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Electrophysiological characterisation of tachykinin receptors in the rat nucleus of the solitary tract and dorsal motor nucleus of the vagus *in vitro*

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1 Recent studies have shown antagonists at the NK_1 subtype of receptor for tachykinins are anti-emetics and suggested that this may result from blockade of tachykinin-mediated synaptic transmission at a central site in the emetic reflex.

2 We have used intracellular recording *in vitro* to study the pharmacology of tachykinins in the nucleus of the solitary tract (NST) and dorsal motor nucleus of the vagus (DMNV).

3 Neurones in the NST were depolarized by substance P (SP), the presumed endogenous ligand for the NK_1 receptor and these effects were mimicked by the NK_1 agonists, SP-O-methylester (SPOMe), GR73632 and septide; however, SP was nearly an order of magnitude less potent than the latter two agonists.

4 In the DMNV, SP and NK_1 receptor agonists evoked similar depolarising responses but SP appeared to be more potent than in the NST and was closer in potency to the other agonists.

5 NK_1-receptor antagonists blocked responses to septide and GR73632 in the NST but had little effect on responses to SP and SPOMe. In contrast, in the DMNV the NK_1-receptor antagonists blocked responses to septide and GR73632 but also reduced responses to SP and SPOMe.

6 Neurokinin A (NKA) was almost equipotent with septide and GR73632 in depolarizing both NST and DMNV neurones but these effects were not mimicked by a specific NK_2-receptor agonist. Responses to NKA were unaffected by an NK_2-receptor antagonist; however, the depolarizing effects of NKA were blocked by NK_1-receptor antagonists.

7 Neurones in both DMNV and NST were unaffected by the endogenous NK_3-receptor ligand, neurokinin B and by a specific agonist for this site, senktide.

8 The results with NK_1 receptor agonists and antagonists suggest that the septide-sensitive NK_1 site is involved in the excitation of both NST and DMNV neurones. The 'classical' NK_1 receptor may play more of a role in the DMNV and a third unknown site may be responsible for the depolarizing response to SP in the NST. The effects of NKA are best interpreted as an action at the septide-sensitive NK_1 site. This raises the possibility that anti-emetic action of the NK_1 antagonists may be due to blockade of NKA transmission at the septide-sensitive site.

Keywords: Substance P; neurokinin A; nucleus of the solitary tract; dorsal motor nucleus of the vagus; emesis

Introduction

The use of 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonists has been a major advance in the management of nausea and vomiting (Aapro, 1991). These agents are effective against the acute phase of emesis but less so against the delayed phase that occurs after chemo- or radiotherapy (for a review see Andrews, 1994). In addition, they do not block vagally-dependent emesis induced by certain agents (Bhandari & Andrews, 1991) or emesis induced by centrally acting stimuli (Bermudez *et al*., 1989; Bhandari *et al*., 1992). Further advances in the control of emesis are desirable and there is considerable interest in therapies aimed at interrupting the flow of sensory information from the vagal afferents that initiate the emetic reflex. One approach which is receiving attention is the use of antagonists of tachykinin neurotransmitters, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). Based on rank orders of potency, these endogenous peptides have been suggested to be prototypic ligands for three receptor subtypes, the NK_1, NK_2 and NK_3 receptors, respectively (Regoli *et al*., 1989, 1994). This classification of tachykinin receptors is currently being revised to incorporate subtypes of receptors (see Maggi *et al*., 1993; Mussap *et al*., 1993; Regoli *et al*., 1994). In particular, discrepancies in binding and functional studies have lead to speculation that two distinct NK_1 receptors exist (Petitet *et al*., 1992; Maggi *et al*., 1993; Meini *et al*., 1994).

In 1993, Bountra *et al*. and Tattersal *et al*. demonstrated that systemic administration of CP-99,994, a specific antagonist at the NK_1 receptor, reduced retching in ferrets challenged with a range of emetogens. Gardner *et al*. (1994) suggested a central site of action of this anti-emetic effect as the effects of systemic administration were mimicked by direct injection of the drug into the brainstem. More recently, Watson *et al*. (1995) also suggested that the anti-emetic effects of CP-99,994 depended on blockade of NK_1 receptors within the brainstem, with the most likely site being the nucleus of the solitary tract (NST).

It has long been suspected that the NST is involved in the emetic reflex. Borison & Wang (1949, 1953) showed that vomiting can be induced by electrical stimulation in the solitary complex of decerebrate cats and that lesions of this region rendered dogs refractory to emetic agents. A recent paper which examined the connectivity of the solitary complex in the ferret suggested that the motor emetic centre was located in the NST (Strominger *et al*., 1994). The NST is the first relay of visceral sensory afferents in the CNS, receiving inputs from multiple modalities, including those originating from gastrointestinal structures and conveyed via the vagus nerves. Such

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fibres also project to the area postrema (AP) which in turn provides further input to the NST. The NST is therefore a convergence point of the two inputs known to be activated by emetic stimuli. Output from the NST diverges to many brainstem regions including the dorsal motor nucleus of the vagus (DMNV) which is the main source of vagal output (e.g. see Ter Horst & Streefland, 1994). Although this picture is oversimplified these pathways form an 'emetic loop' which may be intimately involved in the perception of nausea and the vomiting reflex.

There is good evidence to support a role for SP in these pathways. SP is a transmitter of vagal or glossopharyngeal primary sensory neurones projecting to the brainstem (Baude *et al*., 1989) and is present in vagal afferent fibres and in axon varicosities in the NST, DMNV and AP (Ljungdahl *et al*., 1978; Gillis *et al*., 1980; Chiba & Murata, 1982; Leslie, 1985). These regions exhibit high concentrations of tachykinin receptors (Mantyh *et al*., 1989; Nakaya *et al*., 1994; Maubach *et al*., 1995a; Watson *et al*., 1995) and SP has been shown to be released from the NST *in vivo* (Morilak *et al*., 1988) and *in vitro* (Helke *et al*., 1981). Electrophysiological studies have demonstrated powerful excitatory effects of tachykinins on neurones of the dorsal vagal complex (Carpenter *et al*., 1983; Morin-Surun *et al*., 1984; Plata-Salaman *et al*., 1988; Jacquin *et al*., 1989; Martini-Luccarini *et al*., 1996). There is also evidence linking tachykinins to the emetic reflex. The capsaicin analogue, resiniferatoxin, abolished the emetic response to peripherally and centrally-acting stimuli in ferrets and it was suggested that this may be due to depletion of SP at a central site (Andrews & Bhandari, 1993). Systemic administration of SP can induce emesis in dogs (Carpenter *et al*., 1984) and ferrets (Knox *et al*., 1993). As already mentioned, several studies have demonstrated the anti-emetic effects of the NK_1-receptor antagonist, CP-99,994 (Bountra *et al*., 1993; Tattersal *et al*., 1993; Watson *et al*., 1995).

