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Involvement of neurokinins in the non-cholinergic response to activation of 5-HT_3 and 5-HT_4 receptors in guinea-pig ileum

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1 The involvement of neurokinins in the non-cholinergically-mediated contractile response induced by stimulation of 5-HT_3 and 5-HT_4 receptors has been examined in the longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum.

2 The 5-HT_3 receptor agonist, 2-methyl-5-hydroxytryptamine (2-methyl-5-HT), showed a lower potency in this preparation than the more selective 5-HT_4 receptor agonist 5-methoxytryptamine. The effect of both drugs was markedly reduced by atropine.

3 Substance P (SP) and neurokinin B (NKB) produced biphasic concentration-response curves in the preparation. Neurokinin A (NKA), the NK_1 receptor agonist, [Sar^9,$Met(O_2)^{11}$]SP and the NK_3 receptor agonist, senktide yielded monophasic concentration-response curves.

4 After desensitization of the NK_1 receptor with SP or [Sar^9,$met(O_2)^{11}$]SP, in the presence of atropine, the contractile response to 2-methyl-5-HT was entirely blocked. Desensitization of NK_3 receptors with NKB, also in the presence of atropine, fully suppressed the 5-HT_4 receptor-mediated contraction evoked by 5-methoxytryptamine.

5 In preparations prelabelled with [^{3}H]-choline, SP produced a concentration-dependent increase in tritium overflow, an index of [^{3}H]-acetylcholine release, while an inverse relationship was found with NKB. At low neurokinin concentrations, the releasing effect of NKB was much more marked.

6 It is suggested that in the response to 5-HT_3 receptor stimulation, there is a role for SP and acetylcholine. NKB appears to be preferentially involved in the release of acetylcholine elicited by stimulation of 5-HT_4 receptors.

Keywords: 5-HT; 5-HT_3 receptor; 5-HT_4 receptor; neurokinins; substance P; guinea-pig ileum

Introduction

The contractile effect of 5-hydroxytryptamine (5-HT) in the guinea-pig isolated ileum involves both myogenic and neural mechanisms (Gaddum & Picarelli, 1957). The activation of neuronally-located receptors by 5-HT gives rise to a biphasic concentration-response curve (Buchheit *et al.*, 1985; Butler *et al.*, 1988; Fozard, 1990). The first, high potency phase of the curve, seems to be mediated by the 5-HT_4 receptor (Clarke *et al.*, 1989; Craig & Clarke, 1990), while the second phase, obtained with higher concentrations of 5-HT, would correspond to the activation of the 5-HT_3 receptor (Buchheit *et al.*, 1985; Sanger & Nelson, 1989).

It has been reported that the neuronal actions of 5-HT are mediated by both acetylcholine and substance P release (Buchheit *et al.*, 1985). Substance P (SP) and the structurally related peptides, neurokinin A (NKA) and neurokinin B (NKB), belong to a family of biologically active peptides known as tachykinins. The mammalian members of the tachykinin family are called neurokinins and their actions are mediated through three different types of receptors, NK_1, NK_2 and NK_3 (Henry, 1987; Quirion & Dam, 1988; Guard & Watson, 1991) which are preferentially activated by SP, NKA and NKB respectively (Laufer *et al.*, 1985; Buck & Burcher, 1986; Guard & Watson, 1991), although each peptide can activate all three receptor subtypes to a certain extent.

By using the longitudinal muscle-myenteric plexus (LMMP) preparation we have studied the involvement of the different neurokinins in the contractile response elicited by activation of either 5-HT_3 or 5-HT_4 receptors. The 5-HT_3 receptor agonist 2-methyl-5-hydroxytryptamine (2-Me-5-HT) (Richardson *et al.*, 1985; Bradley *et al.*, 1986) as well as 5-methoxytryptamine (5-MeOT), a more selective agonist for 5-HT_4 than for 5-HT_3 receptors (Eglen *et al.*, 1990; Hill *et al.*, 1990; Fozard, 1990) were used in this study. Neurokinin receptor desensitization by the endogenous peptides or by some more selective synthetic analogues was used as a means of studying the role of the different neurokinins in the contractile response to 5-HT. The ability of the neurokinins to increase tritium overflow from preparations prelabelled with [^{3}H]-choline, an index of [^{3}H]-acetylcholine release (e.g. Wikberg, 1977), was also studied.

