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

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1 We studied the effect of the natural tachykinins and of synthetic agonists selective for the tachykinin NK_1, NK_2 and NK_3 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 NK_1 and NK_3 receptor-selective agonists.

3 Among the natural tachykinins neurokinin B ($EC_{50}=3.2$ 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 ($EC_{50}=121.6$ nM; 95% c.l. = 94–157; $P<0.01$; $n=4$) and neurokinin A ($EC_{50}=83.4$ nM; 95% c.l. = 62–112; $P<0.01$; $n=4$), respectively. Among the synthetic analogues the NK_3 receptor-selective agonist senktide ($EC_{50}=1.1$ nM; 95% c.l. = 0.7–1.8; $n=8$) was the most potent, being about 120, 110 and 20 fold more potent than [Sar^9]substance P sulfone (NK_1 receptor-selective) ($EC_{50}=130.4$ nM; 95% c.l. = 99–172; $P<0.01$; $n=8$), [βAla^8]NKA (4–10) (NK_2 receptor-selective) ($EC_{50}=120.1$ nM; 95% c.l. = 95–151; $P<0.01$; $n=8$) and septide (NK_1 receptor-selective) ($EC_{50}=22.6$ 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 NK_1 or NK_2 receptor-selective agonists, whereas it reduced the E_{max} of senktide by about 50%, without affecting its potency (EC_{50}). Tetrodotoxin (1 μM) totally blocked senktide-induced contractions, as did the combined pretreatment with atropine plus the tachykinin NK_1 and NK_2 receptor-selective antagonists GR 82334 and MEN 11420 (1 μM each), respectively.

5 GR 82334 (1 μM) blocked with apparent competitive kinetics septide- (apparent $pK_B=7.46\pm0.10$; $n=5$) and [Sar^9]substance P sulfone- (apparent $pK_B=6.80\pm0.04$; $n=4$) induced contractions. MEN 11420 (30–300 nM), a novel potent NK_2 receptor antagonist, potently antagonized [βAla^8]NKA (4–10), with competitive kinetics ($pK_B=8.25\pm0.08$; $n=12$: Schild plot slope = −0.90; 95% c.l. = −1.4; −0.35). The NK_3 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 NK_1, NK_2 and NK_3 receptors. The responses obtained by activating NK_1 and NK_2 receptors are atropine-resistant. The contraction obtained by stimulating NK_3 receptors is totally neurogenic, being mediated by the release of endogenous acetylcholine and tachykinins; the latter act, in turn, on postjunctional tachykinin NK_1/NK_2 receptors. The role of the NK_3 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 NK_1, NK_2 and NK_3 (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 NK_3 receptor (Krause *et al*., 1997). With regard to the NK_1 receptor, the existence of NK_1 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 NK_1 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 &

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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 NK_2 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 NK_1, NK_2 and NK_3 receptors, and the following tachykinin receptor-selective antagonists: GR 82334 (NK_1 receptor-selective; Hagan *et al*., 1991), MEN 11420 (NK_2 receptor-selective; Santicioli & Maggi, 1997) and SR 142801 (NK_3 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% O_2 and 4% CO_2) Krebs solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.5, $CaCl_2$ 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 [Sar^9]substance P sulfone (NK_1 receptor), [βAla^8]NKA (4–10) (NK_2 receptor) and senktide (NK_3 receptor), were studied either in the absence or in the presence of atropine (1 μM). 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 NK_1 and NK_3 receptor-selective agonists. To prevent this phenomenon, cumulative curves to NK_1 and NK_3 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 NK_3 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 μM 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 NK_2 receptors, the experiments with this compound were conducted in the presence of the NK_1 receptor-selective antagonist SR 140333 (Emonds-Alt *et al*., 1993) (0.1 μM, 30 min before) to prevent stimulation of NK_1 receptors by the agonist used ([βAla^8]NKA (4–10)), at high concentrations (see Patacchini *et al*., 1994). Each antagonist was also tested against the cholinoceptor 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.

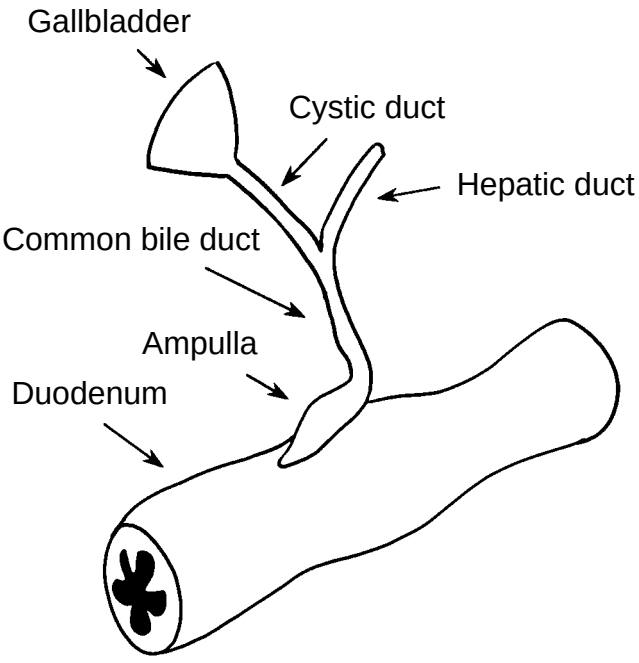

Figure 1 Schematic diagram of the choledochoduodenal junction in the guinea-pig.