If we are to understand further the putative role of tachykinins in the emetic pathways we need to define precisely the effects of the peptides on neurones in the dorsal vagal complex, the receptors involved and the likely endogenous ligand. An array of potent and selective agonists and antagonists for tachykinin receptors have been developed (Regoli *et al*., 1988; McLean *et al*., 1992, 1993). We have used a number of these agents and intracellular recording in a rat brain slice preparation *in vitro* to investigate further the physiology and pharmacology of responses to tachykinins in the NST and DMNV. Some of these results have been published in abstract form (Maubach *et al*., 1994a,b, 1995b).

Methods

Male Wistar rats (120 g) were anaesthetized with ketamine hydrochloride (100 mg kg^{-1} i.m.) and decapitated. The brain was removed and placed in cold (4°C) artificial cerebrospinal fluid (ACSF; composition in mM: NaCl 126, KCl 3.25, $MgSO_4$ 2, NaH_2PO_4 1.25, $NaHCO_3$ 24, $CaCl_2$ 2 and D-glucose 10). A block of the brainstem was dissected away and coronal slices (400 μm thick) containing the dorsal vagal complex were cut in the dorsal to ventral direction using a Campden Vibroslice. Five to six slices were taken starting approximately 1.5 mm caudal to the obex and continued to approximately 1.5 mm rostral to the obex. In this way we included most of the caudal NST in our slices. Some of the more rostral slices would therefore also contain rostral (gustatory) NST. The slices were transferred directly to the recording chamber where they were maintained at the interface between a continuous flow (1.2 ml min^{-1}) of ACSF at 34°C and moistened carbogen gas (95% O_2/5% CO_2). The perfusion medium was continuously bubbled with carbogen and had a pH of 7.4 at the recording temperature. The slices were allowed to equilibrate in the recording chamber for at least 1 h before recording was commenced.

Conventional techniques were used to make intracellular voltage recordings from neurones in the NST and DMNV using electrodes (40–120 megohms) filled with K-acetate (3 M) and an Axoclamp 2A amplifier in bridge mode. In the case of NST neurones, recording were made from both medial and lateral locations within the nucleus. Membrane potential (E_m) was continuously monitored on a digital recording oscilloscope, displayed on a chart recorder and recorded on digital audio tape for subsequent analysis. In the majority of recordings, negative current pulses (100 ms, 0.05–0.2 nA) were injected via the recording electrode every 2–3 s. The amplitude of the hyperpolarizations evoked was used to monitor apparent input resistance.

All drugs were applied by bath perfusion by switching the bath perfusion from drug free to drug containing ACSF. Peptide solutions contained a cocktail of peptidase inhibitors (phosphoramidon, bestatin, chymostatin and leupeptin, all at 1 μM) to help prevent biodegradation of peptides. A period of 1 min was required for solution changes to reach the bath. Once the new solution reached the bath, perfusion was continued for 60 s before switching back to drug free solutions. In this way, receptor agonists were perfused for 2 min periods. In the majority of cases this was sufficient for any change in membrane potential to reach a steady state. When antagonists were applied, a period of at least 15 min was allowed for equilibration before agonists were re-applied. To avoid inaccuracy in calculating changes in membrane potential due to the presence of action potentials, experiments where quantitative comparisons were required (e.g. agonist potency estimates) were conducted in the presence of tetrodotoxin (TTX, 1 μM). Statistical comparisons where made where appropriate with a Students *t* test.

All salts used in making the ACSF were obtained from BDH and were of AnalaR grade or better. The peptidase inhibitors, phosphoramidon, bestatin, leupeptin and chymostatin were obtained from Sigma Chemical Company. TTX was obtained from Calbiochem. All tachykinin agonists and antagonists were a kind gift from Glaxo Wellcome Research and Development Ltd. Substance P, neurokinin A, neurokinin B, substance P-O-methylester (SPOMe), septide (pGlu-Phe-Phe-L-Pro-Leu-Met-NH_2) and senktide (Succ-Asp-Phe-MePhe-Gly-Leu-Met-NH_2) were originally purchased from Peninsula Laboratories, Europe Ltd. The compounds GR73632 (δ-Ava-Phe-Phe-Pro-MeLeu-Met-NH_2), GR82334 (Gly-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-D-Pro[spiro-γ-lactam]Leu-Trp-NH_2), CP-99,994 ((+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine), GR159897 ((R)-1-[2-(5-fluoro-1H-indol-3-yl)ethyl]-4-methoxy-4[(phenylsulfinyl)methyl]-piperidine) and GR64349 (Lys-Asp-Ser-Phe-Val-Gly[R-γ-lactam]-Leu-Met-NH_2) were synthesised in the Department of Medicinal Chemistry, Glaxo Wellcome Research and Development Ltd, Ware. All drugs were dissolved in distilled water (unless otherwise stated), made up to a stock concentration of 1 mM and stored as small aliquots in a freezer (−20°C) until use. SP, NKA, SPOMe and senktide were dissolved in a minimal volume of acetic acid (5 mM), and NKB and septide were dissolved in a minimal volume of dimethylsulphoxide, before making up to volume with distilled water. Stock solutions were diluted to final desired concentration in ACSF immediately prior to application.

Results

Neurones studied

Neurones in the AP are small and so far it has proved impossible to maintain impalements for periods long enough to conduct detailed pharmacological studies; therefore, all the studies have been conducted in the NST and DMNV. Neurones in both nuclei were characterised by relatively depolarized membrane potentials, fast action potentials, large and prolonged after-hyperpolarizations, slow membrane time constants, high apparent input resistances and a high degree of

spontaneous activity (Bradley & Sweazey, 1990; King *et al*., 1993; Tell & Bradley, 1994). Although it is likely that the neurones studied in either nucleus formed a heterogeneous population, no attempt has been made to separate them on the basis of electrophysiological properties or morphology in the present experiments. Neurones with membrane potentials positive to −55 mV and in which spontaneous action potentials could not be prevented by the injection of small amounts of hyperpolarizing current were excluded from analysis. Applying these arbitrary criteria, the mean (±s.e.mean) resting E_M, action potential amplitude (from rest) and input resistance of a sample ($n=47$) of NST neurones were −65.6±1.5 mV, 92.2±1.2 mV and 122.1±4.7 MΩ, respectively. Similar values were seen in DMNV neurones (−66.0±0.8 mV, 99.1±1.2 mV and 105.9±4.9 MΩ, respectively, $n=59$).

Effects of tachykinin agonists on NST neurones

NK_1 receptor agonists The effects of SP were tested on 20 neurones in the NST and changes in membrane potential were recorded in 15 of these (75%). In the remaining 25% even high concentrations (up to 5 μM) had no detectable effect. In those that did respond, application of SP produced a slow depolarization of the membrane potential which in many cells reached threshold for the generation of action potentials. Examples of such responses can be seen in Figures 1 and 5. The depolarization could be accompanied by an increase in apparent input resistance although this was variable, some cells showing little in the way of a resistance change ($n=7$). In a sample of eight neurones the mean depolarization to SP at 500 nM was 4.8±0.6 mV (mean±s.e.mean) and input resistance increased by 25.5±2.9%. Concentration–response curves were constructed in three neurones (e.g. Figure 2) and from these it was possible to estimate a mean EC_{50} of 773±146 nM (Figure 3). The depolarising responses to the SP and other NK_1 receptor agonists (see below) persisted in the presence of TTX indicating a postsynaptic action. When action potential generation was prevented by TTX there was often an apparent enhancement of the depolarization due to the removal of the spikes and their associated strong afterhyperpolarizations. Finally, there was no evidence of desensitisation when repeated applications were made to the same neurone.

