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European Journal of Pharmacology 352 (1998) 103–109

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An investigation of tachykinin NK_2 receptor subtypes in the rat

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Received 16 April 1998; accepted 24 April 1998

Abstract

The heterogeneity of tachykinin NK_2 receptor subtypes was examined in five tissues from the rat, using binding and functional techniques. Initial experiments with the selective radioligand [^{125}I][Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) showed no specific binding to rat spinal cord membranes or sections. However, this radioligand exhibited high specific binding (80–95% of total) in membranes from the rat fundus, colon, bladder and vas deferens. Dissociation constants (K_D) were lower in bladder and colon (0.4 nM) than in fundus (1.9 nM) or vas deferens (1.4 nM). Neurokinin A, neuropeptide γ, [Lys^5,MeLeu^9,Nle^{10}]NK(4–10), SR 48968 [(*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamine], GR 94800 [PhCO-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2] and MEN 10627 [cyclo(Met-Asp-Trp-Phe-Dap-Leu)cyclo(2β–5β)] displayed high affinity (pIC_{50} 8.4–9.5) as competitors, with no significant difference in potency between these four tissues. [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) contracted the isolated fundus (EC_{50} 117 nM) and bladder (EC_{50} 10 nM) and these responses were similarly inhibited by the tachykinin NK_2 receptor antagonists, SR 48968 and MEN 10627 (pA_2 values 7.6–8.2). In spite of differences in K_D seen in some tissues, these results do not provide compelling evidence for tachykinin NK_2 receptor heterogeneity in smooth muscle-containing tissues in the rat. The absence of detectable binding in rat spinal cord may be due to very low expression of tachykinin NK_2 receptors, or to existence of a different receptor subtype.

Keywords: Tachykinin; NK_2 receptor subtype; Fundus, rat; Vas deferens, rat; Colon, rat; Bladder, rat; Spinal cord, rat; Receptor binding

1. Introduction

To date, three main tachykinin receptor types have been described and classification is based on their relative affinities for the natural tachykinins. Substance P (SP) has the greatest potency at tachykinin NK_1 receptors; neurokinin A at tachykinin NK_2 receptors; and neurokinin B at tachykinin NK_3 receptors (Maggi et al., 1993; Mussap et al., 1993). A question of potential therapeutic significance is: are there subtypes of tachykinin NK_1, NK_2 and NK_3 receptors within one species?

Many pharmacological studies have demonstrated species-related variants of the tachykinin NK_2 receptor. The tachykinin NK_2 receptor antagonists L659,877 and R369 had a higher affinity for rat colon or vas deferens and hamster trachea, than for guinea-pig or rat trachea, rabbit trachea, aorta and artery (Williams et al., 1988; Ireland et al., 1991). Alternatively, the tachykinin NK_2 receptor antagonists MEN 10207 and MEN 10376 (Rovero et al., 1990a,b) displayed high affinity for tachykinin NK_2 receptors in rabbit pulmonary artery and a low affinity for hamster trachea. Binding studies revealed that L659,877 was more potent in hamster urinary bladder than bovine stomach, while MEN 10207 was more potent (100-fold) in cell lines transfected with bovine tachykinin NK_2 receptors than in hamster urinary bladder (van Giersbergen et al., 1991). [^{125}I]iodohistidyl neurokinin A failed to label tachykinin NK_2 receptors in guinea-pig lung and rabbit pulmonary artery, but bound with high affinity in hamster, rat and dog urinary bladder (Geraghty et al., 1992; van Giersbergen et al., 1992; Xiao et al., 1992; Mussap et al., 1996). The peptide analogue MDL 28564, selective for tachykinin NK_2 receptors (Harbeson et al., 1990), behaved as a full agonist in some species (guinea-pig trachea and rabbit pulmonary artery) but as a competitive antagonist elsewhere (rat vas deferens, hamster trachea and bladder) (Buck et al., 1990; Patacchini et al., 1991).

These pharmacological findings originally suggested two main receptor types, one expressed in rat, hamster and

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0014-2999/98/$19.00

dog and the other in guinea-pig, rabbit, bovine and human (reviewed in Maggi, 1994). However, molecular data show that the size and sequence of tachykinin NK_2 receptors differ widely, in different species (reviewed in Section 4). The aim of the present study was to undertake a systematic comparison in one species (the rat) using the spinal cord and several different tissues (fundus, colon, bladder and vas deferens) reported to express tachykinin NK_2 receptors (Burcher et al., 1986; Burcher and Buck, 1986; Tsuchida et al., 1990; Takeda and Krause, 1991). Tachykinin NK_2 receptor heterogeneity observed in tissues from the same species would give an indication of the existence of true receptor subtypes. We used the radioligand [^{125}I][Lys^5,Tyr(I_2)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10), which displays both high selectivity and affinity for tachykinin NK_2 receptors in the rat, in contrast to the guinea-pig (Burcher et al., 1993; Badgery-Parker et al., 1993; Zeng et al., 1994).

