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Tachykinin receptors in the guinea-pig renal pelvis: activation by exogenous and endogenous tachykinins

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1 The contractile response to substance P, neurokinin A, selective agonists for the NK_1, NK_2 and NK_3 tachykinin receptors and the activity of receptor-selective antagonists has been investigated in circular muscle strips of the guinea-pig isolated renal pelvis in the presence of indomethacin (3 μM).

2 Neurokinin A was the most potent agonist tested, being about 32 times more potent than substance P. The action of both substance P and neurokinin A was enhanced by peptidase inhibitors (bestatin, captopril and thiorphan, 1 μM each). The selective NK_2 receptor agonist [βAla^8] neurokinin A (4-10), was slightly less potent and effective than neurokinin A itself. The selective NK_1 receptor agonist [Sar^9] substance P sulphone was effective at low (nM) concentrations but its maximal effect did not exceed 30% of maximal response to substance P or neurokinin A. The NK_3-selective agonist [MePhe^7] neurokinin B was effective only at high (μM) concentrations.

3 The pseudopeptide derivative of neurokinin A(4-10), MDL 28,564, displayed a clear-cut agonist character, although it was less potent than neurokinin A.

4 The responses to roughly equieffective (25–35% of maximal response) concentrations of [βAla^8] neurokinin A (4-10), MDL 28,564 and [MePhe^7] neurokinin B were antagonized to a similar extent by MEN 10,376 (3 μM), a selective NK_2 tachykinin receptor antagonist, while the response to [Sar^9] substance P sulphone was unchanged.

5 The response to [Sar^9] substance P sulphone was inhibited by the NK_1 receptor-selective antagonist, GR 82,334 (3 μM) while the response to [βAla^8] neurokinin A (4-10) was unchanged.

6 The selective NK_2 receptor antagonists MEN 10,376, L 659,877 and R 396 antagonized competitively the response to [βAla^8] neurokinin A (4-10) with the following rank order of potency (pA_2 values in parentheses): MEN 10,376 (7.41) > L 659,877 (7.15) > R 396 (6.43). MEN 10,376 and L 659,877 also competitively antagonized the response to neurokinin A, although with lower potency as compared to the selective NK_2 receptor agonist.

7 MEN 10,376, L 659,877 and R 396 reduced in a concentration-dependent manner the contractile response produced by electrical field stimulation (1 Hz, 100 V, 0.25 ms pulse width, trains of 10 s). The rank order of potency of NK_2 receptor antagonists in blocking the response to electrical stimulation (MEN 10,376 > L 659,877 > R 396) closely mimicked their potency in antagonizing exogenous tachykinins.

8 The inhibitory effect of MEN 10,376 toward responses produced by electrical field stimulation was significantly reduced when tested in the presence of peptidase inhibitors, which increased significantly the response to nerve stimulation.

9 GR 82,334 (3 μM) did not significantly affect the response to nerve stimulation in untreated preparations and slightly reduced it in the presence of peptidase inhibitors.

10 We conclude that both NK_1 and NK_2 receptors mediate the contractile effect of tachykinins in the circular muscle of the guinea-pig renal pelvis and that the response ascribable to NK_2 receptor stimulation is larger than that ascribed to NK_1 receptor stimulation. The NK_2 receptor in the guinea-pig renal pelvis belongs to the same subtype previously identified in the rabbit pulmonary artery. NK_2 receptors play a dominant role in the physiological response determined by the release of endogenous tachykinins and a contribution of NK_1 receptors becomes evident after inhibition of peptide degradation.

Keywords: Guinea-pig renal pelvis; tachykinins; tachykinin receptors; NK_2 receptor subtypes; tachykinin antagonists

Introduction

In mammals, the renal pelvis and ureter receive a dense afferent peptidergic innervation, which signals ureteral pain. The major part (91%) of afferents in the guinea-pig ureter belong to that subpopulation of sensory neurones which are capsaicin-sensitive (Cervero & Sann, 1989). In both the renal pelvis (Maggi *et al.*, 1992a) and ureter (Maggi & Giuliani, 1991), the capsaicin-sensitive primary afferents participate in the local modulation of ureteral motility through neuropeptide release from their peripheral nerve endings. In the guinea-pig isolated renal pelvis, the local activation of neuropeptide release from capsaicin-sensitive primary afferents mediates a contractile response which reinforces the spontaneous activity sustained by natural pacemakers (Maggi *et al.*, 1992a; Golenhofen & Hannappel, 1973).

This contractile effect is ascribable to the release of endogenous tachykinins, such as substance P and neurokinin A (Maggi *et al.*, 1992a). Both substance P- and neurokinin A-like immunoreactivity have been shown to be present in the guinea-pig renal pelvis and are simultaneously released following application of capsaicin. Neurokinin A is more

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potent than substance P in producing contraction of the isolated renal pelvis, suggesting the presence of tachykinin NK_2 receptors at this level. However, the receptors involved in tachykininergic control of renal pelvis motility have not been fully characterized. Furthermore, recent studies have provided evidence for the heterogeneity of tachykinin NK_2 receptors, possibly reflecting the existence of NK_2 receptor subtypes (Maggi *et al.*, 1990; 1991a; Buck *et al.*, 1990; Van Giersbergen *et al.*, 1991; Patacchini *et al.*, 1991). The aim of this study was twofold: (a) to assess the type of tachykinin receptors mediating contraction of the guinea-pig isolated renal pelvis and (b) to assess the relative contribution of different tachykinin receptors to the response produced by endogenous tachykinins released during nerve stimulation.