The NK_1 receptor agonist, GR73632, was more effective than SP and depolarized 36 of 40 neurones (e.g. see Figures 1, 5). As with SP, the depolarisation readily reached threshold for action potential generation and was accompanied by an increase in apparent input resistance in many neurones. GR73632 was at least an order of magnitude more potent than SP, gen-

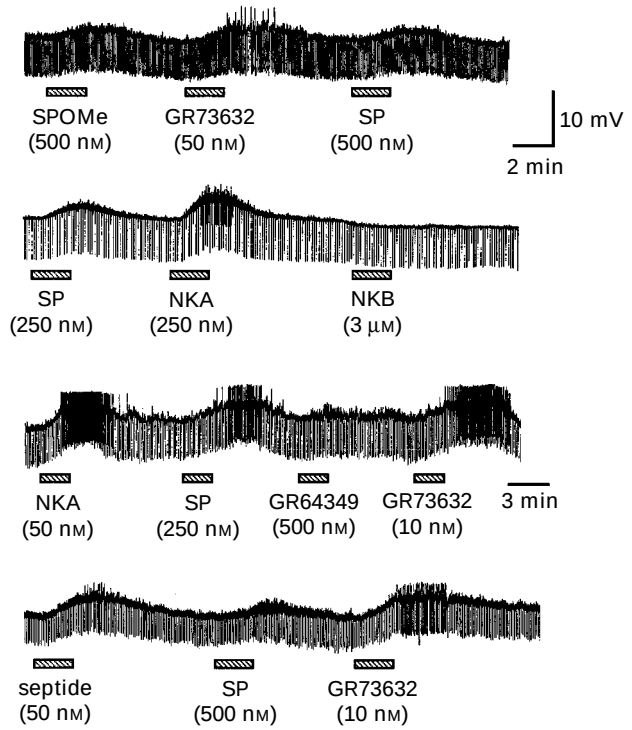

Figure 1 Effects of tachykinin agonists on NST neurones. The traces are chart recordings of membrane potential from four different neurones. The downward deflections are responses to injection of negative current pulses used to monitor neuronal input resistance. The large deflections superimposed on some of the depolarizing responses (e.g. in the third line) are action potentials which have been truncated by filtering of the chart recorder. Agonists were applied by bath perfusion. The bars below the traces represent the time from which the drug solution reached the bath to the time when the return to drug free solution began.

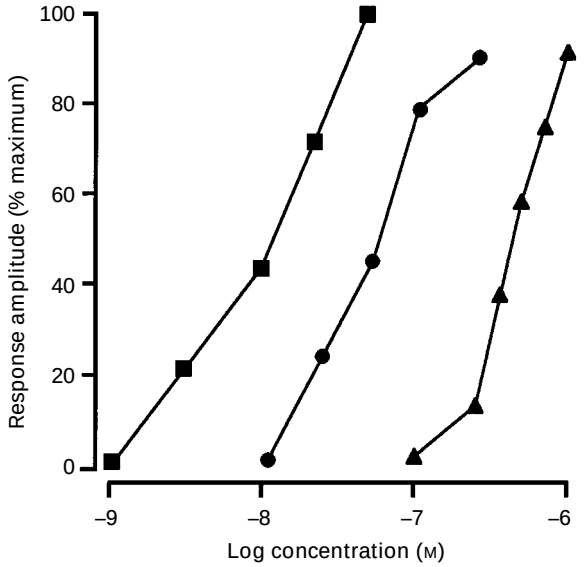

Figure 2 Concentration–response curves to septide (■), NKA (●) and SP (▲) constructed on a single NST neurone. Responses were quantified by measuring the peak amplitude of depolarizations evoked in the presence of TTX to prevent the generation of action potentials.

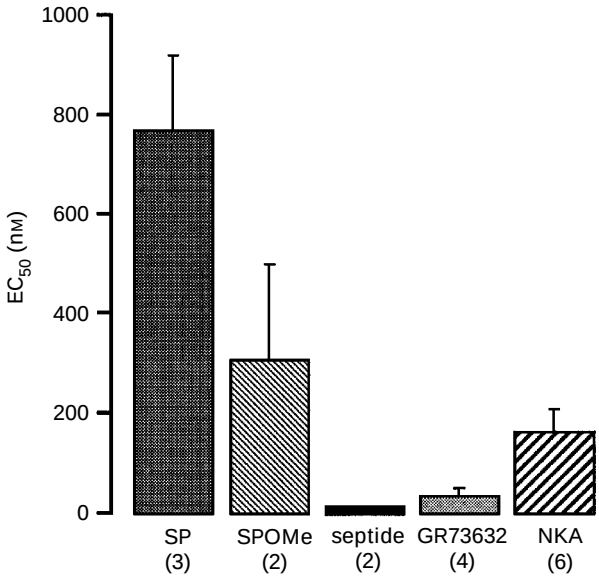

Figure 3 Comparison of EC_{50} values for tachykinin agonists on NST neurones. Values were calculated from concentration–response curves (see Figure 2) in a number (indicated in parentheses) of neurones and an average value determined.

erating a depolarization of 9.3±0.7 mV at 50 nM ($n=27$). The EC_{50} for GR73632 calculated from concentration–response curves in four neurones was 40±13 nM (Figure 3). The difference in potency between SP and GR73632 was highly significant ($P<0.001$). Septide was tested on eight neurones and depolarised seven (Figure 1). The characteristics of the responses were similar to those described for SP and GR73632. Like the latter, septide was more potent than SP and this is illustrated by the concentration–response curves in Figure 2. Similar depolarizing responses were also evoked by SPOMe (Figures 1, 5). This agonist was less potent than GR73632 and septide, but more potent than SP. EC_{50} values calculated from concentration–response curves (Figure 2) were 18 and 23 nM for septide ($n=2$) and 120 and 500 nM for SPOMe ($n=2$). Mean EC_{50} values are illustrated in Figure 3 and suggest a rank order of potency for depolarization of NST neurones by NK_1 receptor-selective agonists of septide ⩾ GR73632 > SPOMe ⩾ SP.

NK_2 and NK_3 receptor agonists GR64349, the selective NK_2-receptor agonist, had no effect over the concentration range tested (0.1–5 μM) on nine NST neurones (Figure 1). Surprisingly, NKA, which has long been accepted as the endogenous ligand for the NK_2 receptor, was very effective (see Figure 1). It depolarised 16 of 18 neurones (89%) and evoked a depolarisation of 9.8±1.9 mV at 100 nM ($n=5$). The EC_{50} value (167±44 nM, $n=6$) was significantly less than that of SP ($P<0.001$) but greater than that of GR 73632 ($P<0.05$) (Figures 2, 3).

