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Temperature and agonist dependency of tachykinin NK_1 receptor antagonist potencies in rat isolated superior cervical ganglion

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Abstract

Using rat isolated superior cervical ganglion we have further characterised tachykinin NK_1 receptors and investigated the possible existence of tachykinin NK_1 receptor subtypes. At 37°C, tachykinin NK_1 receptor antagonists GR82334 ([D-Pro^9[spiro-γ-lactam]Leu^{10},Trp^{11}]physalaemin-(1–11)), CP-99,994 ((+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine) and (±)-RP67580 (7,7-diphenyl-2[1-imino-2(2-methoxy-phenyl)-ethyl]perhydroisoindol-4-one (3*aR*,7*aR*)) antagonised more potently depolarisation responses evoked by GR73632 (δAva[L-Pro^9,*N*-MeLeu^{10}]SP-(7–11)), septide ([pGlu^6,Pro^9]SP-(6–11)) and neurokinin A than those evoked by substance P, substance P *O*-methyl ester and [Sar^9,Met(O$_2$)^{11}]substance P. GR73632 and substance P *O*-methyl ester evoked depolarisation responses of similar magnitude, unaffected by addition of tetrodotoxin, but which cross-desensitised. At 22°C, the ability of GR82334 and (±)-RP67580 to inhibit substance P *O*-methyl ester-evoked but not GR73632-evoked responses was enhanced greatly. These results suggest a single population of tachykinin NK_1 receptors in this preparation. The agonist and temperature dependency of tachykinin NK_1 receptor antagonist potency in rat isolated superior cervical ganglion may reflect different conformational changes in the tachykinin NK_1 receptor induced by partial or full sequence substance P analogues.

Keywords: Substance P; Septide; Tachykinin NK_1 receptor antagonist; Superior cervical ganglion, rat, isolated; (Subtype); (Conformation)

1. Introduction

The mammalian tachykinins act via three pharmacologically distinct receptor types designated NK_1, NK_2 and NK_3, which preferentially bind substance P, neurokinin A and neurokinin B, respectively. Development of potent, non-peptide tachykinin NK_1 receptor antagonists such as CP-96,345 (Snider et al., 1991) and RP67580 (Garret et al., 1991) has indicated that species differences exist for tachykinin NK_1 receptors. CP-96,345 has a higher affinity for tachykinin NK_1 receptors from human and guinea pig than from rat (Beresford et al., 1991), whereas RP67580 has greater affinity for rat than for guinea-pig tachykinin NK_1 receptors (Garret et al., 1991). Sequence comparison of human and rat tachykinin NK_1 receptors has shown that there are 22 divergent residues (Fong et al., 1992c). Substitution of divergent residues in the human tachykinin NK_1 receptor for their rat homologues has demonstrated that two conservative substitutions within the transmembrane domain at positions 116 and 290 are necessary and sufficient to reverse the species selectivity of the two aforementioned antagonists (Fong et al., 1992c).

In addition to interspecies differences in tachykinin NK_1 receptors, there have been several reports suggesting the possible existence of intraspecies subtypes. Seabrook et al. (1993) and Carruette et al. (1992) reported that CP-96,345 and RP67580 have different potencies in different tissues from the guinea pig and suggested that this could indicate the existence of tachykinin NK_1 receptor subtypes. Several groups of workers, using a range of tissues from several species, have demonstrated that both peptide and non-peptide tachykinin NK_1 receptor antagonists antagonise more potently the C-terminal hexapeptide substance P ana-

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logue septide ([pGlu^6,Pro^9]substance P-(6–11)) compared to responses evoked by full sequence analogues of substance P. This has been observed in human cultured astrocytoma cells (U373MG) (Palma et al., 1994; Oury-Donat et al., 1994); in isolated intestinal (Carruette et al., 1992; Chassaing et al., 1992; Petitet et al., 1992; Maggi et al., 1993, 1994) and tracheal (Carruette et al., 1992; Longmore et al., 1994) preparations of the guinea pig, rat isolated urinary bladder (Montier et al., 1994; Meini et al, 1994) and portal vein (Carruette et al., 1992), and rabbit pulmonary artery (Carruette et al., 1992). This phenomenon has also been observed in vivo for tachykinin NK_1 agonist-induced increases in insufflation pressure in guinea-pig lung (Floch et al., 1993; Boni et al., 1994), tachykinin NK_1 agonist-induced scratching in mice (Sakurada et al., 1991) and scratching and salivation in rats (Jung et al., 1994). It has been suggested that these results indicate that septide acts at either a novel tachykinin NK_1 receptor subtype or at a separate site on the tachykinin NK_1 receptor.