Methods

Male albino guinea-pigs (250–300 g b.wt.) were killed by a blow on the back of the head and exsanguination. The whole kidney and attached ureter were removed and placed in oxygenated (96% O_2 and 4% CO_2) Krebs solution, as described previously (Maggi *et al.*, 1992a). The renal pelvis was carefully dissected from the renal parenchyma, separated from the ureter, cut and connected to threads to record motility along the circular axis. The preparation was suspended in a 5 ml organ bath and mechanical activity recorded by means of an isotonic transducer (load 0.15 mN). Transmural electrical field stimulation was applied by means of platinum wire electrodes placed at the top and bottom of the organ bath and connected to a GRASS S 11 stimulator. Square wave pulses (pulse width 0.25 ms, 100 V) were delivered in trains of 10 s duration at a frequency of 1 Hz.

Indomethacin (3 μM) was added to the Krebs solution in order to reduce the amplitude of spontaneous activity and obtain a better quantitative evaluation of the contractile response to stimulants. All experiments started after a 60–90 min equilibration period when the amplitude and frequency of spontaneous activity had reached a steady state (about 15% of maximal contractile responses).

Concentration-response curves to the agonists were constructed in a cumulative manner, the next concentration being added when the effects of the preceding one had reached a steady state.

Preliminary experiments had shown that the contractile response to natural tachykinins and receptor selective synthetic agonists in this preparation do not exhibit significant desensitization. The only exception was the selective NK_1 receptor agonist, [Sar^9] substance P sulphone (Dion *et al.*, 1987) for which non-cumulative concentration-response curves were constructed, because of desensitization. For this agonist, non-cumulative concentration-response curves were constructed by addition of increasing concentrations of the agonist at 15–20 min intervals with washouts intervening between doses.

The effect of antagonists toward contractions produced by agonists were investigated after a contact time of 15 min which, in preliminary experiments, was shown to allow expression of maximal inhibitory effect.

Data evaluation

Previous experiments (Maggi *et al.*, 1992a) had shown that tachykinins produce complex motor response in the spontaneously contracting guinea-pig renal pelvis. The first effect, observed with low concentrations of the agonist is a pure enhancement of amplitude of contractions, accompanied, at higher concentrations, by an increased frequency of contractions. Finally, high concentrations of tachykinins which produce maximal increase in contraction amplitude, also produce an increase in tone. In the text and figures all the effect of agonist and antagonists describe changes in amplitude of contractions produced by test substances as compared to the original baseline.

All changes in mechanical activity were expressed as % of maximal contracture produced by application of barium chloride (10 mM).

Statistical analysis

All values in the text and figures are mean ± s.e. mean. Statistical analysis was performed by means of Student's *t* test for paired or unpaired data or by means of analysis of variance, when applicable. The effect of tachykinin antagonists on the contractile response produced by electrical field stimulation was evaluated by one way analysis of variance and Dunnett's test.

To estimate affinities of NK_2 receptor antagonists toward the responses produced by agonists, concentration-response curves were constructed in the absence and the presence of antagonists and Schild plots constructed accordingly. For slopes of Schild plots not significantly different from unity, the constrained plot method was used to calculate pA_2 values.

Drugs

Drugs used were: indomethacin, captopril, bestatin and thiorphan (Sigma), GR 82,334 (Bachem). Neurokinin A, MDL 28,564, [βAla^8] neurokinin A (4-10), [MePhe^7] neurokinin B, L 659,877 and MEN 10,376 were synthesized by conventional solid phase methods. [Sar^9] substance P sulphone was from Peninsula. R 396 was a kind gift of Prof. D. Regoli, Department of Pharmacology University of Sherbrooke, Canada. The amino acid sequence of peptides investigated is shown in Table 1.

Table 1 Amino acid sequence of peptides used in this study

Agonists	
Substance P	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
[Sar^9]SP sulphone	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2
NKA	H-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
[βAla^8]NKA (4-10)	H-Asp-Ser-Phe-Val-βAla-Leu-Met-NH_2
NKB	H-Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2
[MePhe^7]NKB	H-Asp-Met-His-Asp-Phe-Phe-MePhe-Gly-Leu-Met-NH_2
MDL 28,564	H-Asp-Ser-Phe-Val-Gly-Leuψ[CH_2NH]Leu-NH_2
Antagonists	
MEN 10,376	H-Asp-Tyr-DTrp-Val-DTrp-DTrp-Lys-NH_2
L 659,877	c(Leu-Met-Gln-Trp-Phe-Gly)
R 396	Ac-Leu-Asp-Gln-Trp-Phe-Gly-NH_2
GR 82,334	Pyr-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-DPro(spiro-γ-lactam)Leu-Trp-NH_2

NKA = neurokinin A; NKB = neurokinin B.