NKB and the NK_3-receptor agonist, senktide were tested on four and seven neurones, respectively. NKB (0.1–3 μM) failed to alter membrane potential or input resistance (Figure 1) and likewise senktide (0.1–3 μM) was without effect (data not shown). Thus, of the agonists tested on NST neurones, the rank order of potency can be summarised as: GR73632 ⩾ septide > NKA > SPOMe ⩾ SP > > GR64349 = senktide = NKB.

Effects of tachykinin agonists on DMNV neurones

NK_1 receptor agonists SP produced a concentration-dependent depolarization of membrane potential in 24 of 40 neurones (e.g. Figure 6) and had no effect in the remainder (up to 3 μM). The mean depolarisation to SP (500 nM) in 16 neurones was 6.3±0.5 mV. Again, the depolarization could be accompanied by an increase in input resistance (+20.6±2.8%, $n=8$). Concentration–response curves ($n=6$) gave an EC_{50} of 140±41 nM. There was no apparent difference in the form of responses to SP in the DMNV compared with the NST but it was more potent ($P<0.01$) in the former (Figures 3, 4).

Other NK_1 receptor-selective agonists also depolarised DMNV neurones. GR73632 was extremely effective and depolarised 42 of 48 neurones (see Figure 6) with only six unaffected. As in the NST, GR73632 was more potent than SP ($P<0.005$), generating a mean depolarisation of 9.8±0.9 mV at 50 nM ($n=29$) with an EC_{50} estimated from concentration–response curves in 10 neurones of 34±10 nM. Septide was tested on six neurones and depolarised all of them. Although the EC_{50} for septide (102±21 nM, $n=3$) was significantly greater than that of GR73632 ($P<0.005$) it was not significantly different to SP. SPOMe depolarised 9 of 13 neurones tested (69%). EC_{50}s estimated in two neurones were 29 and 320 nM. Thus, in contrast to the NST, NK_1 receptor agonists were very similar in their ability to depolarize DMNV neurones. Examples of responses to these agonists can be seen in Figure 6 and these results are summarized in Figure 4.

As in the NST, there was little evidence of any desensitization to any of the NK_1-receptor agonists, repeated applications evoking responses of similar amplitude and duration. Again, suppression of action potentials by TTX (1 μM) could result in an apparent enhancement of the underlying depolarisation induced by tachykinin receptor agonists.

NK_2 and NK_3 receptor agonists The effects of the NK_2-receptor agonist, GR64349, was tested on 12 neurones and the NK_3 agonists, NKB and senktide on seven and nine neurones, respectively. These agonists had virtually no effect over the concentration range tested (up to 3 μM). In contrast, NKA was very effective, depolarizing 18 of 22 neurones (e.g. Figure 8) and evoking a mean response of 6.9±0.6 mV at 100 nM ($n=10$). The EC_{50} for NKA (123±9 nM, $n=4$) was similar to that of the NK_1 receptor agonists and very similar to that observed in the NST. The potency differences between these agonists on DMNV neurones is shown in Figure 4. The EC_{50} for NKA was not significantly different from that of SP or septide but significantly greater than that of GR73632 ($P<0.001$).

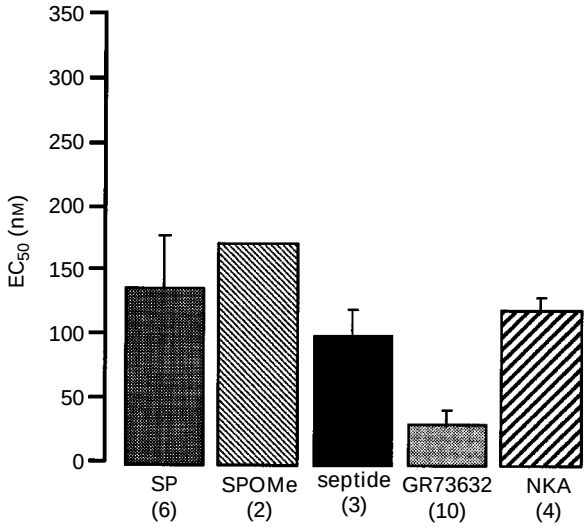

Figure 4 Comparison of EC_{50} values for tachykinin agonists on DMNV neurones. Details as in Figure 3.

Effects of tachykinin receptor antagonists on NST neurones

To examine the effects of antagonists against a range of concentrations of different agonists on the same neurone is not feasible in intracellular studies due the long duration of recording which would be necessary to achieve this. Therefore, we employed the alternative, although less satisfactory approach, of testing the antagonists against single submaximal concentrations of agonists which evoked similar responses. During perfusion with antagonists of either NK_1 or NK_2 receptors there were no discernible direct effects on membrane potential or input resistance of either NST and DMNV neurones.

NK_1-receptor antagonists GR82334, a peptide NK_1-receptor antagonist, significantly reduced responses to GR73632. The agonist (50 nM, $n=6$) evoked a mean depolarization of 8.7±0.6 mV in the absence of the antagonist. During perfusion with GR82334 (5 μM) this was reduced to 1.4±0.5 mV ($P<0.005$). The antagonist had similar effects on responses to septide (50 nM, $n=3$). The mean response amplitude before GR82334 was 7.0±0.6 mV and this was reduced to 0.4±0.4 mV in the presence of the drug ($P<0.005$). Thus, responses to both agonists were almost abolished by this concentration of antagonist.

In contrast, GR82334 (5 μM) had little effect on responses to SPOMe (500 nM, $n=3$, Figure 6). Mean depolarizations in the absence and presence of the antagonist were 5.3±0.3 mV and 5.1±0.5 mV, respectively. Likewise, the antagonist failed to affect responses to SP (500 nM) in two neurones. Figure 5a shows a study where GR82334 abolished the response of an NST neurone to GR73632 with little alteration in responses to either SP or SPOMe.

We have also tested the non-peptide NK_1 receptor antagonist, CP-99,994 (5 μM), and in all respects its effects resembled

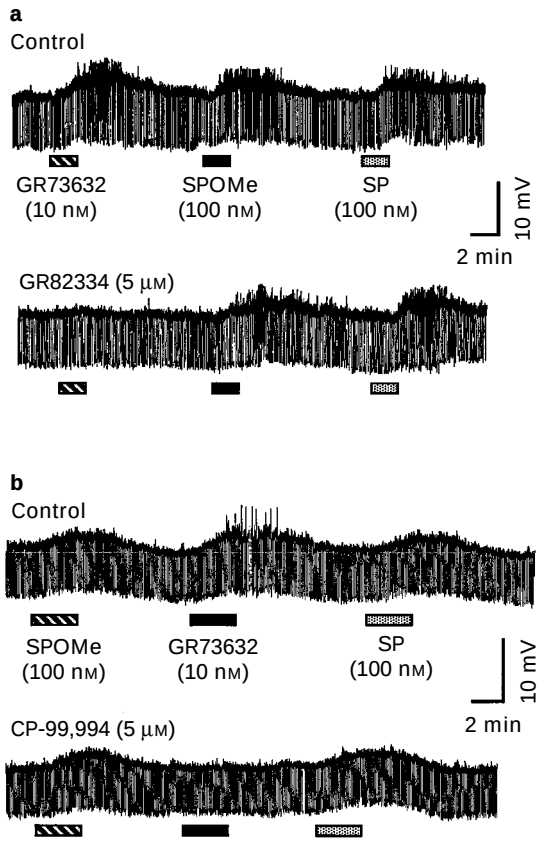

Figure 5 Effect of NK_1-receptor antagonists on responses of NST neurones. (a) Responses to GR73632 were abolished by the peptide antagonist, GR82334, while depolarizations evoked by SP and SPOMe were apparently unaffected. The antagonist had been perfused for 19 min prior to the retesting of GR73632. (b) Similar study on a second neurone using the non-peptide antagonist, CP-99,994. The latter was perfused for 17 min prior to the re-testing of SPOMe.