The results suggest the participation of SP and acetylcholine in the response to 5-HT_3 receptor stimulation, as well as a conspicuous role for NKB in the response elicited by activation of the 5-HT_4 receptor.

Methods

Guinea-pigs of either sex weighing 300–400 g were stunned by a blow to the head and bled. The ileum was excised approximately 10 cm from the ileo-caecal junction and longitudinal muscle strips with the myenteric plexus attached (LMMP) were prepared as described by Paton & Vizi (1969).

Contractility studies

LMMP strips were suspended in a 10 ml organ bath containing Tyrode solution (composition in mM: NaCl 136, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.5) aerated with 95% O_2/5% CO_2 and maintained at 37°C. Contractile responses were recorded isometrically with a resting tension of 0.5 g. Before the experiments were started, tissues were equilibrated for 30 min.

Concentration-response curves were constructed in a non-cumulative fashion with an agonist exposure period of 30 s on a 10 min dose-cycle. In studies with antagonists, each strip

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was used to record two concentration-response curves: the first for the agonist alone and the second for the agonist in the presence of the antagonist, each strip serving as its own control. Antagonists were allowed to pre-equilibrate for 15 min prior to the addition of the agonists.

Characterization of the concentration-response curves

Responses were measured as an increase in the isometric tension and expressed as a percentage of the maximum response. In the presence of an antagonist, results were expressed as a percentage of the maximal response obtained with the agonist alone. Monophasic curves were characterized by the agonist concentration yielding a half-maximal effect, EC_{50}. Biphasic curves were described as follows: for each part, the fractional response contributing to the total response was calculated and the maximum indicated as E_{max1} and E_{max2}. The agonist concentrations which produced half maximal effects were denoted by EC_{50}^1 and EC_{50}^2 respectively.

Desensitizing studies

For desensitization studies, the LMMP preparation was treated repeatedly with high concentrations (0.5 μM) of the neurokinin until the response faded to baseline level. The preparation was then washed and the response to the 5-HT receptor agonist was recorded. Results are expressed as a percentage of the response obtained prior to desensitization.

Release experiments

The method employed to measure the release of acetylcholine was a modification of that described by Kilbinger & Wessler (1980). Briefly, LMMP strips weighing approximately 30 mg were suspended isometrically under a tension of 0.5 g in a 5 ml organ bath and superfused with Tyrode solution containing 1 μM choline. After 30 min incubation with [methyl- ^{3}H]-choline (8 μCi ml^{-1}) and continuous stimulation during this time through platinum electrodes with square wave pulses (0.2 Hz, 1 ms, 13.5 V), the strips were superfused with Tyrode solution containing 10 μM hemicholinium-3 to prevent reuptake of choline. After a washout period of 60 min, aliquots were collected in 3 min fractions. Strips were stimulated twice (S_1, S_2) by field stimulation (1 Hz, 1 ms, 13.5 V), the two pulses being spaced 21 min apart (Torocsik & Vizi, 1991). Drugs were added to the superfusion fluid 21 min after S_2. The spontaneous outflow was calculated by fitting a linear regression line based on the samples taken before and during S_1 stimulation.

Tritium content of the superfused samples was measured by liquid scintillation spectrometry. Under the present experimental conditions, the validity of assuming total tritium as a measure of [^{3}H]-acetylcholine released has been extensively documented (Szerb, 1976; Wikberg, 1977; Yau *et al.*, 1991).