Results

Effect of agonists

Addition of tachykinins increased amplitude and frequency of spontaneous contactions of the guinea-pig isolated renal pelvis. For low concentrations of tachykinins the effect on contraction amplitude dominated and a clear-cut increase in frequency became evident when the positive inotropic effect had reached its maximum. At this stage, the increased frequency of contractions produced by added tachykinins was also accompanied by an increase in tone (Figure 1). For quantitative evaluation of drug effects the increased amplitude of contractions as compared to the original baseline was determined.

Either substance P or neurokinin A produced a concentration-dependent contraction (Figures 1 and 2) which reached the same maximum produced by addition of barium chloride (10 mM). Neurokinin A was about 32 times more potent than substance P (Table 2).

The response to both substance P and neurokinin A was significantly enhanced by peptidase inhibitors (captopril, bestatin and thiorphan, 1 μM each, 15 min before): in the presence of peptidase inhibitors substance P and neurokinin A were 8.5 and 3.7 times more potent than in control experiments, respectively (Table 2). The selective NK_1 receptor agonist, [Sar^9] substance P sulphone had a low threshold (10 nM) and its maximal effect (at 3 μM) did not exceed 30% of maximal response of the preparations. It was noted that the effect of [Sar^9] substance P sulphone exhibits marked desensitization and the curve shown in Figure 2 has been obtained following non-cumulative addition of the peptide. The action of [Sar^9] substance P sulphone was not affected by peptidase inhibitors (Figure 2).

The selective NK_2 receptor agonist, [βAla^8] neurokinin A (4-10) was less potent and less effective than neurokinin A (Figure 2). The maximal response to [βAla^8] neurokinin A (4-10) did not exceed 85% of maximal barium response with an EC_{50} of 95 nM (49–236 nM are 95% c.l.). The action of [βAla^8] was not significantly affected by peptidase inhibitors (Figure 2). The pseudopeptide, MDL 28,564 displayed a clearcut agonist character, although it was less potent than neurokinin A (threshold concentration 3 μM). At the highest concentration tested (100 μM) the effect of MDL 28,564 approached 75% of maximal response (Figure 2).

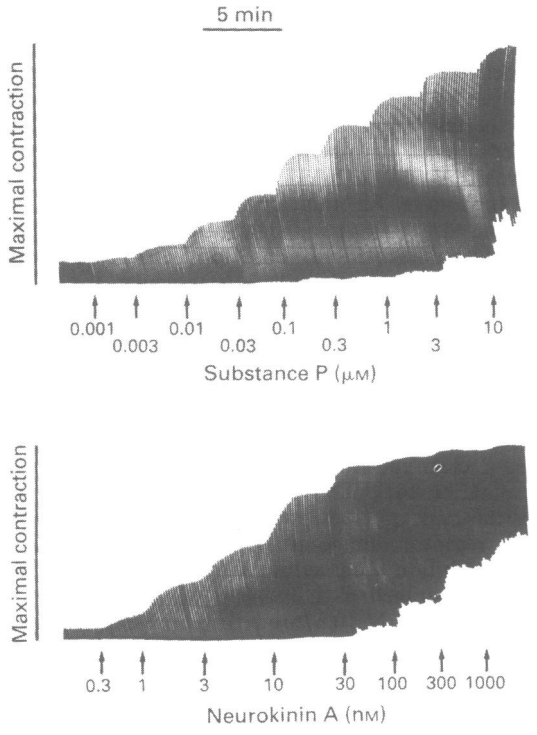

Figure 1 Typical tracing illustrating the contractile effect produced by cumulative addition of substance P or neurokinin A on the spontaneous activity of the guinea-pig isolated renal pelvis.

Table 2 EC_{50}s of substance P and neurokinin A in producing positive inotropic response of the guinea-pig isolated renal pelvis in the absence and presence of peptidase inhibitors

	EC_{50} (nM, 95% c.l. in parentheses)	
	Control	*Peptidase inhibitors*
Substance P	239 (92-602)	28 (16-60)
Neurokinin A	7.4 (6.0–9.6)	2 (1.5–2.4)

Concentration-response curves to substance P or neurokinin A were constructed in the absence (control) or presence of peptidase inhibitors (bestatin, captopril and thiorphan, 1 μM each).