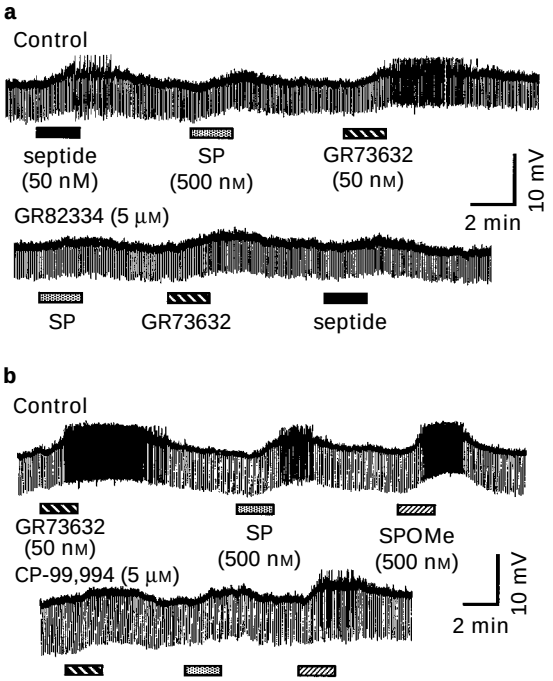

Figure 6 Effect of NK_1-receptor antagonists on responses of DMNV neurones. (a) Responses to septide and GR73632 were clearly reduced by GR82334 and the smaller response to SP was also reduced. (Note different order of agonist applications in presence of antagonist). (b) The non-peptide antagonist, CP-99,994, had a very similar effect, reducing responses to both GR73632 and SP and also to SPOMe on this occasion.

those of GR82334. In three neurones GR73632 (at 50 nM) evoked a mean depolarisation of 8.7 ± 0.3 mV and this was reduced to 1.6 ± 0.9 mV in the presence of CP-99,994 ($P < 0.005$). Similar effects were seen when responses to septide (50 nM) were challenged with CP-99,994 ($n = 2$). Before the addition of the antagonist the response amplitude was 8 (7, 9) mV and this was reduced to 2.8 (2.5, 3) mV in the presence of the antagonist. CP-99,994 had no discernible effect on responses to SPOMe ($n = 2$) or SP ($n = 2$). An example is shown in Figure 5b where responses to GR73632 were abolished by the antagonist but those to SPOMe and SP were unaltered. Thus, in the NST neither NK_1-receptor antagonist reduced responses to SP and SPOMe. This contrasts with their effects on responses to GR73632, septide and NKA which were all reduced to similar extents. These effects are summarized in Figure 7a.

Both CP-99,994 and GR82334 also reduced responses to NKA (100 nM). In the control situation, NKA evoked a mean depolarization of 7.5 ± 0.3 mV ($n = 4$). In the presence of GR82334 (5 μM) this was reduced to 1.4 ± 0.7 mV ($P < 0.005$). With CP-99,994, the depolarizing response was reduced from 6.5 ± 0.3 mV to 1.9 ± 0.1 mV ($P < 0.005$, $n = 5$). Similar effects were seen in the DMNV (see below) and are illustrated in Figure 8.

NK_2-receptor antagonist The effect of the NK_2-receptor antagonist, GR159897 (5 μM), was studied on responses to GR73632 (50 nM, $n = 4$), septide (50 nM, $n = 2$), SP (500 nM, $n = 2$) and SPOMe (500 nM, $n = 2$). Responses to all four NK_1-receptor agonists were unaffected by the antagonist. GR159897 was also tested on responses of three NST neurones to NKA. The responses to NKA were unaffected by the antagonist (Figures 7a and 8a).

Effects of tachykinin receptor antagonists on DMNV neurones

NK_1-receptor antagonists GR82334 (5 μM) reduced responses to GR73632 (50 nM) from a mean of 7.2 ± 0.8 mV to 1.2 ± 0.6 mV ($P < 0.0005$, $n = 5$). Similar effects were seen in two neurones when responses to septide (50 nM) were challenged with GR82334. Response amplitudes were 6 mV and 8 mV in the control situation and in both cases the responses were abolished by the antagonist. CP-99,994 (5 μM) also reduced responses to GR73632 and septide. In the presence of CP-99,994 a mean depolarization of 7.5 ± 0.7 mV to GR73632 was reduced to 3.7 ± 0.3 mV ($P < 0.01$). CP-99,994 reduced responses to septide in a single DMNV neurone from 6.0 mV to 1.5 mV. Examples are shown in Figure 6 and results are summarized in Figure 7. Both antagonists also proved effective against responses to NKA. GR82334 reduced responses to NKA in four neurones from 7.8 ± 0.8 mV to 0.7 ± 0.7 mV ($P < 0.0005$). Similarly, CP-99,994 reduced responses from 7.0 ± 1.0 mV to 2.0 ± 0.4 mV ($P < 0.05$, $n = 3$) (see Figure 8b).

In contrast to the NST, the NK_1 antagonists also reduced responses to SP and SPOMe in the DMNV. GR82334 reduced the response to SP (500 nM) from 5.3 ± 0.7 mV to 2.9 ± 0.6 mV ($P < 0.01$, $n = 3$). Similarly, the responses to SP were reduced from 6.3 ± 0.9 mV to 4.3 ± 0.6 mV ($P < 0.05$, $n = 3$) by CP-99,994. Responses to SPOMe were reduced by GR82334 in one neurone from 8 mV to 3 mV and in a further two neurones by CP-99,994 (6 mV and 8 mV to 3 and 5 mV, respectively; Figure 6, summarized in Figure 7b). Thus, these results have highlighted a difference between the two brainstem nuclei, with responses to SP and SPOMe being reduced by the NK_1-receptor antagonists in the DMNV, but not in the NST. As the effects of both antagonists were similar we have pooled the data with the two in order to make a comparison between DMNV and NST. The mean amplitude of responses to GR 73632 (as a percentage of control) during perfusion with NK_1-receptor antagonists in NST was $15.8 \pm 4.8\%$ ($n = 9$) and