The effect of the drugs studied (D) on the outflow of ^{3}H-label was expressed as a percentage of the increase in the output of ^{3}H-label evoked by the second electrical stimulation (S_2). The increase in the output of ^{3}H-label in response to either the drug or the second electrical stimulation was calculated by subtracting the output during the preceding resting period of 3 min from the output during the stimulation.

Data analysis

The significance between groups was assessed by Student's *t* test (two groups) or ANOVA followed by Scheffe test (several groups). To characterize the concentration-response curves, log EC_{50} was calculated with GraphPAD, ISI Software.

Drugs used

The following drugs were purchased from the suppliers indicated: 5-hydroxytryptamine creatinine sulphate, substance P acetate, atropine sulphate, choline bromide, hemicholinium-3 bromide (Sigma, U.S.A.); 2-methylserotonin maleate (2-methyl-5-HT), 5-methoxytryptamine hydrochloride (Heterocyclic Research, U.S.A.); neurokinin A, neurokinin B (Bachem, Switzerland); [Sar^9, Met(O$_2$)^{11}]SP, senktide (RBI, U.S.A.); choline chloride[methyl-^{3}H] (NEN-Du Pont, Boston, MA, U.S.A.). Methysergide maleate was a gift from Sandoz, Switzerland. Peptides were made up in a stock 0.1 mM concentration in 0.1 N acetic acid; aliquots were stored frozen and diluted before use. All other drugs were dissolved in distilled water.

Results

Contractility studies

The concentration-response curves to 5-MeOT and to 2-Me-5-HT were monophasic. The agonist potencies (− log EC_{50}) were 6.0 ± 0.1 and 5.0 ± 0.2 respectively. Atropine, 0.1 μM, blocked by more than 50% the maximum effect of both 5-HT receptor agonists (Figure 1). The blockade was substantially identical when atropine 1 μM was used.

The concentration-response curve to the neurokinin NKB was biphasic, the initial phase occurred between concentrations of 0.5 nM and 5 nM, and the second phase between

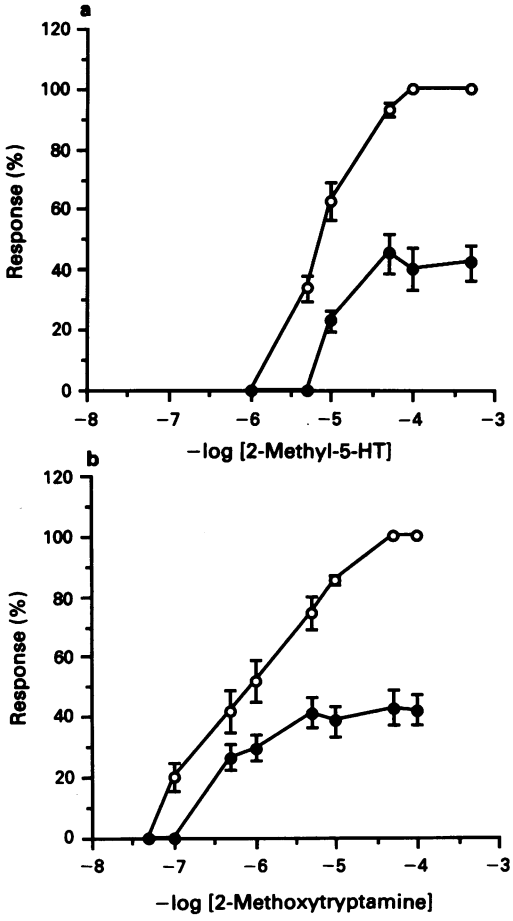

Figure 1 Concentration-response curve to 2-methyl-5-HT (a) and 5-methoxytryptamine (b) in the LMMP preparation of the guinea-pig ileum in absence (○) or in the presence (●) of 0.1 μM atropine. Values are means ± s.e. from eight preparations and are expressed as a percentage of the maximum control response.