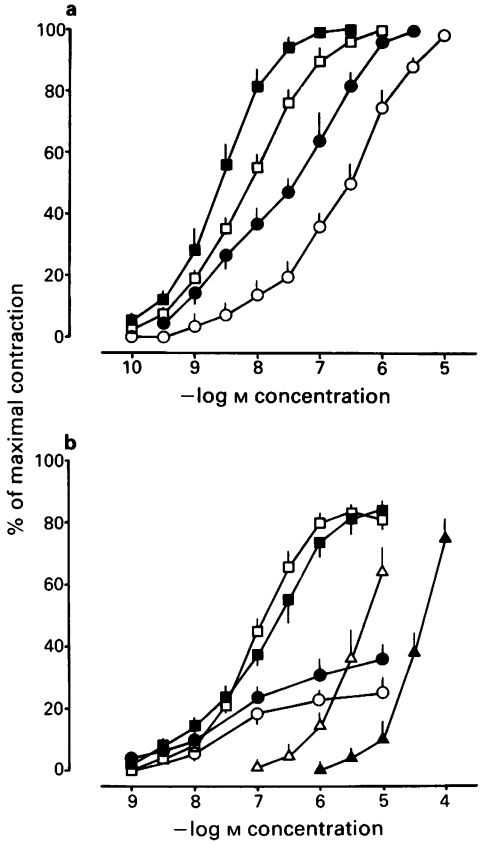

Figure 2 (a) Concentration-dependent contractile effect produced by substance P (SP, circles) or neurokinin A (NKA, squares) in the guinea-pig isolated renal pelvis. Open symbols refer to experiments obtained in the absence of peptidase inhibitors, solid symbols refer to experiments performed in the presence of bestatin, captopril and thiorphan 1 μM each, added 15 min beforehand. Each point is the mean of 12–14 experiments; vertical lines show s.e.mean. (b) Concentration-dependent contractile effect produced by receptor-selective tachykinin agonists in the guinea-pig isolated renal pelvis. For the NK_1 receptor-selective agonist [Sar^9] SP sulphone (circles) and the NK_2 receptor-selective agonist [βAla^8] NKA (4-10) (squares) experiments were performed either in the absence (open symbols) or presence (solid symbols) of peptidase inhibitors (bestatin, captopril and thiorphan 1 μM each, 15 min before). For the NK_2 receptor-selective agonists, MDL 28,564 (▲) and the NK_3 receptor-selective agonists [$MePhe^7$] neurokinin B (△) experiments were performed in the absence of peptidase inhibitors. Each point is mean of 5–8 experiments; vertical lines show s.e.mean.

Also the NK_3 receptor selective ligand, [MePhe^7] neurokinin B displayed agonist activity, although in a concentration-range (0.3–10 μM), much higher than that reported to be effective (low nM) in stimulating NK_3 receptors (Dion *et al.*, 1987).

Effect of antagonists on the response to neurokinin A and [βAla^8] neurokinin A (4-10)

The above experiments indicate that NK_2 receptors prevail in the guinea-pig renal pelvis although the presence of NK_1 and NK_3 receptors may not be ruled out. To address this point further, we studied the effect of MEN 10,376 (3 μM) an NK_2 receptor selective antagonist (Maggi *et al.*, 1991b) toward the response produced by roughly equieffective (25–35% of maximal response) concentrations of [Sar^9] substance P sulphone (3 μM), [βAla^8] neurokinin A (4-10) (30 nM), MDL 28,564 (30 μM) and [MePhe^7] neurokinin B (3 μM). Data in Figure 3 show that MEN 10,376 nearly abolished or greatly reduced the response to [βAla^8] neurokinin A (4-10), MDL 28,564 and [MePhe^7] neurokinin B while leaving the response to [Sar^9] substance P sulphone totally unaffected.

To assess whether the action of [Sar^9] substance P sulphone might involve activation of NK_1 receptors, the NK_1 receptor antagonist, GR 82,334 (3 μM) (Hagan *et al.*, 1991) was studied toward the response produced by [Sar^9] substance P sulphone (3 μM) and [βAla^8] neurokinin A (4-10) (30 nM). Data in Figure 4 show that GR 82,334 significantly inhibited (43%) the response to [Sar^9] substance P sulphone while leaving that to [βAla^8] neurokinin A (4-10) unaffected.

To assess further the nature of the NK_2 receptor involved in the response of the guinea-pig renal pelvis, full concentration-response curves to neurokinin A and [βAla^8] neurokinin A (4-10) were constructed and the effect of different concentrations of MEN 10,376, L 659,877 and R396 was investigated. For each antagonist, at least 4 different concentrations were tested and Schild plots constructed; for each agonist/antagonist combination tested, the slopes of Schild plots were not significantly different from unity (Table 3), and pA_2 values were calculated using the constrained Schild plot method (Tallarida *et al.*, 1979). None of the antagonists tested affected significantly spontaneous contractions of the pelvis. Data shown in Table 3 indicate that both MEN 10,376 and L 659,877 were distinctly more potent (about 10 times for each antagonist) in blocking the action of [βAla^8] neurokinin A (4-10) than in blocking the action of neurokinin A. Antagonist potency toward the effect of the selective NK_2 receptor agonist followed the rank order: MEN 10,376 > L 659,877 > R 396 (Table 3).