Evaluation of data

Agonist activity was expressed as EC_{50}. 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 ± 95% confidence limits (95% c.l.), or ± 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(2β-5β)}, substance P and [βAla^8]NKA(4–10) were synthesized at Menarini Laboratories (Florence, Italy) by conventional solid-phase methods. Meth-acholine, thiorphan, bestatin and captopril were from Sigma (St. Louis, MO, U.S.A.); neurokinin A, neurokinin B, senktide, septide and [Sar^9]substance P sulfone from Peninsula Laboratories (St. Helens, U.K.); atropine from Serva (Heidelberg, Germany); tetrodotoxin from Sankyo (Japan); GR 82334

(or:(D-Pro^9[spiro-γ-lactam][Leu^{10}, Trp^{11}]physalaemin (1–11)) from Bachem (Bubendorf, Switzerland).

The nonpeptide antagonists SR 140333 [(**S**)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-n-3yl]ethyl}-4-phenyl-1-azoniabi-cyclo[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±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 NK_1 and NK_3 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 stubstance 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 NK_3 receptor-selective agonist senktide was the most active, being about 120, 110 and 20 fold more potent than [Sar^9]substance P sulfone, [βAla^8]NKA (4–10) and septide, respectively (Table 1). Between the two NK_1 receptor-selective agonists used, septide was about 6 fold more potent than [Sar^9]substance P sulfone, while the latter agonist was as potent as substance P (cf Table 1).

The selective stimulation of either NK_1, NK_2 or NK_3 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 NK_1 or NK_2 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 NK_1 and NK_2 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

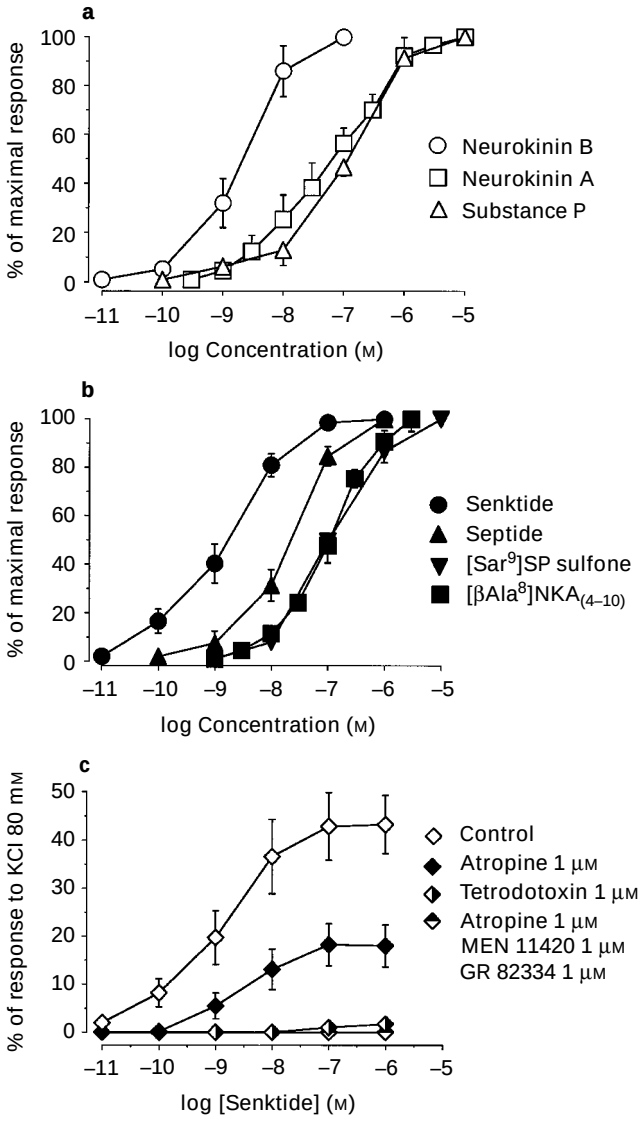

Figure 2 Log concentration–response curves to (a) natural and (b) synthetic receptor-selective tachykinins on the guinea-pig isolated common bile duct. (c) The effects of tetrodotoxin, atropine, and atropine plus the NK_1 and NK_2 receptor-selective antagonists GR 82334 and MEN 11420, respectively, on contractile responses produced by senktide. Each value in the figure is mean of 4–8 experiments; vertical lines show s.e.mean.