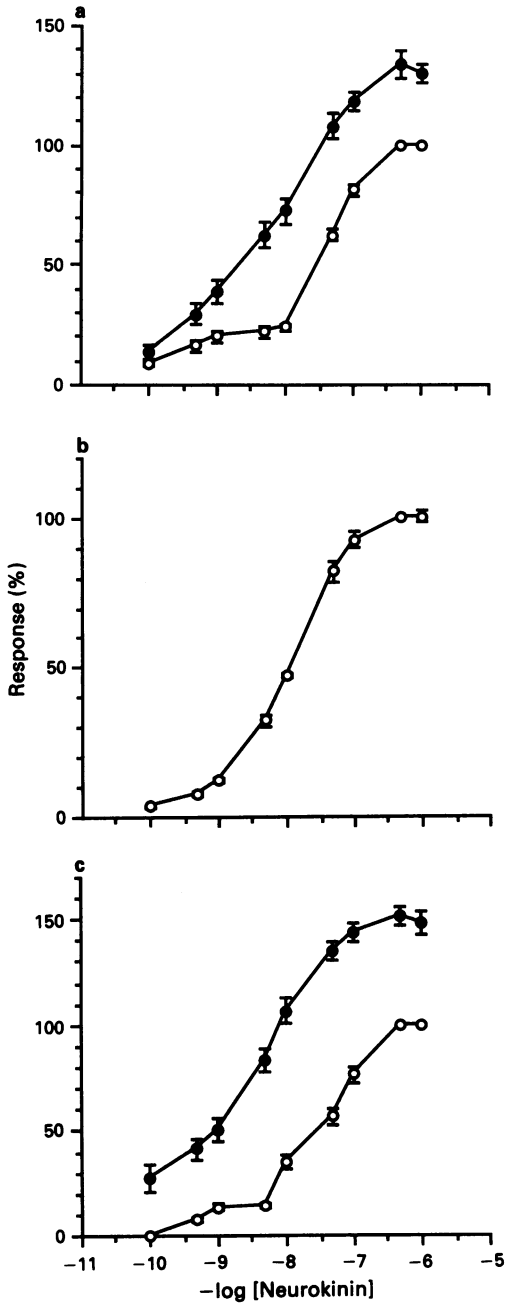

Figure 2 Concentration-response curves to neurokinins in the LMMP preparation of guinea-pig ileum. (a) (○) Substance P (SP) and (●) [Sar^9,met(O$_2$)^{11}]SP; (b) (○) neurokinin A (NKA); (c) (○) NKB and (●) senktide. Values are means ± s.e. from 10–12 preparations and were expressed as percentage of the maximum response obtained with SP (a), NKA (b) or NKB (c).

5 nM and 1 μM ($-\log EC_{50}^1 = 9.3 \pm 0.1$; $-\log EC_{50}^2 = 7.1 \pm 0.01$). The concentration-response curve to SP was also biphasic ($-\log EC_{50}^1 = 8.6 \pm 0.1$; $-\log EC_{50}^2 = 7.3 \pm 0.1$) (Figure 2). NKA gave rise to a monophasic concentration-response curve ($-\log EC_{50} = 7.9 \pm 0.1$). The selective neurokinin receptor agonists, [Sar^9, Met(O$_2$)^{11}]SP and senktide, also yielded monophasic concentration-response curves, with $-\log EC_{50}$ values of 8.2 ± 0.1 and 8.3 ± 0.1 respectively (Figure 2).

Desensitization studies

The application to the LMMP preparation of a high concentration of any neurokinin (0.1–0.5 μM) produced a contraction that faded to baseline after a contact time of 6–12 min. When a desensitization to a particular neurokinin was produced, the tissue became insensitive to further doses of the same neuropeptide, although other neurokinins were still active in the preparation (Table 1). For example, after desensitization to NKB, approximately 40% of the response to SP remained and *vice versa*. The response to any of the three neurokinins was completely abolished in tissues preincubated with NKA, 0.5 μM (not shown).