The aim of the present study was firstly to investigate in a neuronal preparation whether tachykinin NK_1 receptor antagonists antagonise preferentially responses evoked by C-terminal fragments of substance P. Following demonstration of such preferential antagonism, our second aim was to investigate whether this reflected the existence of multiple tachykinin NK_1 receptors. Substance P has been shown to depolarise the rat isolated superior cervical ganglion (Hawcock et al., 1982), and this was subsequently demonstrated to be mediated mainly through an interaction with tachykinin NK_1 receptors (Seabrook et al., 1992). We investigated the abilities of peptide and non-peptide tachykinin NK_1 receptor antagonists to antagonise depolarisation responses evoked by substance P, a range of full sequence analogues and C-terminal hexapeptide analogues of substance P, and neurokinin A. Our results indicated that tachykinin NK_1 receptor antagonists were substantially more potent to antagonise responses evoked by the dodecapeptide neurokinin A and the synthetic hexapeptides septide and GR73632 compared to those evoked by substance P and the undecapeptide analogues substance P *O*-methyl ester and [Sar^9, Met(O_2)^{11}]substance P. To investigate further whether this may reflect the existence of multiple tachykinin NK_1 receptors in the rat superior cervical ganglion, we attempted to dissociate the responses evoked by the two groups of agonists by using tetrodotoxin and performing cross-desensitisation experiments. In addition, as tachykinin agonists have been shown to evoke two depolarisation responses in rat isolated coeliac-superior mesenteric ganglia which could be selectively manipulated by altering the temperature of the preparation (Konishi et al., 1992), we compared agonist and antagonist responses at 37 and 22°C.

2. Materials and methods

2.1. Methods

Male Lister-Hooded rats (300–350 g; Glaxo, Rodent Breeding Unit) were stunned by a blow to the head, decapitated and the superior cervical ganglia dissected from the surrounding tissue. Each ganglion was desheathed under a dissecting microscope and arranged in a two-compartment chamber such that the ganglion body was isolated from the post-ganglionic trunk by a notched Perspex barrier coated in silicone grease. Each ganglion rested on filter paper and was perfused with Krebs-Henseleit solution, pre-equilibrated with 5% CO_2 in O_2 and maintained at either 37°C or 22°C. The composition of the perfusion solution was (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 1.25, $MgSO_4$ 1.6, $NaHCO_3$ 24.9 and glucose 5.6. Each compartment was perfused separately at a rate of 0.5 ml min^{-1} and potential differences across the two compartments recorded with Ag/AgCl electrodes, one positioned in contact with each compartment. Agonist and antagonist drugs were added to the solution perfusing the ganglion body compartment. To prevent peptide breakdown, the peptidase inhibitors bacitracin (100 μM), captopril (5 μM) and phosphoramidon (1 μM) were added to the Krebs-Henseleit solution perfusing the ganglion body compartment. Up to six ganglion preparations were used in each experiment. To prevent tachyphylaxis, agonists were applied serially using an inter-dose interval of 30 min, each concentration remaining in contact with the tissue for 5 min.

Agonist studies

Agonist concentration-response curves were constructed by applying varying concentrations of either GR73632 (δAva[L-Pro^9*N*-MeLeu^{10}]substance P-(7–11)) or test agonist and expressing the responses as a percentage of the maximum response to GR73632 attained in that tissue. Concentration-response curves to GR73632 and one test agonist only were constructed in each tissue. In cross-desensitisation studies, concentrations of GR73632, substance P *O*-methyl ester and the non-tachykinin control agonist bethanechol, which induced repeatable sub-maximal control responses, were reapplied after desensitising the preparations to either GR73632 or substance P *O*-methyl ester and responses compared to those obtained in a non-desensitised time-control tissue. Desensitisation was achieved by applying a continuous perfusion of either GR73632 (1 μM) or substance P *O*-methyl ester (1 μM) to the ganglion body. When the baseline was regained, after approximately 45 min, agonists were re-applied. In experiments using tetrodotoxin, two agonist concentration-response curves were constructed, the first serving as a control for the second. Tetrodotoxin (0.1 μM) was

perfused over the tissues for 30 min before constructing the second concentration-response curve. Data were expressed as a percentage of the control maximum response.

