 1994). Each antagonist was also tested against the cholinergic agonist methacholine, to check its selectivity for tachykinin receptors in the present preparation.

The contractile response to KCl (80 mM) was used as the internal standard in all experiments.

Evaluation of data

Agonist activity was expressed as EC₅₀. Antagonist affinity was expressed as pK_B, when 'Schild plot' analysis (Arunlakshana & Schild, 1959) showed no significant departure from unity slope. In this case the pK_B value was estimated as the mean of the individual values obtained with the equation:

$$pK_B = \log [\text{dose ratio} - 1] - \log [\text{antagonist concentration}]$$

(Jenkinson, 1991; Kenakin, 1993). The antagonist potency of SR 142801 was tentatively estimated as the ratio between two agonist concentrations (A'/A) producing 30% of the control maximal response, in the presence (A') and in the absence (A) of the antagonist, respectively.

Statistical analysis

The values in the text, tables or figures are expressed as means \pm 95% confidence limits (95% c.l.), or \pm s.e.mean. Statistical analysis was performed by means of Student's *t* test for paired or unpaired data or by means of two-way analysis of variance (ANOVA), when applicable. Regression analysis of log concentration–effect curves was performed by the least squares method, the curves being considered linear between 20 and 80% of the maximal response.

Drugs

MEN 11420 (or: c{[(β -D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2 β -5 β)}, substance P and [β Ala⁸]NKA(4–10) were synthesized at Menarini Laboratories (Florence, Italy) by conventional solid-phase methods. Methacholine, thiorphan, bestatin and captopril were from Sigma (St. Louis, MO, U.S.A.); neurokinin A, neurokinin B, senktide, septide and [Sar⁹]substance P sulfone from Peninsula Laboratories (St. Helens, U.K.); atropine from Serva (Heidelberg, Germany); tetrodotoxin from Sankyo (Japan); GR 82334

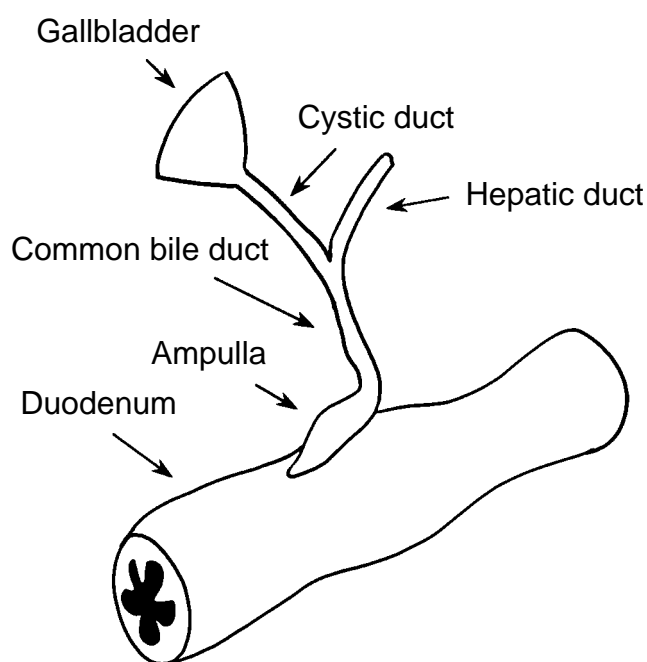


Figure 1 Schematic diagram of the choledochoduodenal junction in the guinea-pig.

(or:(D-Pro⁹[spiro- γ -lactam][Leu¹⁰, Trp¹¹]physalaemin (1–11)) from Bachem (Bubendorf, Switzerland).

The nonpeptide antagonists SR 140333 [(S)-1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-*n*-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]octane chloride] and SR 142801 [(S)-(N)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-N-methylacetamide] were kindly provided by Drs X. Emonds-Alt and G. Le Fur, Sanofi (Montpellier, France).