Desensitization with the selective NK$_1$ agonist, [Sar^9, Met(O$_2$)^{11}]SP, abolished the response to low concentrations of SP, corresponding to the first phase of the biphasic concentration-response curve, while the response to higher SP concentrations was only partially inhibited. Responses to different concentrations of NKB were not modified by NK$_1$ receptor desensitization (Table 1).

Inactivation of the neurokinin receptors did not affect the muscarinic receptors of the preparation, since the response to carbachol, 1 μM, was not modified after desensitization with the neurokinins (not shown).

As described above, a contractile response to either 5-MeOT or 2-Me-5-HT was still present after atropine (Figure 1). A fixed concentration of both 5-HT receptor agonists, approximately the EC_{50} was used for the subsequent experiments. In the presence of atropine 0.1 μM, the remaining response to 5-MeOT, 1 μM, was blocked after preincubation of the preparation with NKB, whereas the remaining effect of 2-Me-5-HT, 10 μM, was blocked after preincubation with either SP or NKB. The contractile response to both 5-HT receptor agonists was entirely (2-Me-5-HT) or almost entirely (5-MeOT) suppressed in tissues desensitized with NKA (0.5 μM) in the presence of atropine (Table 2).

By use of selective neurokinin receptor agonists, the non-cholinergic contraction elicited by 2-Me-5-HT was abolished after desensitization of the preparation with [Sar^9, Met(O$_2$)^{11}] SP. The same desensitization procedure also reduced significantly the atropine-resistant response to 5-MeOT (Table 2). Desensitization with the NK$_3$ agonist, senktide, did not modify the non-cholinergic response induced by stimulation of either 5-HT$_3$ or 5-HT$_4$ receptors (Table 2).

Another experiment was carried out in which the contractile response to 5-MeOT was recorded, in the absence of

Table 1 Effect of desensitization of NK$_1$ and NK$_3$ receptors on the response to endogenous neurokinins in the LMMP preparation of the guinea-pig ileum.

		Desensitization of NK$_1$ receptors		*Desensitization of NK$_3$ receptors*	
Neurokinin		*SP*	*[Sar^9,Met(O$_2$)^{11}]SP*	*NKB*	*Senktide*
SP	100 nM	0	39.1 ± 6.0	41.8 ± 4.4	100
	10 nM	0	0	–	100
	1 nM	0	0	–	100
NKB	100 nM	36.5 ± 3.9	44.8 ± 3.8	0	64.2 ± 18.0
	10 nM	–	74.4 ± 8.4	0	0
	1 nM	–	100	0	0

Desensitization was obtained by the repeated addition of the neurokinin or synthetic analogue (0.5 μM) until the response faded to baseline level. Results are expressed as percentage of the values obtained before desensitization (mean ± s.e. of 6–8 determinations).

Table 2 Effect of desensitization to neurokinins on the response to 2-methyl-5-HT and 5-methoxytryptamine in the presence of atropine (0.1 μM) in the LMMP preparation of the guinea-pig ileum.

Desensitization procedure	2-Methyl-5-HT (10 μM) Before desensitization	2-Methyl-5-HT (10 μM) After desensitization	5-Methoxytryptamine (1 μM) Before desensitization	5-Methoxytryptamine (1 μM) After desensitization
NK_1 receptors:				
SP	61.0 ± 5.0	0	75.3 ± 5.3	67.8 ± 7.9
[Sar^9,Met(O$_2$)^{11}]SP	53.6 ± 4.0	0	71.3 ± 6.1	25.4 ± 1.8
NK_2 receptors:				
NKA	61.0 ± 5.0	0	76.7 ± 5.9	16.7 ± 3.9
NK_3 receptors:				
NKB	61.0 ± 5.0	0	77.5 ± 6.2	0
Senktide	54.2 ± 4.5	50.9 ± 4.5	71.0 ± 6.0	67.4 ± 7.8

Desensitization was obtained by the repeated addition of the neurokinin or synthetic analogue (0.5 μM) until the response faded to baseline level. Results are expressed as percentage of the contraction obtained with the 5-HT receptor agonist in the absence of atropine (mean ± s.e. of 6–8 determinations).