2. Materials and methods

2.1. Radioligand binding studies

The radioligand [^{125}I] [Lys^5,Tyr(I_2)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) was prepared (specific activity approximately 3800 Ci/mmol) as previously described, by iodination of [Lys^5,Tyr^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) and purification using reversed-phase high-performance liquid chromatography (HPLC) (Burcher et al., 1993).

Male outbred Wistar rats (200–500 g) were killed by stunning, followed by cervical dislocation. The fundus, colon, bladder and vas deferens were all removed and rinsed in 0.9% saline. The mucosa and the epithelium were removed from the colon and bladder, respectively. Spinal cords were removed from a different group of male Wistar rats. Membrane homogenates were prepared as previously described (Burcher et al., 1993) and were finally resuspended in the incubation buffer consisting of 50 mM Tris (pH 7.4, 25°C), $MnCl_2$ (3 mM), bovine serum albumin (0.02%) and peptidase inhibitors phosphoramidon (1 μM), chymostatin (4 μg/ml) and leupeptin (4 μg/ml).

Aliquots of the homogenate (final concentration 2% wet weight) were incubated at 25°C with 50 pM [^{125}I] [Lys^5,Tyr(I_2)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) in incubation buffer (final volume of 500 μl). At equilibrium (60 min), reactions were terminated by rapid filtration and washing through Whatman GF/B glass fibre filters (presoaked in 0.5% bovine serum albumin). Filter-bound radioligand was washed with 3×4 ml 50 mM Tris–HCl (pH 7.4, 4°C) containing $MnCl_2$ (3 mM) and bovine serum albumin (0.02%), dried under vacuum and quantified using a Model 1470 Wallac Wizard autogamma counter at 79% efficiency. Non-specific binding was defined using 1 μM unlabelled [Lys^5,Tyr(I_2)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10).

HOT saturation studies were undertaken using increasing radioligand concentrations, in membranes from bladder, fundus, colon and vas deferens. Competition binding studies were performed, with varying amounts of competitor incubated with the radioligand and homogenate. Data were analysed using the computer programs, EBDA and LIGAND. Statistical analysis (one-way analysis of variance, ANOVA) was carried out using SPSS.

2.2. Autoradiographic studies

Rats were killed as above and the spinal cord was removed. Frozen sections (20 μm) were incubated with radioligand (100 pM) in incubation buffer containing 50 mM Tris–HCl (pH 7.4, 25°C), $MnCl_2$ (3 mM), bovine serum albumin (0.02%) and the peptidase inhibitors phosphoramidon (1 μM) and chymostatin (1 μM) for 90 min, as previously described (Strigas and Burcher, 1996). Non-specific binding was defined by coincubation of adjacent sections with 1 μM [Lys^5,Tyr(I_2)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10). Sections were fixed by exposure to paraformaldehyde vapour (30 min at 60°C) and then dipped in liquid LM-1 photographic emulsion (Amersham), exposed in the dark (4°C, 14 days), and then developed, fixed and stained.

2.3. Functional studies

Male Wistar rats were killed as above. The bladder and fundus were removed and rinsed in Krebs–Henseleit solution. The epithelium was removed from the bladder. Strips (2 mm $\times$ 5 mm) were mounted in 2 ml silanised organ baths, bathed in Krebs–Henseleit solution, bubbled with 95% O_2 and 5% CO_2 and containing phosphoramidon (1 μM), atropine (1 μM), mepyramine (5 μM), indomethacin (1 μM) and the NK_1 receptor antagonist, RP 67580 (1 μM) at 37°C. The preparations were equilibrated under a resting tension of 1 g for 60 min, and then an isometric supramaximal response to carbachol (100 μM)

Table 1
Tachykinin NK_2 receptor affinity and number, derived from HOT saturation curves with [^{125}I][Lys^5,Tyr(I_2),MeLeu^9,Nle^{10}] neurokinin A-(4–10)