Results

General

Most preparations (about 80–90%) developed an irregular spontaneous phasic contractile activity within 60–90 min from set-up, averaging $10.8 \pm 2\%$ ($n = 18$) of the maximal response to KCl 80 mM, with a mean frequency of 18.9 ± 1.5 contractions min^{-1} ($n = 18$). This spontaneous activity was maintained for several hours, although in many preparations it decreased or even stopped after strong contractions had been elicited by addition of tachykinins or KCl. In some preparations a slowly-developing increase of tone was also noted. Atropine (1 μM) produced a relaxation of the latter preparations, while leaving both the amplitude and frequency of the spontaneous contractions unaffected.

Effect of tachykinin receptor agonists

All the tachykinins tested (both natural and synthetic) produced a concentration-dependent increase of the tone of the guinea-pig isolated common bile duct (Figure 2). The contractile responses produced by tachykinins underwent a marked tachyphylaxis: this phenomenon was particularly evident for the responses elicited by NK₁ and NK₃ receptor-selective agonists. However, reproducible responses could be obtained by using the procedures described in Methods. Among the natural tachykinins neurokinin B was by far the most potent, being about 40 and 25 fold more potent than substance P and neurokinin A, respectively (Figure 2; Table 1). All the three natural tachykinins produced a similar E_{max} averaging about 50% of that produced by KCl (80 mM) (Table 1).

Among the synthetic tachykinins, the NK₃ receptor-selective agonist senktide was the most active, being about 120, 110 and 20 fold more potent than [Sar⁹]substance P sulfone, [β -Ala⁸]NKA (4–10) and septide, respectively (Table 1). Between the two NK₁ receptor-selective agonists used, septide was about 6 fold more potent than [Sar⁹]substance P sulfone, while the latter agonist was as potent as substance P (cf Table 1).

The selective stimulation of either NK₁, NK₂ or NK₃ receptors by the synthetic agonists used produced similar E_{max} values, with no significant departure from the maxima obtained with natural tachykinins (cf Table 1).

Atropine (1 μM , 30 min before) did not affect the responses to NK₁ or NK₂ receptor-selective agonists, whereas it reduced by about 50% the E_{max} of senktide, without significantly affecting its potency (Table 1 and Figure 2). Tetrodotoxin (1 μM , 30 min before) completely prevented senktide-induced contractions (Figure 2). To investigate whether the atropine-resistant component of the response to senktide could be mediated by endogenous tachykinins, further experiments were performed in which senktide was administered to preparations pretreated with atropine plus the tachykinin NK₁ and NK₂ receptor-selective antagonists GR 82334 and MEN 11420 (1 μM , 15 min before each), respectively. Under the latter conditions senktide was unable to produce contractions, up to 1 μM (Figure 2).

Effect of tachykinin receptor antagonists

None of the selective antagonists tested on the isolated common bile duct produced intrinsic agonist responses. The