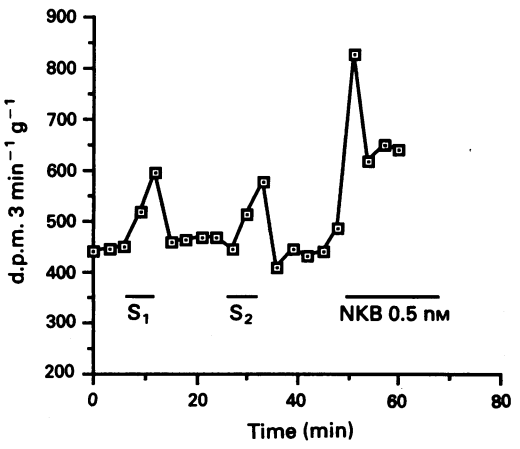

Figure 3 Representative experiment showing the effect of neurokinin B (NKB 0.5 nM) on tritium release used as an index of [^{3}H]-acetylcholine release from the LMMP preparation of guinea-pig ileum. Strips preincubated with [methyl-^{3}H]-choline were subsequently superfused with Tyrode solution containing 10 μM hemicholinium-3. Electrical stimulation (1 Hz, 1 ms, 13.5 V) was performed 6 min (S_1) and 27 min (S_2) after the end of the 60 min washout period. Superfusion with NKB started 21 min after S_2.

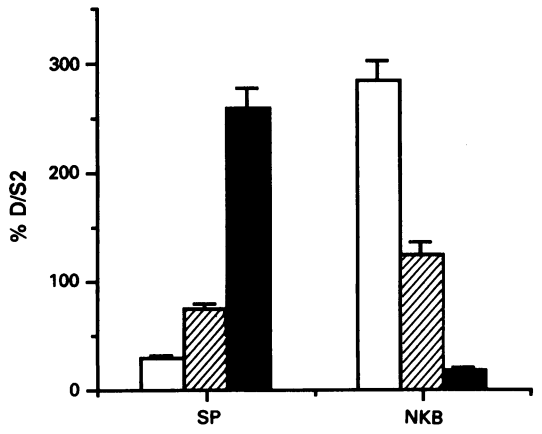

Figure 4 Effect of different concentrations of substance P (SP) and neurokinin B (NKB) on ^{3}H-overflow from LMMP preparations of guinea-pig ileum preloaded with [^{3}H]-choline (□) 0.5 nM, (▨) 5 nM, and (■) 0.1 μM. The effect of the neurokinins (D) on the outflow of ^{3}H-label is expressed as a percentage of the increase in the output of ^{3}H-label evoked by the second electrical stimulation (S_2). Values are mean ± s.e. from 6–8 preparations.

atropine, after the usual desensitization procedure with senktide. The response to 5-MeOT was then 68.4 ± 5.9% (mean ± s.e. of eight experiments) of the control response to the 5-HT$_4$ receptor agonist.

Effect of SP and NKB on tritium overflow from preparations prelabelled with [^{3}H]-choline

Both SP and NKB were able to promote ^{3}H-overflow from the LMMP preparation preloaded with [^{3}H]-choline. A representative experiment is shown in Figure 3. The neurokinins were tested at three different concentrations (0.5, 5 and 100 nM), corresponding to the high- or low-affinity phase of the concentration-response curve. SP produced a concentration-dependent increase in [^{3}H]-overflow, while an inverse relationship was found with NKB, i.e. lower concentrations produced a much higher ^{3}H-overflow (Figure 4).