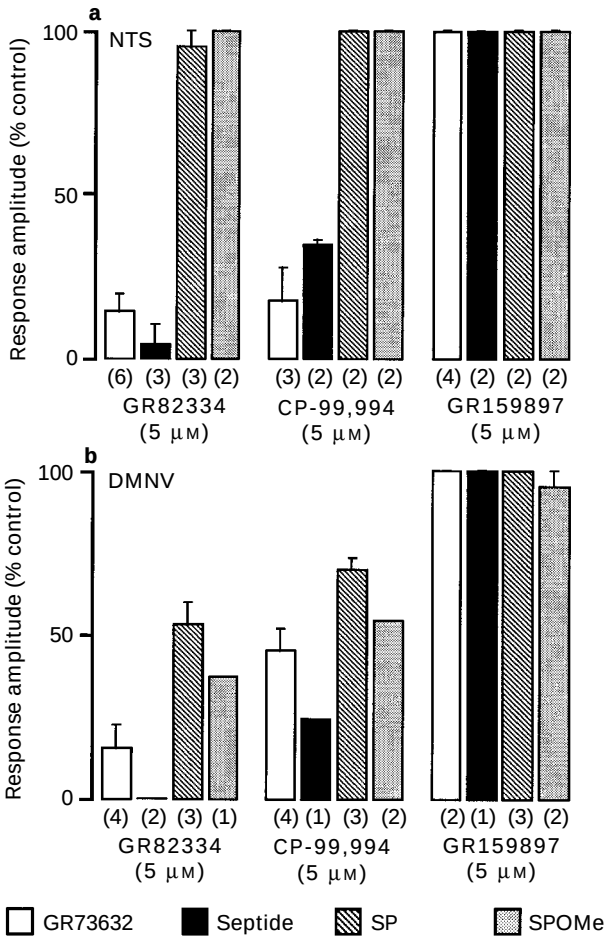

Figure 7 Summary of effects of NK_1 and NK_2 antagonists. (a) Mean percentage reduction of responses of NST neurones to GR73632 (50 nM), septide (50 nM), SP (500 nM) and SPOMe (500 nM) in the presence of the peptide and non-peptide NK_1 antagonists and the NK_2 antagonist, GR159897. (b) Corresponding values for DMNV neurones. Numbers of neurones tested are shown in parentheses.

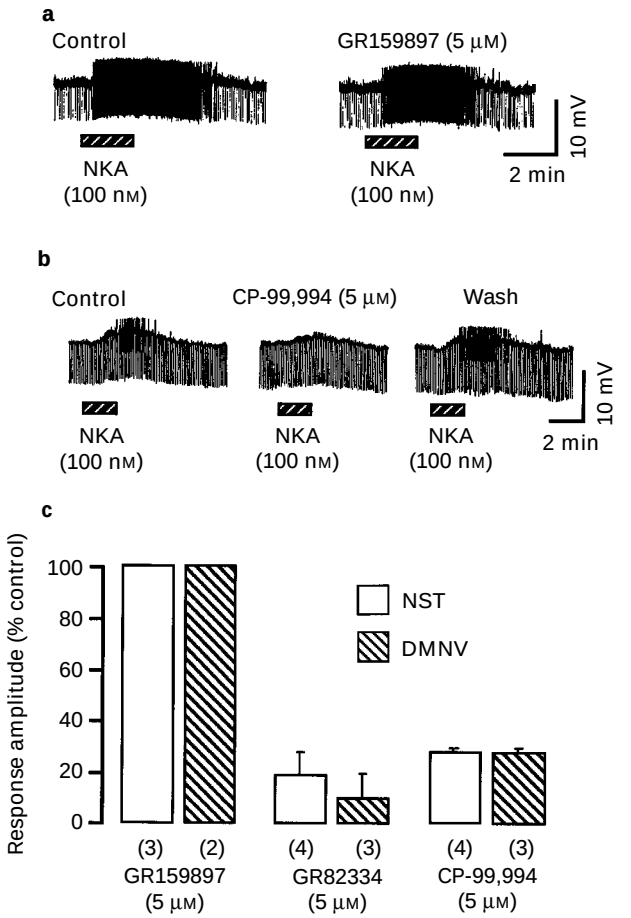

Figure 8 Pharmacology of responses to NKA. (a) Response of this DMNV neurone to NKA was unaffected by the NK_2-receptor antagonist. In contrast, (b) shows that the non-peptide NK_1-receptor antagonist was able to reduce responses to NKA. (c) Summary of the effects of the antagonists on responses of neurones in the NST and DMNV neurones to NKA (100 nM). Numbers of neurones tested are shown in parentheses.

$29.1 \pm 7.4\%$ in the DMNV ($n=9$) and the difference was not significant. Likewise the reduction of septide responses was not significantly different in the two nuclei ($18.8 \pm 8.7\%$ vs $8.3 \pm 10.2\%$, $n=5$ and 3, respectively) nor was the effect on NKA responses ($23.4 \pm 5.1\%$ vs $17.6 \pm 6.9\%$, $n=8$ and 7, respectively). In contrast, there was a significantly ($P<0.005$) greater effect of the antagonists on SP responses in the DMNV ($62.2 \pm 8.9\%$, $n=6$) compared with NST ($98.4 \pm 1.1\%$, $n=4$). Finally, a similar picture was seen with SPOMe (DMNV: $49.3 \pm 7.8\%$, $n=4$: NST: $95.8 \pm 4.9\%$, $n=3$: $P<0.001$).

NK_2-receptor antagonist Results with the NK_2-receptor antagonist were the same as in the NST. Thus, GR159897 (5 μM) failed to affect depolarizations evoked by GR73632 ($n=2$), septide ($n=1$), SP ($n=3$) or SPOMe ($n=3$). In addition, the NK_2 antagonist again had no effect on responses to NKA in two DMNV neurones. An example is shown in Figure 8a. Results from these antagonist studies are summarized in Figures 7b and 8c.

Discussion

Responses of NST and DMNV neurones to SP

The activation of both NST and DMNV neurones by SP seen in our experiments is in agreement with previous reports (Morin-Surun *et al*., 1984; Plata-Salaman *et al*., 1988; Jacquin *et al*., 1989; King *et al*., 1993; Martini-Luccarini *et al*., 1996). Our data suggested that SP was around five fold more effective at depolarizing neurones in the DMNV than in the NST (see later for Discussion). The excitatory effects of SP (and other tachykinins) were, in all likelihood, due to a direct postsynaptic action on the recorded neurones as they were not blocked by TTX. An alternative explanation would be that SP was causing the release of another excitatory transmitter from presynaptic terminals and it has become clear in recent years that a large proportion of spontaneous release of transmitter from presynaptic terminals is TTX resistant. The most likely candidate would be glutamate which has a widespread role as an excitatory transmitter in the NST and DMNV (see Andresen & Kunze, 1994). However, the excitatory responses to SP were unaffected by glutamate antagonists (data not shown) and indeed, TTX resistant, spontaneous release of glutamate in the NST is unaffected by SP (Maubach *et al*., 1995c).