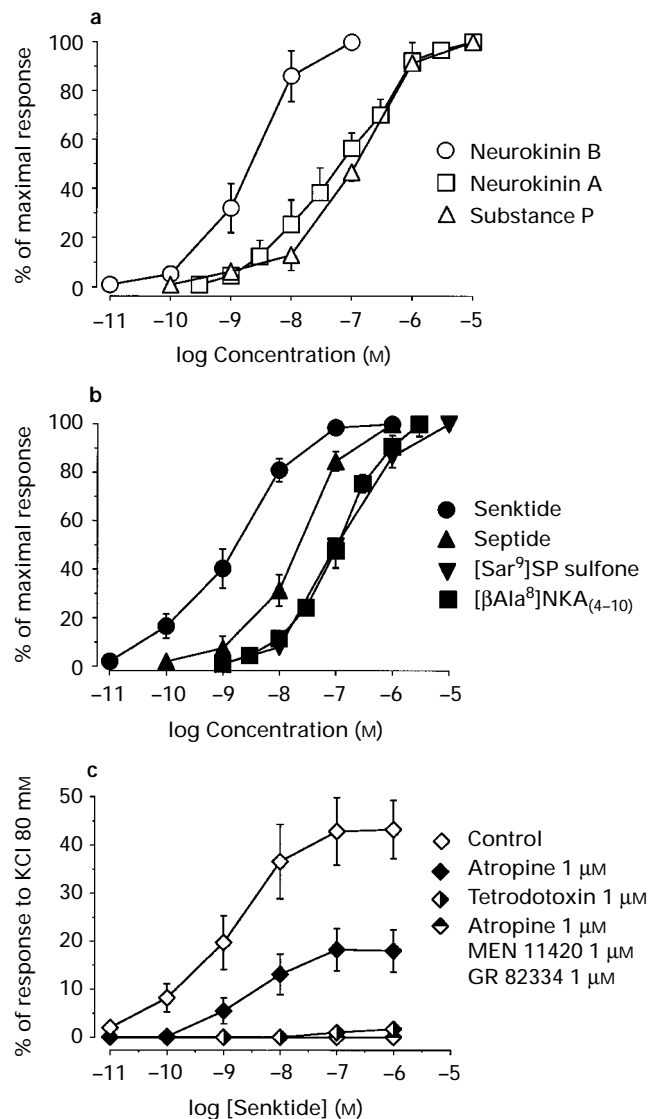


Figure 2 Log concentration–response curves to (a) natural and (b) synthetic receptor-selective tachykinins on the guinea-pig isolated common bile duct. (c) The effects of tetrodotoxin, atropine, and atropine plus the NK₁ and NK₂ receptor-selective antagonists GR 82334 and MEN 11420, respectively, on contractile responses produced by senktide. Each value in the figure is mean of 4–8 experiments; vertical lines show s.e.mean.

NK₂ receptor-selective antagonist MEN 11420 (30–300 nM) was tested against [β -Ala⁸]NKA (4–10) in preparations pretreated with SR 140333 (0.1 μM ; 30 min before), to rule out any involvement of NK₁ receptors in the response to the agonist. MEN 11420 potently ($pK_B = 8.25 \pm 0.08$; $n = 12$) inhibited [β -Ala⁸]NKA (4–10)-induced responses, producing parallel rightward shifts of the agonist curves without depressing the E_{max} (Figure 3). The competitive nature of MEN 11420-induced blockade of the NK₂ receptor was confirmed by the Schild plot slope: -0.90 (95% confidence limits: -1.4 ; -0.35). The NK₁ receptor-selective antagonist GR 82334 (1 μM) antagonized with apparent competitive kinetics both septide (apparent $pK_B = 7.46 \pm 0.10$; $n = 5$), and [Sar⁹]substance P sulfone, (apparent $pK_B = 6.80 \pm 0.04$; $n = 4$) (Figure 3). The NK₃ receptor-selective antagonist SR 142801 (30 nM, 60 min before) produced insurmountable antagonism toward senktide-induced contractions: the E_{max} was depressed by $64 \pm 7\%$; $n = 5$ (Figure 3). The ratio (A/A_0) between two concentrations of senktide producing 30% of the control maximum in the absence and in the presence of

Table 1 Contractile effects produced by natural and synthetic tachykinins in the guinea-pig isolated common bile duct, in the absence or in the presence of atropine

Peptide	Control		Atropine (1 μ M)	
	EC ₅₀ (nM)	E _{max} (% of KCl 80 mM)	EC ₅₀ (nM)	E _{max} (% of KCl 80 mM)
Neurokinin A	83.4 (62–112)	57.6 \pm 10	NT	NT
Neurokinin B	3.2 ^a (2.0–5.1)	42.7 \pm 8	NT	NT
Substance P	121.6 (94–157)	63.7 \pm 5	NT	NT
Septide	22.6 (18–28)	42.3 \pm 9	22.7 (17–30)	51.5 \pm 8
[Sar ⁹] SP sulfone	130.4 ^b (99–172)	44.7 \pm 8	90.3 (67–122)	40.7 \pm 8
[β Ala ⁸]NKA (4–10)	120.1 (95–151)	43.7 \pm 4	140.2 (104–189)	41.5 \pm 4
Senktide	1.1 ^c (0.7–1.8)	43.2 \pm 7	2.7 (1.8–3.9)	18.2 ^d \pm 4

Each value is mean \pm s.e. mean, or 95% confidence limits (in parentheses), or 4–8 determinations. E_{max} = maximal response expressed as % of that to KCl 80 mM. ^aSignificantly different from EC₅₀ values of neurokinin A and substance P: $P < 0.001$. ^bSignificantly different from the EC₅₀ of septide: $P < 0.05$. ^cSignificantly different from the EC₅₀ of septide, [Sar⁹] SP sulfone and [β Ala⁸]NKA (4–10): $P < 0.01$. ^dSignificantly different from the control response: $P < 0.01$. NT = not tested.