Antagonist studies

In the antagonist studies, two agonist concentration-response curves were constructed using each preparation, the first curve serving as a control for the second. Antagonists were perfused over the tissues for 30 min before construction of the second concentration-response curve. Data were expressed as a percentage of the control maximum response achieved in the first curve. A control tissue receiving the test agonist alone was included in each experiment.

2.2. Data analysis

Agonist potency was expressed as pD_2, the negative logarithm of the effective concentration inducing 50% of the maximum response (EC_{50}). EC_{50} values were measured graphically. Maximum responses (relative to GR73632 or an initial control curve) were also measured. To measure antagonist potency, lateral displacements of the concentration-response curves were estimated at the control EC_{50} level, to give concentration ratios between antagonist-treated and control curves. Where appropriate, antagonist potency was estimated by Schild analysis and expressed as mean (±S.E.M.) pK_B values obtained from a Schild slope constrained to unity. Otherwise, concentration ratios estimated from individual ganglion preparations were used to calculate the negative logarithm of the apparent dissociation constant (pK_B) for the antagonists using the equation, $pK_B = \log(\text{dose ratio} - 1) - \log(\text{antagonist concentration})$. Significance was determined using the unpaired Student's *t*-test.

2.3. Materials

Substance P, [Sar^9,Met(O$_2$)^{11}]substance P, substance P *O*-methyl ester, neurokinin A, and septide ([*p*Glu^6,Pro^9]substance P-(6–11)) were purchased from Peninsula. Bacitracin, captopril, phosphoramidon and tetrodotoxin were purchased from Sigma. GR82334 ([D-pro^9[spiro-γ-lactam]Leu^{10},Trp^{11}]physalaemin-(1–11)) was purchased from Neosystem Laboratories. GR73632 (δAva[L-Pro^9*N*-MeLeu^{10}]substance P-(7–11)) was purchased from Peptide and Protein Research, University of Exeter. CP-99,994 (McLean et al., 1993) ((+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine), CP-100,263 ((−)-(2*R*,3*R*)-3-(2-methoxybenzylamino)-2-phenylpiperidine), racaemic (±)-CP-99,994 and (±)-RP67580 (7,7-diphenyl-2[1-imino-2(2-methoxy-phenyl)-ethyl]perhydroisoindol-4-one (3*aR*,7*aR*)) were synthesized in the Department of Medicinal Chemistry, Glaxo Research and Development.

Table 1
Potency estimates (pD_2) and relative maximum responses (E_{max}) of tachykinin NK$_1$ receptor agonists in rat isolated superior cervical ganglion

Agonist	pD_2	E_{max}
GR73632	8.4±0.1	100
Substance P *O*-methyl ester	8.3±0.1	93±6
Septide	8.4±0.1	87±4
[Sar^{9}Met(O$_2$)^{11}] Substance P	8.2±0.1	87±7

Concentration-response curves to GR73632 and one other agonist were carried out in each preparation. E_{max} data are expressed as % maximum response to GR73632. Each value is mean±S.E.M. (n = 5–18).

3. Results

3.1. Agonist responses

Relative potencies and maximum responses to the undecapeptides substance P *O*-methyl ester and [Sar^9,Met(O$_2$)^{11}]substance P were compared with those evoked by the C-terminal fragments septide and GR73632. All agonists induced concentration-dependent depolarisations of the rat isolated superior cervical ganglion with similar nanomolar potencies and responses of similar maximum size (Table 1, Fig. 1). The endogenous tachykinins substance P and neurokinin A also induced depolarisation responses. They appeared to act as full agonists, but they were not compared with

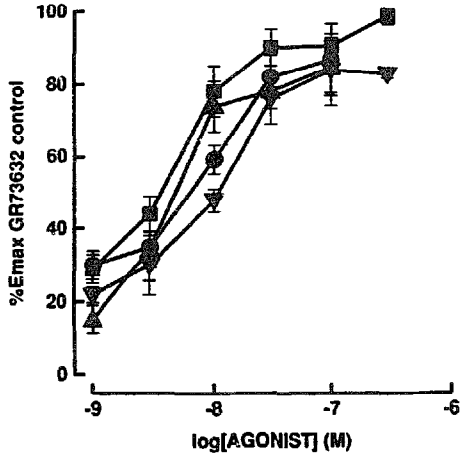

Fig. 1. Concentration-response curves to substance P *O*-methyl ester (●), septide (▲), [Sar^{9}Met(O$_2$)^{11}]substance P (▼) and GR73632 (■). Responses are expressed as a percentage of the maximum response evoked by GR73632 in each tissue. Data are mean±S.E.M. obtained in 3–9 separate experiments.