Discussion

The results of the present study suggest the involvement of two different neurokinins, SP and NKB, in the non-cholinergic response to activation of 5-HT$_3$ and 5-HT$_4$ receptors in guinea-pig ileum. Apparently, only NKB seems to be involved in the response induced by 5-HT$_4$ receptor stimulation.

It is known that the response mediated by the 5-HT$_4$ receptor subtype is atropine-sensitive (Clarke *et al.*, 1989). Accordingly, the monophasic concentration-response curve to 5-MeOT, an agonist more selective for 5-HT$_4$ than for 5-HT$_3$ receptors (Fozard, 1990), was partially antagonized by atropine 0.1 μM. The role of acetylcholine in the response to 5-HT$_3$ receptor activation has not been clearly established (Buchheit *et al.*, 1985; Fox & Morton, 1990), but in the present study, the concentration-response curve to the selective 5-HT$_3$ receptor agonist 2-Me-5-HT (Richardson *et al.*, 1985) was markedly attenuated by atropine, 0.1 μM, suggesting that acetylcholine is also implicated in the response mediated by activation of this 5-HT receptor subtype.

The nature of the atropine-resistant component in the contractile response to both 2-Me-5-HT and 5-MeOT was next considered in the present study. It has been proposed that the contractile effect of 5-HT in the LMMP preparation is mediated, at least in part, by SP (Buchheit *et al.*, 1985), although this point has been controversial (Sanger & Nelson, 1989; Craig *et al.*, 1990; Fox & Norton, 1990). It is known that this preparation contains not only NK_1 receptors for SP, but also NK_3 receptors for NKB (Yau & Youther, 1982; Laufer *et al.*, 1985). Biphasic concentration-response curves were obtained with both neurokinins; the first phase of the curve probably corresponding to the activation of the receptor for which the neurokinin is more specific (SP for NK_1

and NKB for NK_3 receptors) while the second phase, with higher concentrations of the neurokinin, should originate from the activation of both neurokinin receptor subtypes.

To study the implication of the neurokinins in the contractile response evoked by activation of either 5-HT_3 or 5-HT_4 receptors, agonist-induced desensitization was used to block the neurokinin effect. Even though potent competitive antagonists of NK_1 receptors were recently described (e.g. Watling & Krause, 1993), there is not enough evidence on the selectivity of antagonists for other neurokinin receptor subtypes. Agonist-induced desensitization may consequently represent a valid alternative to receptor blockade. In fact, desensitizing studies provided the first indication of neurokinin receptor heterogeneity (Lee *et al.*, 1982; Laufer *et al.*, 1985). After desensitization of the preparation with NKB, a significant percentage of the response to SP remained and *vice versa*. When the desensitization was produced with the selective agonist senktide (Wormser *et al.*, 1986; Guard *et al.*, 1990), the contractile response to low concentrations of NKB was suppressed, as expected, while the response to SP was not affected. The remaining response to high concentrations of NKB after NK_3 receptor desensitization would correspond to stimulation of the myogenic NK_1 receptor also present in the preparation.

The contractile response to 2-Me-5-HT was abolished after desensitization with either SP or NKB in the presence of atropine. To assess which neurokinin was preferentially involved in this response the desensitization procedure was repeated with the selective NK_1 receptor agonist [Sar^9, $Met(O_2)^{11}$]SP (Drapeau *et al.*, 1987). The response to 2-Me-5-HT was fully suppressed, while desensitization with the NK_3 agonist senktide had no effect on the atropine-resistant response. These results suggest the specific involvement of SP in the response to 5-HT_3 receptor activation. Coexistence of acetycholine with neurokinins has been repeatedly described in excitatory enteric neurones (see review by Furness *et al.*, 1992) and it is possible that 5-HT_3 receptor activation releases both of these neurotransmitters which contribute to the contractile response.