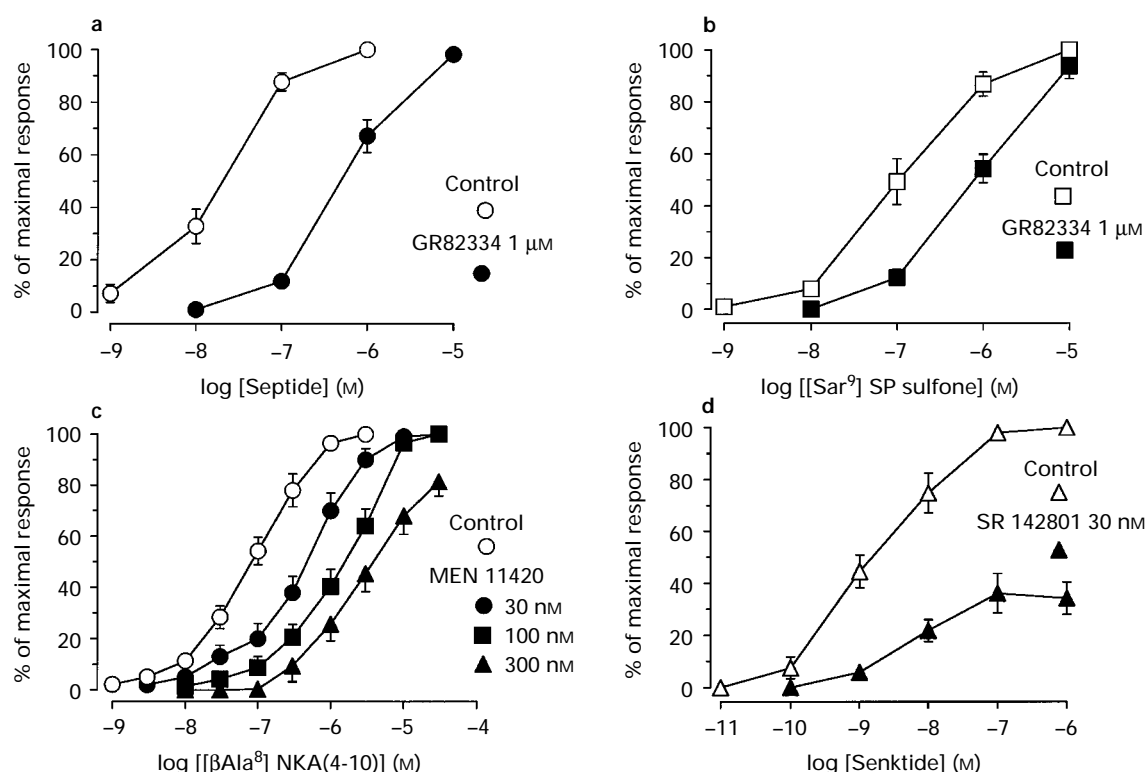


Figure 3 Antagonism by GR 82334 (NK₁ receptor-selective antagonist) (a and b), MEN 11420 (NK₂ receptor-selective antagonist) (c) and SR 142801 (NK₃ receptor-selective antagonist) (d) of contractions produced by corresponding receptor-selective agonists in the guinea-pig isolated common bile duct. The experiments with MEN 11420 were performed on preparations pretreated with SR 140333 (0.1 μ M), to prevent activation of NK₁ receptors by the agonist used ([β Ala⁸]NKA (4–10)) at high concentrations. Each value in the figure is mean of 4–5 experiments; vertical lines show s.e. mean.