Tissue	K_d (nM)	B_{max} (fmol/mg wet weight tissue)
Bladder	0.43 ± 0.04	3.9 ± 0.5
Fundus	1.9 ± 0.7	3.6 ± 0.9
Colon	0.39 ± 0.02	3.4 ± 0.9
Vas deferens	1.4 ± 0.4	1.8 ± 0.4

Values were obtained using EBDA and represent mean ± S.E.M. of data from HOT saturation studies with [^{125}I][Lys^5,Tyr(I_2),MeLeu^9,Nle^{10}]neurokinin A-(4–10) carried out in homogenates from various rat tissues (n = 4–5 animals in each group). The K_d values were significantly different (ANOVA, $F = 4.03$, $P = 0.029$). No significant differences in B_{max} were seen (ANOVA, $F = 1.64$).

Table 2
Potency of tachykinins and analogues as competitors against [^{125}I][Lys^5,Tyr(I$_2$),MeLeu^9,Nle^{10}]neurokinin A-(4–10) binding in membranes from four rat tissues

Ligand	Bladder	Fundus	Colon	Vas deferens
[Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10)	9.09 ± 0.05	9.20 ± 0.23	8.82 ± 0.17	9.40 ± 0.09
Neuropeptide γ	9.52 ± 0.06	9.40 ± 0.04	9.28 ± 0.03	9.45 ± 0.06
Neurokinin A	9.13 ± 0.01	9.05 ± 0.06	9.08 ± 0.20	8.90 ± 0.05
GR 94800	8.70 ± 0.01	8.40 ± 0.09	8.46 ± 0.09	8.90 ± 0.07
MEN 10627	8.71 ± 0.11	8.60 ± 0.12	8.50 ± 0.02	9.43 ± 0.23
SR 48968	8.89 ± 0.15	8.74 ± 0.08	8.71 ± 0.03	9.12 ± 0.13
MDL 28564	6.42 ± 0.10	6.16 ± 0.23	6.27 ± 0.02	6.62 ± 0.05

Data were analysed using EBDA to obtain IC_{50} values and are presented here as pIC_{50} values (means ± S.E.M. of values from 4–16 animals).

was obtained. All subsequent tissue responses were measured as g tension and expressed as a percentage of the maximal response of that preparation to carbachol. Tissues were incubated with or without a tachykinin NK_2 receptor antagonist (MEN 10627 or SR 48968) for 120 min (as determined by preliminary experiments, data not shown), before a concentration-response curve to the agonist [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) was established. Paired preparations from the same animal were used for control and antagonist treatment. Only one concentration-response curve was obtained from each preparation to prevent possible tachyphylaxis. Data were expressed as mean ± S.E.M. and pA_2 values were obtained (Arunlakshana and Schild, 1959; Tallarida et al., 1979).

2.4. Materials

Neurokinin A and neuropeptide γ were purchased from Auspep (Melbourne, Australia). The antagonists, SR 48968 [(*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamine], GR 94800 [PhCO-Ala-Ala-DTrp-Phe-DPro-Pro-Nle-NH_2], RP 67580 [3αR,7αR)-7,7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)-ethyl]perhydroisoindol-4-one] MDL 28564 [Asp-