At variance with the above data, the atropine-resistant response to the 5-HT_4 receptor agonist, 5-Me-OT, was only abolished after desensitization with NKB. Neither SP nor the NK_3 agonist, senktide, modified the response. Interestingly, the contractile response to 5-MeOT was essentially the same after adding atropine to the preparation or desensitizing with senktide in the absence of the muscarinic receptor antagonist. This may be indicative of a direct coupling between NKB-containing neurones and cholinergic neurones in the myenteric plexus. The ability of senktide to release acetylcholine in this preparation has been described (Guard & Watson, 1991; Fox & Morton, 1991; see also below). Surprisingly, a high percentage of the atropine-resistant response to 5-MeOT was eliminated when desensitizing with the NK_1 receptor agonist [Sar^9, $Met(O_2)^{11}$]SP. As shown in Figure 2, the concentration-response curve to NKB is biphasic. The first phase is likely to correspond to the specific activation of NK_3 receptors whereas the second part would represent the activation not only of NK_3 receptors, but also of a muscular NK_1 receptor (Kilbinger *et al.*, 1986) with a presumably higher contribution to the contractile response. Another possible explanation for the remaining effect of 5-MeOT after desensitization with senktide could be the activation of NK_2 receptors, since desensitization of the preparation to NKA markedly blocks the response to 5-MeOT 1 μM. Some authors have suggested the mediation of this neurokinin receptor subtype in the contractile response of the preparation (Jacoby *et al.*, 1986; Dion *et al.*, 1987), while this possibility has been excluded by others (Laufer *et al.*, 1988).

When SP and NKB were compared in preparations preloaded with [^{3}H]-choline for their ability to induce ^{3}H-overflow, an index of [^{3}H]-acetylcholine release (Szerb, 1976; Wikberg, 1977), NKB produced at a low concentration a much more marked ^{3}H-overflow than SP. This result argues in favour of the physiological significance of NKB as an acetylcholine releaser in this preparation. Other studies in different tissues (e.g. Arenas *et al.*, 1991) are also indicative of the potency of NKB in inducing acetylcholine release. As shown in Figure 4, a very high SP concentration was necessary to induce a marked acetylcholine release. This would explain the biphasic concentration-response curve to SP: the first phase of the curve would correspond to stimulation of muscular NK_1 receptors, while the second phase would be the result of both NK_1 receptor stimulation and acetylcholine release after NK_3 receptor activation. The decreased acetylcholine release when increasing the NKB concentrations would in turn originate from the activation of NK_1 receptors at higher concentrations of NKB.

An alternative interpretation of the present results could be an unpredictable direct desensitizing effect of the neurokinins on 5-HT receptors. For example, electrophysiological (Clapham & Neher, 1984), biochemical (Role *et al.*, 1981) and functional (Molinero & Del Río, 1987) experiments indicate that SP reduces the action of acetylcholine at nicotinic receptors and it has been suggested that SP may enhance nicotinic receptor desensitization (Akasu *et al.*, 1984; Clapham & Neher, 1984). While a direct regulatory action of the neurokinins on 5-HT receptors cannot be discounted, it was verified that the muscarinic receptors of the LMMP preparation were not affected by the desensitization procedures used in this study since the contractile response to carbachol was not modified.

In summary, acetylcholine and the neurokinins SP and NKB appear to be involved in the contractile response to either 5-HT_3 or 5-HT_4 receptor activation in the LMMP preparation of the guinea-pig ileum. Whilst SP seems to be more specifically involved in the response to 5-HT_3 receptor activation, it is of particular interest to note the possible involvement of NKB-containing neurones in the cholinergic response elicited by 5-HT_4 receptor stimulation. This possibility should be taken into consideration when analysing the mechanism of action of compounds such as cisapride or metoclopramide, which stimulate 5-HT_4 receptors (Linnik *et al.*, 1991; Meulemans & Schuurkes, 1992), and are widely used for the treatment of different gastrointestinal disorders.

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