SR 142801 (30 nM; 60 min before), averaged 86 ± 12 ($n = 5$). The selectivity of the above mentioned antagonists for tachykinin receptors was assessed by testing them against the cholinergic agonist methacholine. Methacholine (100 nM–300 μ M) produced reproducible contractile responses ($EC_{50} = 6.5$ μ M; 95% c.l. = 4.8–8.8; $E_{max} = 78 \pm 4\%$ of KCl 80 mM; $n = 12$) in the common bile duct, which were not affected by either GR 82334 (1 μ M) ($EC_{50} = 10$ μ M; 95% c.l. = 5.3–20; $E_{max} = 61 \pm 7\%$ of KCl 80 mM, in the presence of the antagonist; $n = 4$), or MEN 11420 (1 μ M) ($EC_{50} = 7.3$ μ M; 95% c.l. = 1.4–36.5; $E_{max} = 75 \pm 6\%$ of KCl 80 mM, in the presence of the antagonist; $n = 4$), or SR 142801 (30 nM; 60 min before) ($EC_{50} = 6.0$ μ M; 95% c.l. = 2.3–15.6; $E_{max} = 77 \pm 4\%$ of KCl 80 mM, in the presence of the antagonist; $n = 4$).

Discussion

To our knowledge this is the first study to demonstrate the effect of tachykinins on the motility of the guinea-pig terminal biliary tract.

Our data indicate that tachykinins produce a contractile response on this preparation by activating all three tachykinin receptors, NK₁, NK₂ and NK₃. This conclusion is based on the following results obtained with tachykinin receptor-selective agonists and antagonists: (1) all the synthetic agonists employed, septide and [Sar⁹] substance P sulfone (NK₁ receptor-selective), [β Ala⁸]NKA (4–10) (NK₂ receptor-selective) and senktide (NK₃ receptor-selective), produced full agonist responses, the maxima of which were not significantly different from those obtained with natural tachykinins. (2) The response

to each synthetic agonist was blocked by the corresponding receptor-selective antagonist, the selectivity of which for tachykinin receptors was indirectly proven by their ineffectiveness against the cholinergic agonist methacholine.

Thus, septide and [Sar⁹]substance P sulfone were antagonized by GR 82334 (NK₁ receptor-selective; Hagan *et al.*, 1991). In the common bile duct GR 82334 showed an apparent affinity against septide and [Sar⁹]substance P sulfone similar to that observed in other guinea-pig tissues against the same agonists (e.g. see Maggi & Patacchini, 1992; Maggi *et al.*, 1994a). The affinity shown by GR 82334 for NK₁ receptors of the common bile duct was greater (apparent $pK_B = 7.46$) when assayed against septide as an agonist than against [Sar⁹]substance P sulfone (apparent $pK_B = 6.80$). Similar findings were previously obtained in other guinea-pig isolated tissues, with GR 82334 and other tachykinin NK₁ receptor-selective antagonists (e.g. Petitet *et al.*, 1992; Maggi *et al.*, 1993a; 1994b; Burcher & Zeng, 1994; Patacchini *et al.*, 1995b), and are the main functional evidence supporting the proposed existence of two subtypes of the NK₁ receptor (Petitet *et al.*, 1992).

The NK₂ receptor-selective agonist [β Ala⁸]NKA (4–10) was potently and competitively antagonized by MEN 11420 (NK₂ receptor-selective; Santicioli & Maggi, 1997), in the present experiments. In contrast, MEN 11420 was found to be ineffective at tachykinin NK₁ and NK₃ receptors present in other guinea-pig preparations, up to 3 μ M (Santicioli & Maggi, 1997, and unpublished observations).