GR73632 in the same tissue, so relative maximum responses could not be estimated (Table 2).

3.2. Antagonist studies

Results of experiments with antagonists are summarised in Table 2. All the antagonists caused concentration-dependent parallel rightward displacements of the agonist concentration-response curves in the rat isolated superior cervical ganglion. When three or more concentrations of antagonist were used, Schild analysis of the data was carried out. All slopes were not significantly different to unity (Fig. 2). The peptide tachykinin NK_1 receptor antagonist GR82334 and non-peptide tachykinin NK_1 receptor antagonists (±)-CP-99,994 and (±)-RP67580 were significantly ($P < 0.0001$) more potent to antagonise depolarisation responses induced by the hexapeptide substance P analogues GR73632 and septide than responses induced by the full sequence analogues substance P, substance P *O*-methyl ester and [Sar^9,$Met(O_2)^{11}$]substance P. CP-99,994 behaved in a similar manner to (±)-CP-99,994 (Table 2), while its less active isomer, CP-100,263, was inactive against GR73632-induced depolarisations of rat isolated superior cervical ganglion ($pK_B < 5.0$, $n = 4$). Responses to neurokinin A were antagonised by GR82334 with similar potency to those observed against GR73632 and septide (Table 2). GR82334 also antagonised responses to substance P *O*-methyl ester more potently than those evoked by substance P ($P < 0.01$; Table 2).

3.3. Tetrodotoxin and desensitisation studies

To investigate whether the hexapeptide and full sequence agonists may be acting via different tachykinin NK_1 receptor subtypes in this preparation we investigated further responses evoked by the full sequence substance P analogue, substance P *O*-methyl ester and the reduced C-terminal sequence substance P analogue, GR73632. The maximum responses and potency estimates for both agonists were unaffected by addition of tetrodotoxin (0.1 μM) to the perfusion medium (Table 3). Desensitisation of the preparation by continuous perfusion of either GR73632 or substance P *O*-methyl ester (1 μM) virtually abolished responses evoked by a sub-maximal concentration (0.1 μM) of both tachykinin NK_1 agonists. In contrast, responses in non-desensitised control tissues were not significantly different to the control depolarisations. Sub-maximal responses to the non-tachykinin agonist bethanechol (3 μM) were largely unchanged in both the desensitised and control tissues (Table 4).

3.4. Investigation of temperature dependency

At 37°C there was no significant difference in the potency (pD_2) or the size of the maximum responses to either GR73632 or substance P *O*-methyl ester. Reducing the experimental temperature from 37°C to 22°C caused a significant ($P < 0.001$) reduction in the magnitude of depolarisation responses evoked by GR73632 and substance P *O*-methyl ester. The maximum responses evoked by GR73632 and substance P *O*-methyl ester were reduced from 650 ± 57 to 357 ± 54 μV, and 648 ± 86 to 346 ± 95 μV, respectively (Fig. 3). There was no significant difference in the potency (Table 5) or the maximum responses to either agonist at 22°C. Conducting experiments at the lower temperature markedly enhanced the ability of the antagonists

Table 2
Agonist potency estimates (pD_2) and antagonist affinity estimates (pK_B) for GR82334, CP-99,994, (±)-CP-99,994 and (±)-RP67580 to antagonise depolarisation responses induced by tachykinin analogues in rat isolated superior cervical ganglion

Agonist	pD_2	Antagonist pK_B ± S.E.M.			
		GR82334	(±)CP-99,994	CP-99,994	(±)-RP67580
GR73632	8.2 ± 0.1	7.0 ± 0.1 (0.8, 0.6–1.1)	6.4 ± 0.1 (0.9, 0.5–1.2)	6.2 ± 0.1	6.4 ± 0.1 (0.9, 0.6–1.3)
Substance P *O*-methyl ester	8.3 ± 0.1	5.9 ± 0.1 (1.0, 0.7–1.4)	< 5.0 [3]	< 5.0 [4]	< 5.0 [3]
[$Sar^9Met(O_2)^{11}$] Substance P	8.2 ± 0.1	5.3 ± 0.3 [3]	< 5.0 [3]	nd	< 5.0 [3]
Substance P	8.0 ± 0.1	5.5 ± 0.1 (0.9, 0.4–1.4)	< 5.0 [4]	nd	< 5.0 [3]
Neurokinin A	7.5 ± 0.2	7.0 ± 0.1 (0.9, 0.6–1.2)	nd	nd	nd
Septide	8.5 ± 0.2	7.1 ± 0.1 (0.9, 0.6–1.2)	6.0 ± 0.1 (1.2, 0.9–1.4)	6.3 ± 0.2 [3]	6.5 ± 0.1 (0.8)