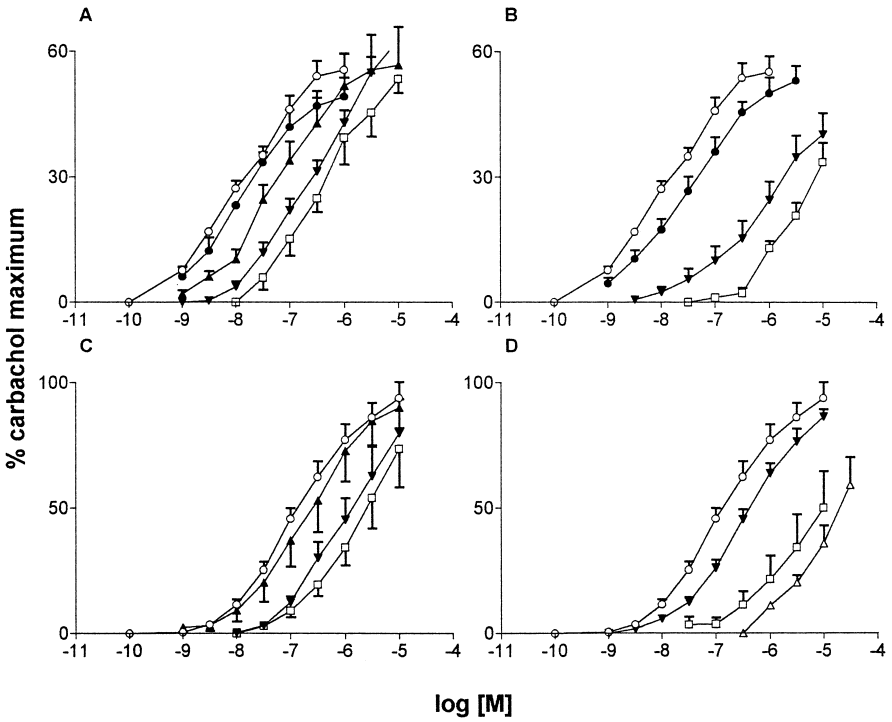

Fig. 1. Effect of tachykinin NK_2 receptor antagonists, SR 48968 (A,C) and MEN 10627 (B,D) on contractile responses to [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) in rat isolated bladder (A,B) and fundus (C,D). Curves were obtained in the absence (○) and presence of antagonist. (A) SR 48968 at 10 nM (●), 30 nM (▲), 100 nM (▼) and 300 nM (□). (B) MEN 10627 at 10 nM (●), 100 nM (▼), and 300 nM (□). (C) SR 48968 at 30 nM (▲), 100 nM (▼) and 300 nM (□). (D) MEN 10627 at 100 nM (▼), 300 nM (□) and 1 μM (Δ). Data are mean ± S.E.M. from 4–6 animals in the presence of phosphoramidon (1 μM), atropine (1 μM), mepyramine (5 μM), indomethacin (1 μM) and RP 67580 (1 μM). The pA_2 values determined from these data are listed in Table 3.

Ser-Phe-Val-Gly-Leu-CH_2NH-Leu-NH_2] and MEN 10627 [cyclo(Met-Asp-Trp-Phe-Dap-Leu)cyclo(2β–5β)] were gifts from Dr. X. Emonds-Alt (Sanofi Recherche, Montpellier, France), Dr. R.M. Hagan (Glaxo, Ware, UK), Dr. C. Garret (Rhone Poulenc Rorer, Vitry-sur-Seine Alfortville, France), Marion Merrell Dow (Cincinnati, USA), and Dr. C.A. Maggi (A. Menarini Pharmaceuticals, Florence, Italy), respectively. [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10), [Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10), [Lys^5,Tyr^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) and GR 94800 were generous gifts from Dr. S. Lavielle (CNRS URA, Paris, France). [^{125}I]NaI was purchased from Du Pont (USA). Phosphoramidon, chymostatin, leupeptin, atropine sulphate, mepyramine, indomethacin, manganous chloride and bovine serum albumin were purchased from Sigma (St. Louis, USA). All other reagents were of analytical grade. Stock solutions of tachykinins and analogues (0.5–5 mM) were made up in 0.01 M acetic acid with 1% β-mercaptoethanol and stored at −20°C until use.

3. Results

3.1. Binding studies

Specific binding of [^{125}I][Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}] neurokinin A-(4–10) (50 pM) reached maximum (80 to 95% of total binding) after 60 min incubation with the membrane homogenates from bladder, fundus, colon and vas deferens. Specific binding in homogenates of spinal cord was too low (3.9 ± 2.4%) to be characterized.

Homogenates from all four smooth muscle tissues showed saturable high affinity specific binding of [^{125}I][Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) (concentration range 0.005–5 nM). Binding affinity (K_d) in bladder and colon was similar and of higher affinity than in fundus and vas deferens (Table 1). Binding capacity (B_{max}) was similar in bladder, fundus and colon, and lowest in vas deferens.

The tachykinin NK_2 receptor agonists, neurokinin A, neuropeptide γ, the synthetic [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) and the three selective tachykinin NK_2 receptor antagonists, SR 48968 (Advenier et al., 1992), GR 94800 (McElroy et al., 1992) and MEN 10627 (Maggi et al., 1994) were all potent competitors for [^{125}I][Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) binding to membranes in rat bladder, fundus, colon and vas deferens, with a pIC_{50} range of 8.4 to 9.5 (Table 2). For each competitor, no significant difference in potency was observed in these four tissues (ANOVA). In contrast, the peptide MDL 28564 displayed 100 to 1000 times lower affinity (pIC_{50} range 6.2 to 6.6) in all four tissues, but was still able to achieve a total inhibition of binding at a concentration of 10 μM (Fig. 1, Table 2). Slope factors not significantly different from unity suggested interaction with one site.