Responses to other tachykinin receptor agonists

Neurones in both the NST and DMNV were also depolarized by other tachykinin receptor agonists but there were differences in relative potencies in the two nuclei. In the DMNV SP, SPOMe and septide were approximately equipotent and GR73632 was generally more effective. In the NST, while septide and GR73632 were equipotent they were nearly an

order of magnitude more effective than SP. A recent study (Martini-Luccarini *et al*., 1996) has examined responses of DMNV neurones to tachykinin agonists and also found that were there not huge differences in potency between SP, septide and another NK_1 agonist, [Sar^9,$Met(O_2)^{11}$]SP. Like us, they reported that NKA also depolarized neurones although, in contrast to us they found that a specific NK_2 agonist was also active. Furthermore, Martini-Luccarini *et al*. (1996) reported that an NK_2 antagonist could (partially) block the effects of NKA whereas we found blockade of NKA responses with an NK_1 but not an NK_2 antagonist. We suggest that NKA is acting as an NK_1 agonist in DMNV (and NST, see below) whereas Martini-Luccarini *et al*. (1996) propose that both NK_1 and NK_2 receptors can mediate excitation in the DMNV. We are not sure why our results differ from theirs although different agonists and antagonists have been used in the two studies and there is a need to cross-check results with the relevant compounds. The studies are in agreement that the lack of effect of NKB and, in our case senktide, showed that NK_3 receptors are unlikely to be involved in direct excitatory transmission in either the NST or DMNV even though there are dense concentrations of receptors to be found there (Ding *et al*., 1996).

The NK_1 agonists that we used do not appear to exhibit large differences in potency in other tissues (e.g. Dion *et al*., 1987; Hall & Morton, 1992) and our results in the DMNV agree with this. In the NST there were considerable differences between agonists and this was greater than expected for an action at the classical NK_1 receptor (Regoli *et al*., 1989). This discrepancy is unlikely to be due to a higher susceptibility to enzymatic destruction as a cocktail of peptidase inhibitors was included in all drug solutions. Peptidase inhibition did potentiate the responses to SP (data not shown), but the potency of SP never approached that of GR73632 or septide. Also, under similar conditions there were no great potency differences in the DMNV. Overall, our rank orders of potency, based on studies in other preparations (Regoli, 1988, 1989), suggest that the NK_1 receptor is the predominant receptor involved in excitation in both NST and DMNV although the discrepant potencies of SP and SPOMe compared with GR73632, septide and NKA raise some interesting possibilities.

A septide-sensitive NK_1 receptor in the brainstem?

The reduced potency of SP and SPOMe compared with septide and GR73632 on NST neurones could be the result of a lower affinity of the former compared with the latter for the same recognition site. They could also be indicative of the existence of an NK_1 receptor subtype. This failure of the NK_1-receptor antagonists to block responses to SP and SPOMe in the NST may be evidence in support of this view. There is evidence from studies in other tissues for an NK_1 receptor which is distinct from the 'classical' NK_1 receptor and which has been termed the septide-sensitive NK_1 receptor (see Glowinski, 1995). For example, Petitet *et al*. (1992) found that septide was equipotent to other NK_1-receptor agonists in contracting the guinea pig ileum but was a much weaker inhibitor of the binding of [^{3}H][Pro^9]SP sulfone (i.e. NK_1 binding). They also showed that the NK_1-receptor antagonist GR71251 (Petitet *et al*., 1992) was more effective at blocking concentrations induced by septide than those induced by [Pro^9]SP. These findings have been confirmed and extended to other preparations (Maggi *et al*., 1993; Meini *et al*., 1994). The anomalies in the pharmacology of septide, which were not easily accommodated in the pharmacology of the 'classical' NK_1 receptor, led to the proposal that two NK_1 receptor exist, one recognized with high affinity by SP and the other with a high affinity for septide. The work of Meini *et al*. (1995) in rat urinary bladder indicated that GR73632, the NK_1 agonist used extensively in the present study, was a preferential agonist at the septide-sensitive receptor.

It should be pointed out that there is an alternative view of the above findings, i.e. that septide and SP are acting at different sites on the same receptor, perhaps activating different G-proteins (see Glowinski, 1995). Whatever the interpretation, the rank order of agonist potencies we found in the NST clearly suggests that the septide-sensitive receptor mediates direct excitation of neurones in this nucleus. The presence of this receptor would also explain the potent effect of septide and GR73632 in the DMNV. The relatively greater potencies of SP and SPOMe in DMNV compared with NST and the results with NK_1-receptor antagonists in the two nuclei are more difficult to fit into the overall picture.

Effects of tachykinin antagonists

Results with GR159897 were straightforward. The failure of this compound to affect responses to NK_1 agonists in NST or DMNV demonstrate that the excitatory responses were not mediated by an action at NK_2 receptors. Its failure to block responses to NKA demonstrate that NK_2 receptors are also unlikely to be involved in the effect of the endogenous ligand (see below). The results with NK_1 antagonists, however, presented a confusing picture. Although effective against septide and GR73632 in both NST and DMNV, they were inactive against SP and SPOMe in NST but partially reduced responses to both agonists in the DMNV. It is difficult to explain these findings without postulating multiple sites of action of agonists/antagonists. The relatively similar potencies of SP, SPOMe, septide or GR73632 in the DMNV and the reduction of responses to all four by GR82334 and CP-99,994 could indicate that the depolarizations are mediated primarily by a combination of the 'classical' and the septide-sensitive receptor, both of which can be blocked by the antagonists. In the NST, however, the reduced potency of SPOMe and SP and lack of effect of the antagonists on responses to them might indicate the presence of an unknown 'atypical' site. It would be necessary to postulate that the classical NK_1 receptor would play a minor role as the antagonists were effective only against septide and GR73632.

What the atypical site would be is a matter for conjecture. It could represent a previously undescribed receptor for SP. There are both inter- and intraspecies differences in NK_1 receptors (e.g. Beresford *et al*., 1992; Clerc *et al*., 1992) and the antagonists used in our studies have a higher affinity for the human/guinea pig receptor than the rat/mouse NK_1 receptor (Snider *et al*., 1991). The concentrations of antagonists used (5 μM) should have been sufficient to block the action of SP at the rat receptor (McLean *et al*., 1993). It is interesting that a recent study (Lepre *et al*., 1996) has shown effects of SP on spinal motorneurones which are mimicked by an NK_1 agonist but are insensitive to blockade by NK_1 antagonists. In autoradiographic studies we have shown that the NST/DMNV area in the rat displays SP binding which is insensitive to displacement by CP-99,994 (Maubach *et al*., 1995a). This could conceivably represent the atypical SP site responsible for depolarization. An alternative explanation could be that the responses in the NST represent an anomalous effect of SP which is not mediated via a member of the tachykinin receptor family. There are indications that SP can interact with the nicotinic acetylcholine receptor (Min *et al*., 1994) and with receptors for the neuropeptide, bombesin (Jensen *et al*., 1984). We have no evidence for effects of SP being mediated via either receptor but these studies do indicate a promiscuity of the peptide and suggest that atypical actions are not without precedent.