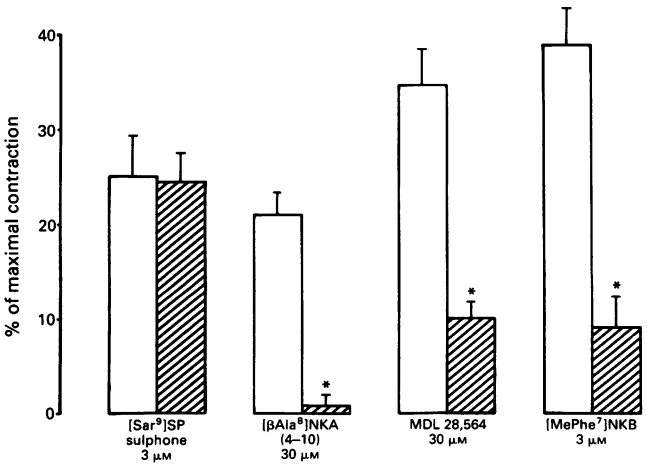

Figure 3 Effect of the NK_2 receptor selective antagonist, MEN 10,376 (3 μM) on the contractile effect produced by roughly equieffective concentrations of receptor-selective synthetic agonists in the guinea-pig isolated renal pelvis. Open columns = control; hatched columns = in the presence of MEN 10,376. Each column is mean of 5–7 experiments; vertical bars show s.e.mean. *Significantly different from the control response, $P<0.05$.

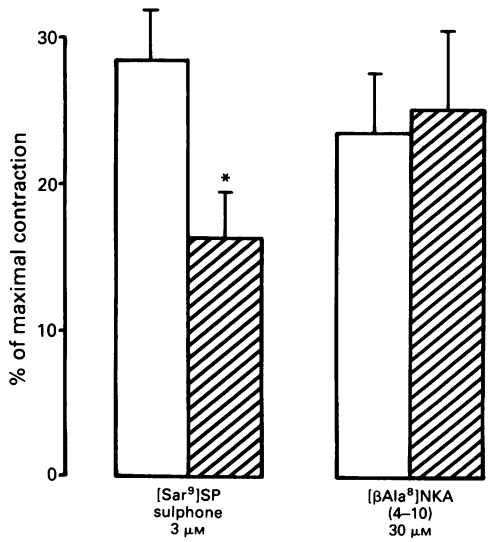

Figure 4 Effect of the NK_1 receptor-selective antagonist, GR 82,334 (3 μM) on the contractile effect produced by an NK_1 and an NK_2 receptor-selective agonist in the guinea-pig isolated renal pelvis. Open columns = control; hatched columns = in the presence of GR 82,334. Each column is mean of 5–6 experiments; vertical bars show s.e.mean. *Significantly different from the control response, $P<0.05$.

Effect of antagonists on the response to electrical field stimulation

The following experiments were designed to assess whether or not the same rank order of potency of NK_2 receptor antagonists observed toward the selective NK_2 receptor agonists [βAla^8] neurokinin A (4-10), occurs for inhibition of the response produced by nerve stimulation. With this aim, the response produced by a train of stimuli (100 V, 0.25 ms pulse width) delivered at a frequency of 1 Hz every 20 min was repeatedly elicited in the presence of increasing concentrations of the test antagonist. Three cycles of stimulation were performed in each strip before addition of the antagonists or of the vehicle.

Data in Figure 5a, indicate that, as compared to the spontaneous decay observed in control strips, MEN 10,376 significantly reduced the response to electrical stimulation at all concentrations tested (0.1–3 μM), whilst the inhibitory effect of L 659,877 and R 396 was statistically significant only at 1–3 μM for L 659,877 and at 3–10 μM for R 396, respectively. In Figure 5b, the effect of antagonists is presented as % inhibition of the evoked responses after the observed values had been corrected for the spontaneous decay observed over repeated stimulation cycles in controls; from this analysis, a clear-cut rank order of potency of antagonists emerges: MEN 10,376 > L 659,877 > R 396 which closely matches the rank order of potency in antagonizing the selective NK_2 receptor antagonist, [βAla^8] neurokinin A (4-10). Maximal inhibition observed with 3 μM MEN 10,376 in these experiments averaged 63 ± 2% ($n = 6$).

The effect of MEN 10,376 on strips pre-exposed to peptidase inhibitors (thiorphan, captopril and bestatin 1 μM each) was also investigated. The amplitude of the response to electrical stimulation (22 ± 4% of barium response, $n = 10$) was significantly enhanced (39 ± 4% of barium response, $n = 10$, $P<0.05$) by peptidase inhibitors. At 0.1–1 μM the inhibitory effect of MEN 10,376 was significantly reduced when tested in the presence of peptidase inhibitors (Figure 6).