NK_2 receptor-selective antagonist MEN 11420 (30–300 nM) was tested against [βAla^8]NKA (4–10) in preparations pretreated with SR 140333 (0.1 μM; 30 min before), to rule out any involvement of NK_1 receptors in the response to the agonist. MEN 11420 potently (pK_B=8.25±0.08; n=12) inhibited [βAla^8]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 NK_2 receptor was confirmed by the Schild plot slope: −0.90 (95% confidence limits: −1.4; −0.35). The NK_1 receptor-selective antagonist GR 82334 (1 μM) antagonized with apparent competitive kinetics both septide (apparent pK_B=7.46±0.10; n=5), and [Sar^9]substance P sulfone, (apparent pK_B=6.80±0.04; n=4) (Figure 3). The NK_3 receptor-selective antagonist SR 142801 (30 nM, 60 min before) produced insurmountable antagonism toward senktide-induced contractions: the E_{max} was depressed by 64±7%; n=5 (Figure 3). The ratio (A′/A) 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

	Control		*Atropine* (1 μM)	
Peptide	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±10	NT	NT
Neurokinin B	3.2[a] (2.0–5.1)	42.7±8	NT	NT
Substance P	121.6 (94–157)	63.7±5	NT	NT
Septide	22.6 (18–28)	42.3±9	22.7 (17–30)	51.5±8
[Sar^9] SP sulfone	130.4[b] (99–172)	44.7±8	90.3 (67–122)	40.7±8
[βAla^8]NKA (4–10)	120.1 (95–151)	43.7±4	140.2 (104–189)	41.5±4
Senktide	1.1[c] (0.7–1.8)	43.2±7	2.7 (1.8–3.9)	18.2[d]±4

Each value is mean±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. [a]Significantly different from EC_{50} values of neurokinin A and substance P: $P<0.001$. [b]Significantly different from the EC_{50} of septide: $P<0.05$. [c]Significantly different from the EC_{50} of septide, [Sar^9] SP sulfone and [βAla^8]NKA (4–10): $P<0.01$. [d]Significantly different from the control response: $P<0.01$. NT = not tested.