Schild slopes, where calculated, are given in parentheses. Each pD_2 and pK_B value is mean ± S.E.M. ($n = 6$–15, unless indicated in square brackets). Schild slopes are expressed as mean with 95% confidence limits. nd = not determined.

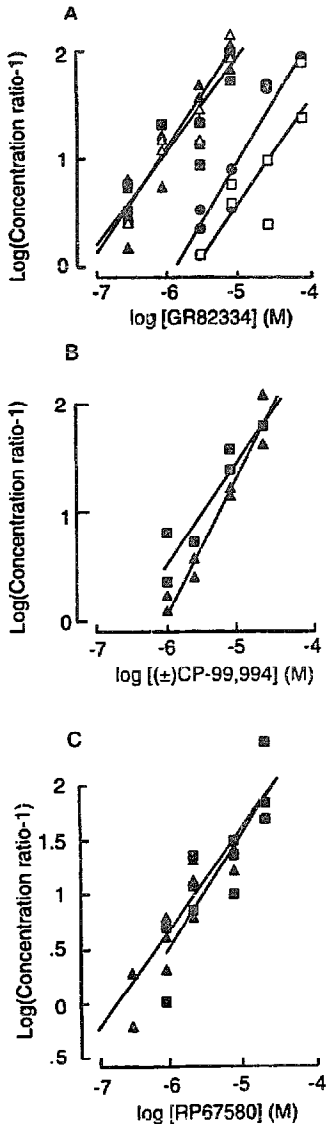

Fig. 2. Schild analysis for antagonism by GR82334 (A), (±)-CP-99,994 (B) and (±)-RP67580 (C) of depolarisation responses evoked by GR73632 (■), septide (▲), neurokinin A (△), substance P *O*-methyl ester (●) and substance P (□). Each data point was obtained from a separate preparation. The gradient of the line of best fit was determined by linear regression.

Table 3
Effect of tetrodotoxin (0.1 μM) on depolarisation responses induced by substance P *O*-methyl ester and GR73632 in rat isolated superior cervical ganglion

Agonist	pD_2		Concentration ratio	E_{max} (%)
	Control	+TTX		+TTX
Substance P-*O* methyl ester	7.9±0.1	8.0±0.1	1.0±0.3	97± 8
GR73632	8.2±0.1	8.2±0.1	1.0±0.4	93±13

Concentration-response curves were constructed in the absence and presence of tetrodotoxin (TTX) in each preparation and data expressed as % control maximum response. Potency estimates (pD_2) and E_{max} were determined for each curve and dose ratio and E_{max} in the presence of tetrodotoxin (TTX) as a % control E_{max} were calculated. Data are mean±S.E.M. (n = 3–4).

Table 4
Effect of desensitisation to either substance P *O*-methyl ester or GR73632 on depolarisation responses to substance P *O*-methyl ester, GR73632 and the non-tachykinin agonist bethanechol, in the rat isolated superior cervical ganglion

Agonist	% Reduction in control depolarisation responses following desensitisation to:		
	Substance P *O*-methyl ester	GR73632	Non-desensitised
Substance P *O*-methyl ester	98±1	94±4	0±3
GR73632	100±0	100±0	4±8
Bethanechol	12±6	8±3	1±5

Depolarisation responses evoked by sub-maximal concentrations of agonists following desensitisation of the preparation to either substance P *O*-methyl ester (1 μM) or GR73632 (1 μM) were compared to control responses evoked in the same preparation, prior to desensitisation. Data are mean±S.E.M. (n = 3).