Table 3
Potency of tachykinin NK_2 receptor antagonists against [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) on isolated preparations of rat bladder and fundus

Antagonist	Bladder		Fundus	
	pA_2	Schild slope	pA_2	Schild slope
SR 48968	7.92 ± 0.12	1.30 ± 0.17	7.96 ± 0.23	1.07 ± 0.22
MEN 10627	8.24 ± 0.19	1.45 ± 0.22	7.56 ± 0.38	1.48 ± 0.48

The values represent mean ± S.E.M. of paired determinations from 4–6 animals. The pA_2 values for antagonists were determined by extrapolation of respective Schild plots to the abscissa (Arunlakshana and Schild, 1959). Slopes and correlation coefficient of Schild plots were determined by linear regression analysis. The slopes of the Schild plots were not significantly different from unity (Student's *t*-test).

3.2. Autoradiographic studies

No specific binding of [^{125}I][Lys^5,Tyr(I$_2$)^7,MeLeu^9, Nle^{10}]neurokinin A-(4–10) was seen in film exposed to radiolabelled sections of rat spinal cord, or to emulsion-dipped sections (data not shown). Control sections of guinea-pig lung showed localised specific binding to bronchial smooth muscle (as described in Strigas and Burcher, 1996).

3.3. Functional studies

Because of possible differences in receptor characteristics revealed in saturation binding studies (above), some functional studies were carried out in isolated bladder (K_d 0.4 nM) and fundus (K_d 1.9 nM) to further investigate possible subtypes. These studies were carried out in the presence of the NK_1 receptor antagonist, RP 67580 in order to eliminate any possible effects at NK_1 receptors.

The selective tachykinin NK_2 receptor agonist, [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) was more potent in contracting isolated strips from the bladder than the fundus (EC_{50} 10 nM and 117 nM, respectively). The response to each concentration was initiated rapidly and reached a plateau within 2 min. The tachykinin NK_2 receptor antagonists, SR 48968 and MEN 10627 caused a concentration-related inhibition of contractile responses to [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) in both rat bladder (antagonist concentration range 10 to 300 nM) and fundus (30 nM to 1 μM), resulting in a rightward shift of the agonist concentration-response curve (Fig. 1). The pA_2 values derived from Schild plots were similar for both tissues (Table 3).

4. Discussion

Tachykinin NK_2 receptors differing in their length and primary sequence have been isolated from a variety of species and tissues such as rat stomach (390 aa residues, Sasai and Nakanishi, 1989), human trachea and jejunum (398 aa, Gerard et al., 1990; Kris et al., 1991), mouse

genoma and bovine stomach (384 aa, Masu et al., 1987; Sundelin et al., 1992), hamster bladder (384 aa, Aharony et al., 1994a), guinea-pig lung (402 aa, Aharony et al., 1994b) and rabbit pulmonary artery lung (384 aa, Aharony et al., 1994b). The human tracheal and jejunal tachykinin NK_2 receptor differ by only one amino acid residue in the intracellular C-terminal tail. Presently, only one copy of the tachykinin NK_2 receptor gene has been detected in the genoma of various species. However, evidence for receptor subtypes could come from alternate splicing from the same gene, or from differences in glycosylation of the receptor protein.

Data from the present study do not provide strong evidence for heterogeneity (intraspecies variation) of rat tachykinin NK_2 receptors. Apart from a lack of specific binding in the rat spinal cord (refer below), binding and functional studies show no obvious pharmacological differences in tachykinin NK_2 receptors in fundus, colon, bladder and vas deferens. The only indication of possible receptor differences was the 3–5-fold higher affinity (K_d) of [^{125}I][Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) in membranes from the bladder and colon, as compared with fundus and vas deferens. This difference in affinity was reflected in a higher potency of the agonist in isolated bladder as compared with fundus preparations. However, in competition binding assays, no agent (including MDL 28564, previously used to reveal interspecies subtypes), was able to discriminate between these four smooth muscle-containing tissues. All tachykinin NK_2 receptor ligands used (except MDL 28564) displayed a high affinity for this receptor.

In support of these binding data, functional studies with antagonists showed no indication of two subtypes of tachykinin NK_2 receptors between tissues (bladder or fundus) in the same tissue. These studies found a concentration-dependent antagonism of [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) by both the non-peptide tachykinin NK_2 receptor antagonist, SR 48968 and the peptide antagonist, MEN 10627. These two antagonists were used as they have previously been shown to interact with different regions of the tachykinin NK_2 receptor (Patacchini et al., 1994). The potency of SR 48968 in rat bladder and fundus in the present study (pA_2 7.9 and 8.0) was similar to that reported in rat vas deferens (pA_2 8.3, Giuliani et al., 1993) but different from the higher pA_2 values of 9.4 and 9.6 reported by Advenier et al. (1992) in rat vas deferens and rat colon, respectively. The pA_2 values for MEN 10627 of 8.2 and 7.6 in rat bladder and fundus, respectively, are similar to values obtained here for SR 48968. To our knowledge, no pA_2 values for MEN 10627 have been reported in rat colon and vas deferens.