Table 3 pA_2 values and slopes of Schild plots of MEN 10,376, L 659,877 and R 396 toward positive inotropic effect produced by neurokinin A or the selective NK_2 tachykinin receptor agonist, [βAla^8] neurokinin A (4-10) on the circular muscle of the guinea-pig isolated renal pelvis

Antagonist	*Neurokinin A*		*[βAla^8] neurokinin A*	
	Slope	*pA_2*	*Slope*	*pA_2*
MEN 10,376	−0.86 (0.67–1.05)	6.56 ± 0.08	−0.75 (0.41–1.19)	7.41 ± 0.09
L 659,877	−1.29 (0.56–2.03)	6.08 ± 0.11	−1.44 (0.80–1.74)	7.15 ± 0.11
R 396	NT	NT	−0.80 (0.59–1.22)	6.43 ± 0.10

Each value was calculated from at least 9 experiments.
pA_2 values were calculated using the constrained Schild plot method
NT = not tested

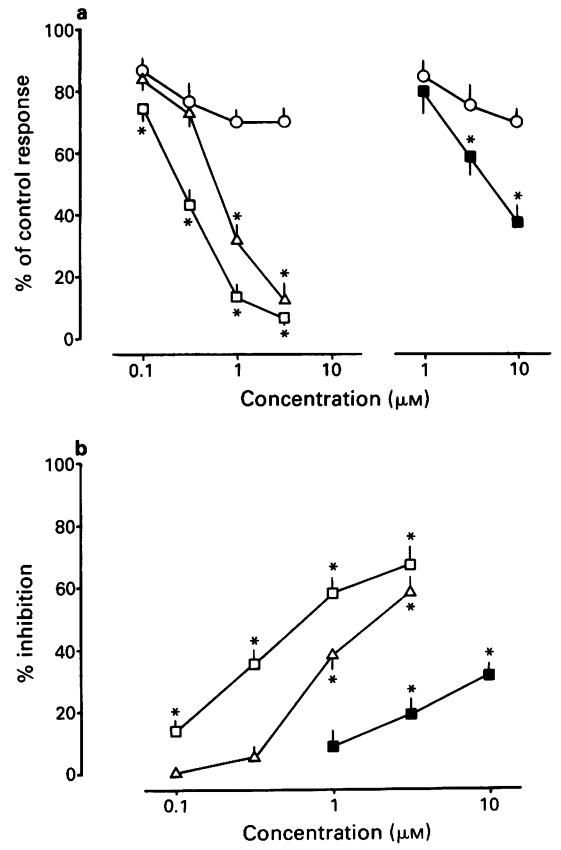

Figure 5 (a) Concentration-dependent inhibitory effect produced by cumulative addition of three NK_2 receptor selective antagonists MEN 10,376 (□), L 659,877 (△) and R 396 (■) on the contractile response to electrical field stimulation in the guinea-pig isolated renal pelvis; (○) indicate the spontaneous decay of the response in control experiments. In (b) the effect of the three antagonists is shown as % inhibition of the response to electrical field stimulation after correction for the spontaneous decay of the response observed in control experiments. Each point is mean of 6 experiments; vertical lines show s.e.mean. *Significantly different from the control response, $P<0.05$.

In a further series of experiments, the effect of MEN 10,376 and GR 82,334 (both at 3 μM) was investigated toward the response produced by electrical field stimulation, in the absence and presence of peptidase inhibitors. MEN 10,376 was effective both in the absence and presence of peptidase inhibitors (65 ± 2 and 49 ± 5% inhibition, respectively). GR 82,334 failed to affect the evoked response in the absence of peptidase inhibitors but slightly reduced it (about 25%) in the presence of bestatin, captopril and thiorphan (Figure 7).

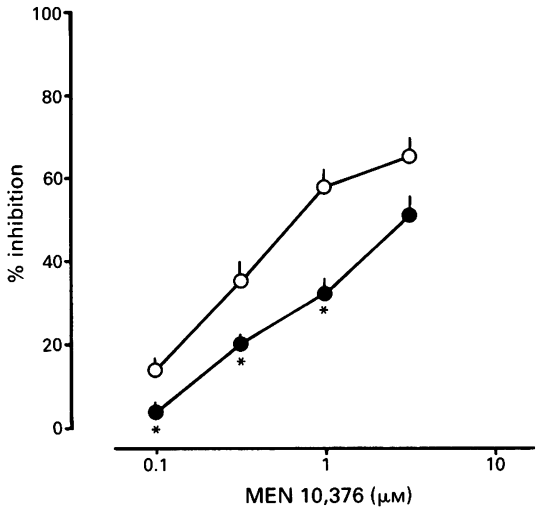

Figure 6 Concentration-dependent inhibitory effect of the NK_2 receptor-selective antagonist, MEN 10,376 on the contractile effect produced by electrical field stimulation in the guinea-pig isolated renal pelvis in the absence (control, ○) and presence of peptidase inhibitors (bestatin, captopril and thiorphan, 1 μM each, ●). Each point is mean of 6–8 experiments; vertical lines show s.e.mean. *Significantly different from the control response, $P<0.05$.