Table 5
Effect of temperature on affinity estimates (pK_B) for GR82334 and (±)-RP67580 to antagonise depolarisation responses induced by tachykinin NK_1 receptor agonists GR73632 and substance P *O*-methyl ester

Agonist	Temperature (°C)	pD_2	Antagonist pK_B	
			(±)-RP67580	GR82334
Substance P *O*-methyl ester	37	8.2±0.1	≤5.0 [n = 3]	5.9±0.1 (1.0, 0.7–1.4)
	22	8.0±0.1	6.4±0.1 (0.9, 0.5–1.3)	7.0±0.1 (0.9, 0.6–1.1)
GR73632	37	8.2±0.1	6.4±0.1 (0.9, 0.6–1.3)	7.0±0.1 (0.8, 0.6–1.1)
	22	8.0±0.1	6.8±0.2	7.3±0.3 (1.0, 0.5–1.5)

Data are mean pK_B±S.E.M. (n = 4–12, unless indicated) and, in parentheses, Schild slopes with 95% confidence limits.

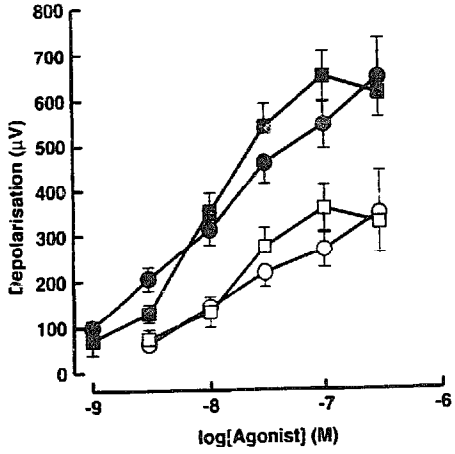

Fig. 3. Concentration-response curves to substance P *O*-methyl ester (○,●) and GR73632 (□,■) at 37°C (filled symbols) and 22°C (open symbols). Responses are expressed as depolarisation (μV). Data are mean±S.E.M. obtained from 15–26 preparations.

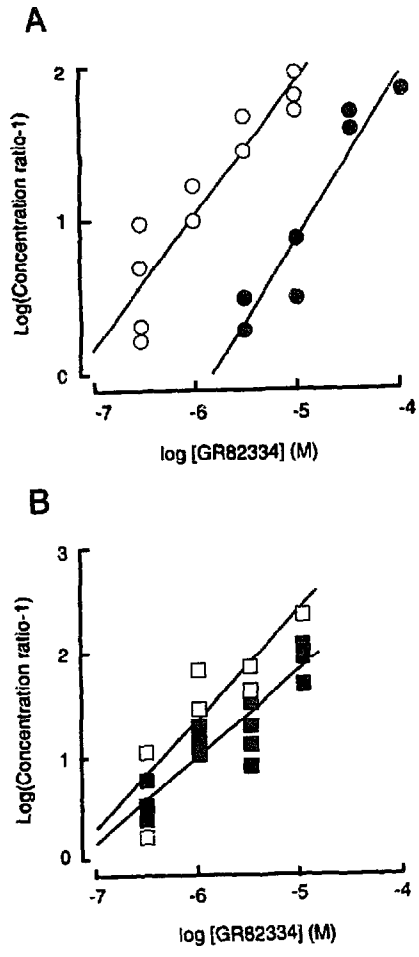

Fig. 4. Schild analysis for antagonism by GR82334 of depolarisation responses evoked by substance P *O*-methyl ester (●,○) (A) and GR73632 (■,□) (B) at 37°C (filled symbols) and 22°C (open symbols). Each data point was obtained from a separate preparation. The gradient of the line of best fit was determined by linear regression.

GR82334 and (±)-RP67580 to antagonise substance P *O*-methyl ester-induced depolarisations ($P < 0.0001$). In contrast, the potency of GR82334 to inhibit GR73632-induced depolarisations was only slightly increased ($P < 0.05$) and (±)-RP67580 was unaffected ($P > 0.05$). Thus at 22°C antagonists inhibited responses evoked by GR73632 and substance P *O*-methyl ester with similar potencies (Table 5). When three or more antagonist concentrations were used, Schild analysis of the data was carried out. Schild slopes, where calculated, were not significantly different to unity (Fig. 4, Table 5).