Senktide was insurmountably blocked by the nonpeptide antagonist SR 142801 (NK₃ receptor-selective; Emonds-Alt *et al.*, 1995), as observed in a previous study with the guinea-pig ileum (Patacchini *et al.*, 1995a). In the present study with the common bile duct, the insurmountable nature of the antagonism produced by SR 142801 became evident at a lower concentration (30 nM) than in the ileum (≥ 100 nM; Patacchini *et al.*, 1995a). Since the insurmountable effect produced by SR 142801 at guinea-pig NK₃ receptors is due to the essentially irreversible antagonism produced by this compound (Patacchini *et al.*, 1995a), we think that the different sensitivity to SR 142801 shown by ileum and common bile duct could depend upon 'tissue factors' (i.e. efficiency of receptor coupling and receptor density). The antagonist potency of SR 142801 (evaluated as the agonist dose-ratio produced at 30 nM after 60 min of incubation) was quite similar in the common bile

duct and ileum ($A'/A = 86 \pm 12$ vs 34 ± 7 , respectively: cf present results and Patacchini *et al.*, 1995a).

It is noteworthy that stimulation of NK₃ receptors, unlike NK₁ and NK₂ receptors, produces a totally indirect contractile response of the common bile duct, as shown by the use of tetrodotoxin against senktide. Part of the response to senktide is ascribable to release of endogenous acetylcholine, while the atropine-resistant part is due to endogenous tachykinins, as suggested by the total blockade obtained by combining atropine plus GR 82334 and MEN 11420. The observation that tetrodotoxin completely abolished the response to senktide, together with the high selectivity of the two tachykinin antagonists used, shows that endogenous tachykinins released upon stimulation of prejunctional NK₃ receptors produce contraction of the common bile duct through activation of tachykinin NK₁ and/or NK₂ receptors, putatively expressed on smooth muscle cells. A similar pattern of indirect excitatory motor responses initiated by stimulation of prejunctional NK₃ receptors, and mediated by endogenous acetylcholine or acetylcholine plus tachykinins, was obtained in the guinea-pig ileum (either in the longitudinal or circular muscle) and colon (Kilbinger *et al.*, 1986; Guard & Watson, 1987; Laufer *et al.*, 1988; Maggi *et al.*, 1990; 1994a; 1997). A recent electrophysiological study performed on isolated neurones from guinea-pig gallbladder ganglia (Mawe, 1995) has shown that tachykinins produce depolarization of the neurones through stimulation of receptors of the NK₃ type only. The present results, along with the latter study, provide evidence that the tachykinin NK₃ receptor is the mediator for the excitatory effect induced by tachykinins on intrinsic neurones of the biliary and intestinal system.

In conclusion, the present study shows for the first time that tachykinins produce excitatory motor responses on the isolated terminal biliary tract of the guinea-pig, through stimulation of NK₁, NK₂ and NK₃ receptors. Excitation of these latter receptors (NK₃) leads to a completely neurogenic response, the final neurotransmitters of which are acetylcholine and tachykinins, probably released from intramural neurones, which in turn activate postjunctional muscarinic and tachykinin NK₁/NK₂ receptors. This latter finding provides further evidence that NK₃ receptors are the main effectors for the excitatory role played by tachykinins on neurones of the gastrointestinal tract.