Discussion

Tachykinin receptors in the guinea-pig renal pelvis

The combined results of this study indicate that the NK_2 receptor is the principal mediator of the contractile effect exerted by tachykinins in the guinea-pig isolated renal pelvis. In fact: (a) neurokinin A is more potent than substance P; (b) the NK_2 agonist, [βAla^8] neurokinin A (4-10) was effective at low concentrations; (c) MDL 28,564 which is perhaps the most selective NK_2 receptor ligand available (Harbeson *et al.*, 1990), displayed agonist activity blocked by the NK_2 receptor antagonist MEN 10,376; (d) selective NK_2 receptor antagonists such as MEN 10,376 (Maggi *et al.*, 1991b), L 659,877 (Williams *et al.*, 1988) and R 396 (Dion *et al.*, 1990) were effective against neurokinin A and [βAla^8] neurokinin A (4-10) (see below for discussion about NK_2 receptor subtypes); (e) the response to [βAla^8] neurokinin A (4-10) is unaffected by GR 82,334, a selective NK_1 receptor antagonist (Hagan *et al.*, 1991).

In the past two years, pharmacological evidence has been provided for the heterogeneity of the tachykinin NK_2 receptor (Maggi *et al.*, 1990; 1991a; Buck *et al.*, 1990; Van Giersbergen *et al.*, 1991; Patacchini *et al.*, 1991). Two criteria which allow the distinction between NK_2 receptor subtypes have emerged: (a) rank order of potency of antagonists and

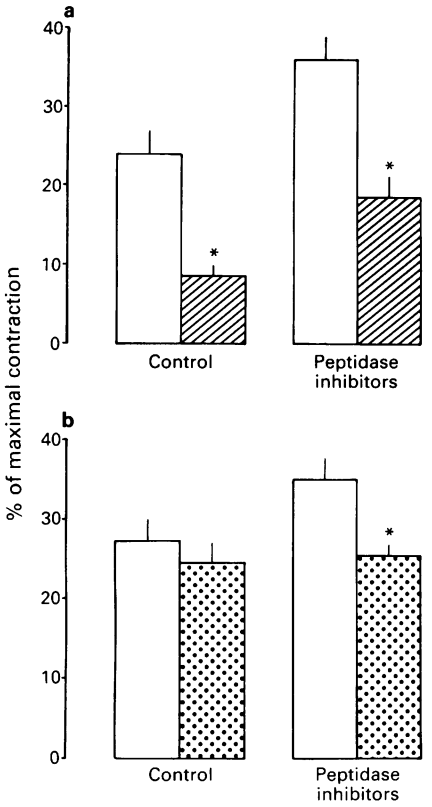

Figure 7 (a) Comparison of the inhibitory effect of the NK_2 receptor-selective antagonist, MEN 10,376 on the contractile response produced by electrical field stimulation in the guinea-pig isolated renal pelvis in the absence or presence of peptidase inhibitors (bestatin, captopril and thiorphan 1 μM each). Open columns = control; hatched columns = in the presence of MEN 10,376. (b) The same as for (a) but with the NK_1 receptor-selective antagonists GR 82,334. Open columns = control; stippled columns = in the presence of GR 82,334. Note that GR 82,334 produced a small inhibitory effect only in the presence of peptidase inhibitors. Each column is mean of 6–8 experiments; vertical lines show s.e.mean. *Significantly different from the control responses, $P<0.05$.

(b) the different agonist/antagonists character of MDL 28,564. On the basis of present findings we conclude that the NK_2 receptor mediating positive inotropic effect in the guinea-pig renal pelvis is of the same subtype occurring in the guinea-pig bronchi (Maggi *et al.*, 1991c) and rabbit pulmonary artery (Maggi *et al.*, 1990).

Our data also consistently indicate the existence of a population of NK_1 receptors mediating contraction of the guinea-pig renal pelvis; this conclusion is based on: (a) the response to [Sar^9] substance P sulphone, which is a highly selective NK_1 receptor agonist (Dion *et al.*, 1987), with negligible or no activity at NK_2 receptors and subtypes (e.g. Maggi *et al.*, 1990); (b) the response to [Sar^9] substance P sulphone was unaffected by MEN 10,376 while it was reduced by GR 82,334.

We can exclude a participation of NK_3 receptors in the contractile response of the pelvis. [MePhe^7] neurokinin B has been shown to stimulate NK_3 receptors in the guinea-pig ileum and rat portal vein at nM concentrations (Dion *et al.*, 1987). This ligand is certainly more selective than neurokinin B itself for NK_3 receptors, but at high concentrations it may act at NK_2 sites (Dion *et al.*, 1987); this probably occurs under our experimental conditions because [MePhe^7] neurokinin B was effective at μM concentrations and its effect was blocked by MEN 10,376 to a similar extent as the response to MDL 28,564 or [βAla^8] neurokinin A (4-10).

Therefore, both NK_1 and NK_2 receptors mediate the response of the guinea-pig isolated renal pelvis to tachykinins. In this study, we concentrated our efforts on the action of test peptides on the amplitude of spontaneous contractions. This parameter is the more sensitive measure of the effect of tachykinins in this preparation. It is evident that, for high concentration of tachykinins, the increased frequency of contractions and the increased tone (possibly related to the failure to relax to baseline because of the increased frequency of contractions) also contribute to the overall increase in amplitude. Although we did not note any major difference in this respect between e.g. neurokinin A and substance P, further studies are needed to assess whether NK_1 and NK_2 receptors might play a differential role in mediating the effect of tachykinins on contraction amplitude and frequency.