4. Discussion

Tachykinin agonists depolarised the rat isolated superior cervical ganglion with the rank order of potency substance P *O*-methyl ester = [Sar^9,Met(O$_2$)^{11}] substance P = substance P > neurokinin A. Substance P *O*-methyl ester, [Sar^9,Met(O$_2$)^{11}]substance P and septide attained similar maximum depolarisations, indicating that they all behave as full agonists in this preparation. The rank order of agonist potency is in broad agreement with that previously reported in this tissue (Seabrook et al., 1992) and consistent with an action of tachykinin agonists predominantly at tachykinin NK$_1$ receptors. While substance P depolarised the rat isolated superior cervical ganglion with similar potency to that reported by Seabrook et al. (1992), other agonists which were also examined by Seabrook and colleagues, including substance P *O*-methyl ester, [Sar^9,Met(O$_2$)^{11}]substance P and neurokinin A, were 6- to 8-fold more potent in the present study. Variations in experimental protocol such as agonist contact time, perfusion rate, inclusion of peptidase inhibitors or experimental temperature could provide an explanation for these potency differences.

In the rat superior cervical ganglion we have demonstrated that both the peptide tachykinin NK$_1$ antagonist GR82334 and the non-peptide tachykinin NK$_1$ antagonists CP-99,994 and (±)-RP67580 were substantially more potent to antagonise responses evoked by septide and neurokinin A compared to those evoked by substance P and full sequence (undecapeptide) analogues such as substance P *O*-methyl ester, and [Sar^9,Met(O$_2$)^{11}]substance P. This observation in a neuronal preparation is in agreement with results from other laboratories observed both in vitro in smooth muscle preparations, and in vivo (see Introduction). We obtained similar findings using an alternative C-terminal hexapeptide substance P analogue, GR73632. The tachykinin NK$_1$ receptor antagonist activity of (±)-CP-99,994 was due entirely to the (+)-enantiomer, which behaved in a similar manner to the racemate, while the (−)-enantiomer was devoid of antagonist activity against GR73632-evoked responses. GR82334 antagonised responses induced by the decapeptide neurokinin A with similar potency to that observed against septide and GR73632, indicating that this preferential antagonism is not simply due to the greatly reduced peptide length of the hexapeptides. Although responses evoked by substance P *O*-methyl ester were antagonised with considerably reduced potency than those evoked by septide, GR82334 antagonised substance P *O*-methyl ester-induced responses more potently than it did responses evoked by substance P ($P < 0.01$). Similarly, in the rabbit isolated iris sphincter, Hall et al. (1994) observed that responses evoked by both substance P *O*-methyl ester and septide were more potently blocked by tachykinin NK$_1$ receptor antagonists than were responses to substance P. However, while in rat isolated superior cervical ganglion GR82334 was only slightly more potent to antagonise substance P *O*-methyl ester-induced responses

than responses evoked by substance P, in rabbit isolated iris sphincter tachykinin NK_1 receptor antagonists were almost as potent to antagonise substance P *O*-methyl ester-induced responses as they were to antagonise responses evoked by septide (Hall et al., 1994). These differences may be related to species differences in tachykinin NK_1 receptors. Indeed, while the rabbit tachykinin NK_1 receptor has not yet been cloned, pharmacological studies have indicated that tachykinin NK_1 receptors in rabbit are similar to those in guinea pig and human and distinct to those in rat and mouse (Beresford et al., 1991).

The potency of GR82334 in rat isolated superior cervical ganglion to antagonise responses induced by analogues of substance P was similar to that reported by Meini et al. (1994) in the rat isolated bladder preparation. However, the potency of (±)-RP67580 against responses evoked by septide in the rat isolated superior cervical ganglion was a log order of magnitude lower than that reported in the rat isolated bladder preparation (Meini et al., 1994; Montier et al., 1994). This observation is at variance with work by Seabrook et al. (1993) who demonstrated that RP67580 was equipotent to antagonise responses evoked by substance P *O*-methyl ester in isolated bladder and superior cervical ganglion preparations of the rat. Differences in experimental protocol described earlier could contribute to the difference in observed potency.

To investigate further the preferential antagonism of C-terminal hexapeptide analogues of substance P by tachykinin NK_1 receptor antagonists, we examined responses evoked by substance P *O*-methyl ester and GR73632 in greater detail. To determine whether the C-terminal hexapeptide analogues of substance P may act at a receptor presynaptic to that acted upon by the undecapeptide substance P analogues, we investigated the effect of tetrodotoxin on responses evoked by GR73632 and substance P *O*-methyl ester. Tetrodotoxin did not modify the response to either agonist, indicating that both agonists are acting at a postsynaptic site and do not require the generation of action potentials to evoke the depolarisation response. We then attempted to separate responses evoked by the two agonists by desensitising different preparations to either substance P *O*-methyl ester or GR73632. Responses to both agonists were abolished by desensitisation to either substance P *O*-methyl ester or GR73632, suggesting that these agonists evoke responses through a common mechanism.