References

- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.
- BARTHÓ, L. & HOLZER, P. (1985). Search for a physiological role of substance P in gastrointestinal motility. *Neuroscience*, **16**, 1–32.
- BURCHER, E. & ZENG, X.-P. (1994). Evidence for a possible 'septide-sensitive' tachykinin receptor in guinea-pig bronchus. *Neuropeptides*, **26**, 29.
- DAHLSTRAND, C., BJORCK, S., EDIN, R., DAHLSTROM, A. & AHLMAN, H. (1988). SP in the control of extrahepatic biliary motility in the cat. *Reg. Peptides*, **20**, 11–24.
- EMONDS-ALT, X., BICHON, D., DUCOUX, J.P., HEAULME, M., MILOUX, B., PONCELET, M., PROIETTO, E., VAN BROECK, D., VILAIN, P., NELIAT, G., SOUBRIÉ, P., LE FUR, G. & BRELIÉRE, J.C. (1995). SR 142801, the first potent nonpeptide antagonist of the tachykinin NK₃ receptor. *Life Sci.*, **56**, PL 27–32.
- EMONDS-ALT, X., DOUTREMEPUICH, J.D., HEAULME, M., NELIAT, G., SANTUCCI, V., STEINBERG, R., VILAIN, P., BICHON, D., DUCOUX, J.P., PROIETTO, E., VAN BROECK, D., SOUBRIÉ, P., LE FUR, G. & BRELIÉRE, J.C. (1993). *In vitro* and *in vivo* biological activities of SR 140,333, a novel potent nonpeptide tachykinin NK₁ receptor antagonist. *Eur. J. Pharmacol.*, **250**, 403–413.
- FEELEY, T.M., CLANACHAN, A.S. & SCOTT, G.W. (1987). Contractility of human gallbladder muscle in vitro. *Alimentary Pharmacol. Ther.*, **1**, 607–616.
- GUARD, S. & WATSON, S.P. (1987). Evidence for neurokinin-3 receptor-mediated tachykinin release in the guinea-pig ileum. *Eur. J. Pharmacol.*, **144**, 409–412.
- GUARD, S. & WATSON, S.P. (1991). Tachykinin receptor types: classification and membrane signalling mechanisms. *Neurochem. Int.*, **18**, 149–165.
- GUO, Y.S., SINGH, P., GOMEZ, G., RAJARAMAN, S. & THOMPSON, J.C. (1989). Contractile response of canine gallbladder and sphincter of Oddi to substance P and related peptides in vitro. *Digestive Dis. Sci.*, **34**, 812–817.
- HAGAN, R.M., IRELAND, S.J., BAILEY, F., MCBRIDE, C., JORDAN, C.C. & WARD, P. (1991). A spiro lactam conformationally-constrained analogue of physalaemin which is a peptidase-resistant selective neurokinin NK₁ receptor antagonist. *Br. J. Pharmacol.*, **102**, 168P.
- HASTRUP, H. & SCHWARTZ, T.W. (1996). Septide and neurokinin A are high-affinity ligands on the NK-1 receptor: evidence from homologous versus heterologous binding analysis. *FEBS Lett.*, **399**, 264–266.
- JENKINSON, D.H. (1991). How we describe competitive antagonists: three questions of usage. *Trends Pharmacol. Sci.*, **12**, 53–56.
- KENAKIN, T.P. (1993). *Pharmacologic Analysis of Drug-Receptor Interaction*. Second Ed. New York: Raven Press.