While natural tachykinins were capable of producing a maximal response, this was not the case for the NK_1 or NK_2 receptor-selective agonists: their maximal effect approached about 30% (for [Sar^9] substance P sulphone) and 85% (for [βAla^8] neurokinin A(4-10)) of that to neurokinin A or substance P. We interpret these findings as further indication that natural tachykinins have the ability to interact, although at different concentrations, with both NK_1 and NK_2 tachykinin receptors. At the same time, it is evident that the relative contribution of NK_2 receptors to the evoked response of the pelvis is quantitatively greater than that of NK_1 receptors, a conclusion supported by experiments with NK_2 receptor antagonists toward the responses elicited by endogenous tachykinins (see below).

Tachykinin receptors and activation by endogenous ligands

In a previous study (Maggi *et al.*, 1992a) we proposed that endogenous tachykinins are responsible for the contractile effect produced by nerve stimulation of the guinea-pig isolated renal pelvis. Having established that both NK_1 and NK_2 receptors are present at this level, the next logical step was to determine the relative contributions of the two receptors in the response produced by release of endogenous ligands for tachykinins receptors.

Our data indicate that the same subtype of NK_2 receptor which is activated by exogenous tacykinins is also responsible for a large fraction of the response to endogenous tachykinins released by electrical stimulation of intramural nerves. The rank order of potency of NK_2 receptor antagonists in reducing the response to electrical stimulation was the same as that found for their ability to antagonize the response to [βAla^8] neurokinin A (4-10). By contrast GR 82,334 at a concentration which selectively reduces the response to [Sar^9] substance P sulphone did not affect the response to electrical stimulation. Accordingly, a more important role for neurokinin A (or for other ligands with high affinity for the NK_2 receptor) than for substance P in the response produced by activating sensory nerves in the renal pelvis can be postulated. Such an hypothesis is also supported by the observation that the maximal effect of the NK_1 receptor-selective agonist is much lower than that produced by the NK_2-receptor selective agonist.

In the presence of peptidase inhibitors, three major changes occurred: (a) the potency of natural tachykinins was enhanced, particularly for substance P; (b) the response produced by electrical stimulation was increased, suggesting degradation of endogenous tachykinins; (c) the effectiveness of MEN 10,376 in antagonizing the respose to nerve stimulation was decreased while (c) a small but a significant inhibitory effect of GR 82,334 became evident. All together, these findings indicate that breakdown by peptidases limits the expression of activity of endogenous tachykinins acting preferentially at NK_1 receptors (putatively substance P).

Owing to the desensitization of the response to the NK_1 receptor selective agonist, and the spontaneous decay of the response to repeated cycles of electrical stimulation (circles in Figure 5a) the possibility cannot be ruled out, on the basis of present findings, that decay of the response to endogenous

tachykinins involves desensitization of NK_1 receptors by endogenous ligands. Even assuming that such a mechanism may entirely account for the spontaneous decay of the tachykinin response to nerve stimulation it is evident that, in preparations not receiving peptidase inhibitors, the contribution of NK_1 receptors could not exceed 20–25% of the overall response. This estimate originates from the intensity of spontaneous decay during repeated cycles of stimulation in control preparations vs. intensity of inhibition produced by MEN 10,376 in matched preparations (Figure 5a, left panel).

The results of this part of the study could be compared with those obtained in other preparations in which tachykinins released from nerves by electrical stimulation have been shown to produce smooth muscle contraction. In the guinea-pig isolated bronchi (Maggi *et al.*, 1991d) and circular muscle of the human ileum (Maggi *et al.*, 1992b) both NK_1 and NK_2 receptors mediate contraction to tachykinins and endogenous tachykinins mediate atropine-resistant excitatory responses to nerve stimulation. In these preparations, the use of receptor-selective tachykinin antagonists has disclosed a major role of NK_2 receptors in mediating the physiological response i.e., the same finding obtained here for the guinea-pig renal pelvis. Furthermore, in the guinea-pig bronchus the relative contribution of NK_1 receptors becomes more evident after addition of peptidase inhibitors (Maggi *et al.*, 1991d), which is the same pattern found in the renal pelvis. From these studies a major contribution of neurokinin A, or anyway of endogenous ligands with high affinity for NK_2 receptors in physiological responses involving smooth muscle contraction by endogenous peptides of this family can be postulated.

In conclusion, the present findings indicate that both NK_1 and NK_2 receptors mediate the contractile response of the guinea-pig renal pelvis to tachykinins. The NK_2 receptors activated by exogenous and endogenous tachykinins is of the same subtype which has been previously identified in the rabbit pulmonary artery. Activity of endogenous tachykinins acting at NK_1 receptors is limited by degradation by peptidases. The present findings support a more important role for neurokinin A than for substance P as physiological mediators of contraction in the renal pelvis.

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