The failure of [^{125}I][Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) to detect tachykinin NK_2 binding sites in the spinal cord may be due to low receptor numbers. Using nuclease protection analysis, Takeda and Krause (1991) found that tachykinin NK_2 receptor expression in rat spinal cord was 500-fold lower than in bladder, with vas deferens expression about one third that of the bladder. Tsuchida et al. (1990) also found high levels of tachykinin NK_2 receptor mRNA in rat peripheral tissues, with bladder > colon > stomach; spinal cord levels were undetectable, similar to results from the present study. In addition, Suzuki et al. (1993) showed that very few polymerase chain reaction-amplified cDNA fragments derived from the neonatal rat spinal cord had a sequence identical to that of tachykinin NK_2 receptor cDNA. However, other studies provide evidence for functional tachykinin NK_2 receptors in rat spinal cord. Xu et al. (1991) found that MEN 10207 possessed a high (pmol) affinity for rat spinal cord, as compared to a low (mmol) affinity in rat smooth muscle preparations (urinary bladder and ileum), suggesting that there may be two different populations of tachykinin NK_2 receptors in the rat, located on smooth muscle and in the nervous system. The highly selective radioligand used in the present study failed to bind in the rat spinal cord, perhaps due to either a low expression of tachykinin NK_2 receptors, or to a discrimination between smooth muscle NK_2 receptors and those in the nervous system. Some earlier autoradiographic studies using less selective radioligands such as [^{125}I]Bolton–Hunter neurokinin A (Buck et al., 1986) or [^{125}I]neurokinin A (Yashpal et al., 1990) have provided evidence for low levels of specific binding sites in the dorsal horn of the rat spinal cord. However, these radioligands are not wholly selective and can detect NK_1 receptors, as demonstrated in the airways (Burcher et al., 1989; Geraghty et al., 1992), thus prompting development of the present highly selective radioligand [^{125}I][Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) (Burcher et al., 1993).

There is only limited evidence for intraspecies heterogeneity of tachykinin NK_2 receptors. In rat urinary bladder, autoradiographic abstracts (Morrison et al., 1990; Nimmo et al., 1992) described two tachykinin NK_2 receptor binding sites, with the antagonist MEN 10207 having pIC_{50} values of 9.0 and 6.5 in subepithelial tissue and smooth muscle, respectively. This low affinity of MEN 10207 in rat bladder muscle was confirmed by Flowers et al. (1991) who reported a pA_2 value of 5.8 for this antagonist against [βAla^8]neurokinin A-(4–10). It was suggested (Morrison et al., 1990) that the receptors present at the epithelial level are expressed on primary afferent nerves, as they disappear in the capsaicin-pretreated rats. However, Banasiak and Burcher (1994) found no change in the distribution of tachykinin NK_2 autoradiographic binding sites in rat bladder after capsaicin. Note that our present studies, indicating high affinity tachykinin NK_2 receptors in rat bladder smooth muscle, were carried out in epithelium-denuded tissue.

Thus, to date, there is minimal evidence for heterogeneity of tachykinin NK_2 receptors in the rat. There is an indication of possible differences between tachykinin NK_2 receptors on smooth muscle and those in the spinal cord.

However, at this stage, we do not know whether true differences exist, as evidenced by the absence of binding of [^{125}I][Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}]neurokinin A-(4–10) in spinal cord, or whether our data simply reflect very low numbers of tachykinin NK$_2$ receptors. One must also consider the possible involvement of various uncharacterised peptidases influencing the results obtained. Further studies using techniques of molecular biology (alternative genetic splicing) together with protein biochemistry (affinity chromatography purification) may provide evidence for the coexistence of true tachykinin NK$_2$ receptor subtypes in the same species (Hall et al., 1993).

Acknowledgements

This work was supported by a grant from the National Health and Medical Research Council. We wish to thank Dr. X. Emonds-Alt, Dr. C. Garret, Dr. R.M. Hagan, Dr. S. Lavielle and Dr. C.A. Maggi for their generous gifts of peptides.

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