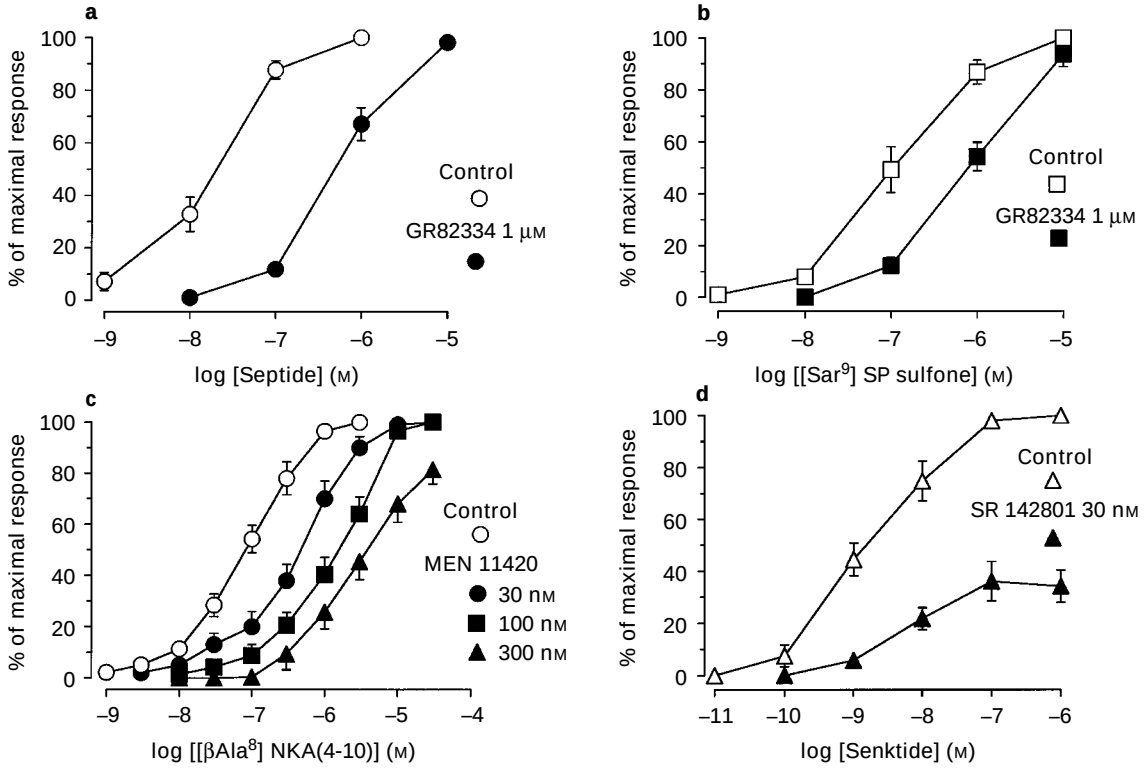

Figure 3 Antagonism by GR 82334 (NK_1 receptor-selective antagonist) (a and b), MEN 11420 (NK_2 receptor-selective antagonist) (c) and SR 142801 (NK_3 receptor-selective antagonist) (d) of contractions produced by corresponding receptor-selective agonists in the guinea-pig isolated common bile duct. The experiments with MEN 11420 were performed on preparations pretreated with SR 140333 (0.1 μM), to prevent activation of NK_1 receptors by the agonist used ([βAla^8]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 cholinoceptor agonist methacholine. Methacholine (100 nM–300 μM) produced reproducible contractile responses ($EC_{50}=6.5$ μM; 95% c.l. = 4.8–8.8; $E_{max}=78\pm4$% of KCl 80 mM; $n=12$) in the common bile duct, which were not affected by either GR 82334 (1 μM) ($EC_{50}=10$ μM; 95% c.l. = 5.3–20; $E_{max}=61\pm7$% of KCl 80 mM, in the presence of the antagonist; $n=4$), or MEN 11420 (1 μM) ($EC_{50}=7.3$ μM; 95% c.l. = 1.4–36.5; $E_{max}=75\pm6$% of KCl 80 mM, in the presence of the antagonist; $n=4$), or SR 142801 (30 nM; 60 min before) ($EC_{50}=6.0$ μM; 95% c.l. = 2.3–15.6; $E_{max}=77\pm4$% 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, NK_1, NK_2 and NK_3. This conclusion is based on the following results obtained with tachykinin receptor-selective agonists and antagonists: (1) all the synthetic agonists employed, septide and [Sar^9]substance P sulfone (NK_1 receptor-selective), [βAla^8]NKA (4–10) (NK_2 receptor-selective) and senktide (NK_3 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 cholinoceptor agonist methacholine.

Thus, septide and [Sar^9]substance P sulfone were antagonized by GR 82334 (NK_1 receptor-selective; Hagan *et al*., 1991). In the common bile duct GR 82334 showed an apparent affinity against septide and [Sar^9]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 NK_1 receptors of the common bile duct was greater (apparent $pK_B = 7.46$) when assayed against septide as an agonist than against [Sar^9]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 NK_1 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 NK_1 receptor (Petitet *et al*., 1992).

The NK_2 receptor-selective agonist [βAla^8]NKA (4–10) was potently and competitively antagonized by MEN 11420 (NK_2 receptor-selective; Santicioli & Maggi, 1997), in the present experiments. In contrast, MEN 11420 was found to be ineffective at tachykinin NK_1 and NK_3 receptors present in other guinea-pig preparations, up to 3 μM (Santicioli & Maggi, 1997, and unpublished observations).

Senktide was insurmountably blocked by the nonpeptide antagonist SR 142801 (NK_3 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 ($\geqslant$100 nM; Patacchini *et al*., 1995a). Since the insurmountable effect produced by SR 142801 at guinea-pig NK_3 receptors is due to the essentially irreversible antagonism produced by this compound (Patacchini *et al*., 1995a), we think that the different sensitivity to SR 142801 shown by ileum and common bile duct could depend upon 'tissue factors' (i.e. efficiency of receptor coupling and receptor density). The antagonist potency of SR 142801 (evaluated as the agonist dose-ratio produced at 30 nM after 60 min of incubation) was quite similar in the common bile duct and ileum ($A'/A = 86 \pm 12$ vs 34 ± 7, respectively: cf present results and Patacchini *et al*., 1995a).

It is noteworthy that stimulation of NK_3 receptors, unlike NK_1 and NK_2 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 tacykinins released upon stimulation of prejunctional NK_3 receptors produce contraction of the common bile duct through activation of tachykinin NK_1 and/or NK_2 receptors, putatively expressed on smooth muscle cells. A similar pattern of indirect excitatory motor responses initiated by stimulation of prejunctional NK_3 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 NK_3 type only. The present results, along with the latter study, provide evidence that the tachykinin NK_3 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 NK_1, NK_2 and NK_3 receptors. Excitation of these latter receptors (NK_3) 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 NK_1/NK_2 receptors. This latter finding provides further evidence that NK_3 receptors are the main effectors for the excitatory role played by tachykinins on neurones of the gastrointestinal tract.

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