Konishi et al. (1992) demonstrated that the tachykinins substance P, neurokinin A and neurokinin B could evoke two distinct depolarisation responses in rat isolated coeliac-superior mesenteric ganglia, one fast and one slow. While both fast and slow responses persisted in the presence of tetrodotoxin, responses could be distinguished by lowering the temperature. Thus, they observed that lowering the temperature markedly attenuated the slow response while the fast response was largely unaffected suggesting that the two responses were either mediated by different subtypes of tachykinin receptor or by a single class of receptor linked to two different intracellular mechanisms. We were therefore interested to investigate whether depolarisation responses to substance P *O*-methyl ester or GR73632 could be differentially affected by modifying experimental temperature. Conducting the experiments at 22°C attenuated responses evoked by both agonists by similar amounts and there remained no significant difference between the potency or maximum depolarisation response of each agonist, which is in agreement with both agonists acting via a second messenger-linked transduction system. However, at 22°C the potencies of the antagonists to inhibit responses to substance P *O*-methyl ester were markedly enhanced whereas their abilities to block GR73632-evoked responses were largely unaffected. These temperature-related effects are unlikely to be associated with increased peptidase activity degrading either agonist or antagonist at 37°C since the agonists evoked larger responses at 37°C than at 22°C, there was no increase in agonist potency at the lower temperature and the potency of the antagonists to block responses evoked by GR73632 was largely unaffected. An alternative explanation could be that at 37°C substance P *O*-methyl ester and GR73632 act via different receptor subtypes, while at 22°C both agonists are acting via a single subtype. A third possibility is that at the higher temperature the receptor has greater conformational freedom and the interaction of substance P *O*-methyl ester, or other full sequence analogues with the receptor causes the adoption of a conformation which is less favourable for antagonist binding. For a number of reasons, we favour the latter hypothesis. Firstly, molecular biology studies in a number of species, including rat, have yielded no indication of intraspecies heterogeneity of the tachykinin NK_1 receptor (see Gerard et al., 1993). Secondly, Pradier et al. (1993), using the rat recombinant tachykinin NK_1 receptor expressed in COS-1 cells, recently described the ability of RP67580 to inhibit septide-induced responses with greater potency than its ability to antagonise responses evoked by substance P. Although these results do not completely rule out the existence of a septide receptor which is distinct from the tachykinin NK_1 receptor, they demonstrate that these pharmacological phenomena can be obtained by interactions at a single population of tachykinin NK_1 receptors. Peptide agonists and non-peptide antagonists have been shown to bind to different segments of the tachykinin NK_1 receptor (Fong et al., 1992a,b, 1993; Gether et al., 1993) while different tachykinin agonists interact in partially different ways with multiple receptor epitopes distributed

throughout the receptor structure (Gether et al., 1993). Thus, tachykinin agonists may interact with multiple epitopes of the tachykinin NK_1 receptor, which are distinct from those recognising tachykinin NK_1 receptor antagonists. Taking these findings together with our own data illustrating the temperature dependency of agonist-antagonist interactions at tachykinin NK_1 receptors, we suggest that GR73632, neurokinin A and septide act at site(s) on the tachykinin NK_1 receptor which are at least partially distinct from that to which the full sequence substance P analogues bind. Substance P *O*-methyl ester, and most likely the other undecapeptide agonists, interact with the receptor in a temperature-dependent manner such that at 37°C their interaction with the receptor causes it to adopt a conformation which is less favourable for antagonist binding.

In summary, the ability of tachykinin NK_1 receptor antagonists to antagonise tachykinin NK_1 agonist-induced depolarisation responses in rat isolated superior cervical ganglia is both agonist and temperature dependent. However, we are unable to find evidence for the existence of multiple subtypes of the tachykinin NK_1 receptor in this preparation. We conclude that the agonist and temperature dependency of tachykinin NK_1 receptor antagonist potencies in the rat isolated superior cervical ganglion most likely reflects different conformational changes in the receptor induced by partial or full sequence substance P analogues. The conformational change induced by full sequence analogues appears to be unfavourable for antagonist binding.

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