- KRAUSE, J.E., STAVETEIG, P.T., MENTZER, J.N., SCHMIDT, S.K., TUCKER, J.B., BRODBECK, R.M., BU, J.K. & KARPITSKIY, V.V. (1997). Functional expression of a novel human neurokinin-3 receptor homolog that binds (3H)senktide and (125-I-MePhe-7) neurokinin B, and is responsive to tachykinin peptide agonists. *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 310–315.
- KILBINGER, H., STAUB, P., ERLHOF, I. & HOLZER, P. (1986). Antagonist discrimination between subtypes of tachykinin receptors in the guinea-pig ileum. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **334**, 181–187.
- LAUFER, R., GILON, C., CHOREV, M. & SELINGER, Z. (1988). Desensitization with selective agonists discriminates between multiple tachykinin receptors. *J. Pharmacol. Exp. Ther.*, **245**, 639–643.
- MAGGI, C.A. (1995). Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. *Prog. Neurobiol.*, **45**, 1–98.
- MAGGI, C.A., GIULIANI, S. & ZAGORODNYUK, V. (1997). Sequential activation of the triple excitatory transmission to the circular muscle of guinea-pig colon. *Neuroscience*, **79**, 263–274.
- MAGGI, C.A. & PATACCHINI, R. (1992). Tachykinin NK₁ receptor in the guinea-pig isolated proximal urethra: characterization by receptor selective agonists and antagonists. *Br. J. Pharmacol.*, **106**, 888–892.
- MAGGI, C.A., PATACCHINI, R., GIACHETTI, A. & MELI, A. (1990). Tachykinin receptors in the circular muscle of the guinea-pig ileum. *Br. J. Pharmacol.*, **101**, 996–1000.
- MAGGI, C.A., PATACCHINI, R., MEINI, S. & GIULIANI, S. (1993a). Evidence for the presence of a septide-sensitive tachykinin receptor in the circular muscle of the guinea-pig ileum. *Eur. J. Pharmacol.*, **235**, 309–311.
- MAGGI, C.A., PATACCHINI, R., MEINI, S. & GIULIANI, S. (1994a). Effect of longitudinal muscle-myenteric plexus removal and indomethacin on the response to tachykinin NK-2 and NK-3 receptor agonists in the circular muscle of the guinea-pig ileum. *J. Auton. Pharmacol.*, **14**, 49–60.
- MAGGI, C.A., PATACCHINI, R., MEINI, S., QUARTARA, L., SISTO, A., POTIER, E., GIULIANI, S. & GIACHETTI, A. (1994b). Comparison of tachykinin NK₁ and NK₂ receptors in the circular muscle of the guinea-pig ileum and proximal colon. *Br. J. Pharmacol.*, **112**, 150–160.
- MAGGI, C.A., PATACCHINI, R., RENZI, D., SANTICIOLI, P., REGOLI, D., ROVERO, P., DRAPEAU, G., SURRENTI, C. & MELI, A. (1989). Effect of thiorphan on response of the guinea-pig gallbladder to tachykinins. *Eur. J. Pharmacol.*, **165**, 51–61.
- MAGGI, C.A., PATACCHINI, R., ROVERO, P. & GIACHETTI, A. (1993b). Tachykinin receptors and tachykinin receptor subtypes. *J. Auton. Pharmacol.*, **13**, 23–93.
- MAWE, G.M. (1995). Tachykinins as mediators of slow EPSPs in guinea-pig gallbladder ganglia: involvement of neurokinin-3 receptors. *J. Physiol.*, **485**, 513–524.
- MELDRUM, L.A., BOJARSKI, J. & CALAM, J. (1987). Effects of substance P and of other neuropeptides on guinea-pig gallbladder muscle. *Digestion*, **37**, 193–199.
- PATACCHINI, R., BARTHÓ, L., HOLZER, P. & MAGGI, C.A. (1995a). Activity of SR 142801 at peripheral tachykinin receptors. *Eur. J. Pharmacol.*, **278**, 17–25.
- PATACCHINI, R., DE GIORGIO, R., GIACHETTI, A. & MAGGI, C.A. (1994). Different mechanism of tachykinin NK₂ receptor blockade by SR 48,968 and MEN 10,627 in the guinea-pig isolated gallbladder and colon. *Eur. J. Pharmacol.*, **271**, 111–119.
- PATACCHINI, R. & MAGGI, C.A. (1992). Effect of newly developed tachykinin agonists and antagonists on the guinea-pig isolated gallbladder. *J. Pharmacol. Exp. Ther.*, **261**, 191–194.
- PATACCHINI, R., QUARTARA, L., ASTOLFI, M., GOSO, C., GIACHETTI, A. & MAGGI, C.A. (1995b). Activity of cyclic pseudopeptide antagonists at peripheral tachykinin receptors. *J. Pharmacol. Exp. Ther.*, **272**, 1082–1087.
- PETITET, F., SAFFROY, M., TORRENS, Y., LAVIELLE, S., CHASSAING, G., LOEUILLLET, D., GLOWINSKI, J. & BEAUJOUAN, J.C. (1992). Possible existence of a new tachykinin receptor subtype in the guinea-pig ileum. *Peptides*, **13**, 383–388.
- REGOLI, D., DRAPEAU, G., DION, S. & D'ORLEANS-JUSTE, P. (1989). Receptors for substance P and related neurokinins. *Pharmacology*, **38**, 1–15.
- SANTICIOLI, P. & MAGGI, C.A. (1997). Electrophysiological study on the activity of the tachykinin NK₂ receptor antagonist MEN 11420 in the guinea-pig colon. *Br. J. Pharmacol.*, **120**, 191P.
- SHOOK, J.E. & BURKS, T.F. (1987). A novel bioassay for the NK-2 neurokinin receptor: the guinea-pig gallbladder. *Life Sci.*, **39**, 2533–2539.

(Received July 31, 1997
Revised September 18, 1997
Accepted September 19, 1997)