Responses to NKA

NKA preferentially interacts with NK_2 receptors but neither NK_2 binding sites nor the mRNA encoding for the NK_2 receptor have been convincingly identified in the adult brain (Guard & Watson, 1991). Both NST and DMNV neurones were strongly depolarized by NKA but our studies with GR159897 and GR64349 provide convincing evidence that NK_2 receptors were not directly involved. Instead, the re-

sponses were readily blocked by the NK_1 receptor antagonists suggesting that the latter sites were the more likely target for NKA. A number of studies using various agonists and antagonists on spinal cord neurones have suggested that excitatory effects of NKA are mediated via the NK_1 rather than the NK_2 receptor (Guo *et al*., 1993; Hosoki *et al*., 1994; Otsuka *et al*., 1994) and similar conclusions were reached in a study of coeliac ganglion neurones (Zhao *et al*., 1995). On the basis of our results it would not be unreasonable to suggest that NKA rather than SP might be the endogenous ligand mediating tachykinin transmission, particularly in the NST. Furthermore, it may be that NKA could exert its effects via the septide-sensitive site on the postsynaptic neurones. In the DMNV, the situation may be different as the potencies of SP and NKA were more similar and the antagonists were effective against both endogenous peptides.

Relevance to emesis

CP-99,994 is an effective anti-emetic agent against a range of emetogenic stimuli and recent studies have speculated that the NK_1-receptor antagonists mediate their anti-emetic effects via a central site of action in the region of the solitary complex (Gardner *et al*., 1994; Watson *et al*., 1995). The present study provides a basis for this speculation and furthermore indicates that blockade of receptors within either the NST or DMNV or both could be involved in the anti-emetic effects of CP-99,994. We would suggest that NKA may play a more predominant role as the endogenous mediator in tachykinin pathways in the NST. Furthermore, as CP-99,994 inhibits the responses to NKA in the NST and DMNV, it is possible that CP-99,994 exerts its anti-emetic properties by inhibiting the action of NKA rather than SP and that this may occur via an action at the septide-sensitive receptor. It is possible that development of agents which specifically target this site or which can specifically interact with the effect of NKA at this site might prove a fruitful approach to anti-emetic treatment. If SP were the endogenous transmitter rather than NKA then our data would suggest that the anti-emetic effect of CP-99,994 would be unlikely to depend on blockade of tachykinin transmission in the NST but could be due to reduction of the motor output from the DMNV. This may indicate that the use of NK_1 antagonists at anti-emetics may be less attractive than initially thought. They could prevent vomiting by suppressing motor output without affecting nausea which would require also suppression of sensory input to the NST.

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References

AAPROS, M.S. (1991). 5-HT_3 receptor antagonists. An overview of their present status and future potential in cancer therapy-induced emesis. *Drugs*, **42,** 551–568.

ANDRESEN, M.C. & KUNZE, D.L. (1994). Nucleus tractus solitarius: gateway to neural circulatory control. *Am. Rev. Physiol.*, **56,** 93–116.

ANDREWS, P.L.R. (1994). $5HT_3$ antagonists and anti-emesis. In *5-HT_3 Receptor antagonists* ed. King, F.D., Jones, B. & Sanger, G.K. pp. 255–317. Boca Raton: CRC Press.

ANDREWS, P.L.R. & BHANDARI, P. (1993). Resiniferatoxin, an ultrapotent capsaicin analogue, has anti-emetic properties in the ferret. *Eur. J. Pharmacol.*, **32,** 779–806.

BAUDE, A., LANOIR, J., VERNIER, P. & PUIZILLOUT, J.J. (1989). Substance P-immunoreactivity in the dorsal medial region of the medulla in the cat: the effects of nodosectomy. *J. Chem. Neuronat.*, **2,** 67–81.

BERESFORD, I.J.M., IRELAND, S.J., STABLES, J.M., STUBBS, C.M., BALL, D., HAGAN, R.M. & BIRCH, P.J. (1992). Investigation into differences in tachykinin NK_1 receptors between and within species using a peptide and non-peptide NK_1 receptor antagonist. *Br. J. Pharmacol.*, **106,** P62.

BERMUDEZ, J., BOYLE, E.A., MINER, W.D. & SANGER, G.J. (1989). The anti-emetic potential of the 5-hydroxytryptamine-3 receptor antagonist, BRL 43694. *Br. J. Cancer*, **58,** 644–651.

BHANDARI, P. & ANDREWS, P.L.R. (1991). Preliminary evidence for the involvement of the putative 5-HT_4 receptor in zacopride and copper sulphate-induced vomiting in the ferret. *Eur. J. Pharmacol.*, **204,** 273–280.

BHANDARI, P., BINGHAM, S. & ANDREWS, P.L.R. (1992). The neuropharmacology of loperamide-induced emesis in the ferret: the role of the area postrema, vagus, opiate and 5-HT_3 receptors. *Neuropharmacology*, **31,** 735–742.

BORISON, H.L. & WANG, S.C. (1949). Functional localization of central coordinating mechanisms for emesis in cat. *J. Neurophysiol.*, **12,** 305–313.

BORISON, H.L. & WANG, S.C. (1953). Physiology and pharmacology of vomiting. *Pharmacol. Rev.*, **5,** 193–230.

BOUNTRA, C., BUNCE, K., DALE, T., GARDNER, C., JORDAN, C., TWISSELL, D. & WARD, P. (1993). Anti-emetic profile of a non-peptide neurokinin NK_1 receptor antagonist, CP-99,994 in ferrets. *Eur. J. Pharmacol.*, **249,** R3–R4.

BRADLEY, R.M. & SWEAZEY, R.D. (1990). *In vitro* intracellular recordings from gustatory neurones in rat solitary nucleus. *Brain Res.*, **508,** 168–171.

CARPENTER, D.O., BRIGGS, D.B. & STROMINGER, N. (1983). Response of neurons of canine area postrema to neurotransmitter and peptides. *Cell. Mol. Neurobiol.*, **3,** 113–126.

CARPENTER, D.O., BRIGGS, D.B. & STROMINGER, N. (1984). Peptide induced emesis in dogs. *Behav. Brain Res.*, **11,** 277–281.

CHIBA, T. & MURATA, Y. (1982). Substance P-like immunoreactive axon varicosities with synaptic junctions in the nucleus tractus solitarius of the rat. *Brain Res.*, **251,** 160–163.

CLERC, F.F., FOUCAULT, F., RIDOUX-SILLY, L. & FARDIN, V. (1992). Affinity and stability of some substance P agonists in rat and guinea-pig: a comparative study in central and peripheral tissues. *Neuropeptides*, **22,** 14.

DING, Y.Q., SHIGEMOTO, R., TAKADA, M., OHISHI, H., NAKANISHI, S. & MIZUNO, N. (1996). Localization of the neuromedin K receptor (NK_3) in the central nervous system of the rat. *J. Comp. Neurol.*, **364,** 290–310.

DION, S., D'ORLEANS-JUSTE, P., DRAPEAU, G., RHALEB, N.E., ROUISSI, N., TOUSIGNANT, C. & REGOLI, D. (1987). Characterisation of neurokinin receptors in various isolated organs by the use of selective agonists. *Life Sci.*, **41,** 2269–2278.

GARDNER, C.J., BOUNTRA, C., BUNCE, K.T., DALE, T.J., JORDAN, C.C., TWISSELL, D.J. & WARD, P. (1994). Anti-emetic activity of neurokinin NK_1 receptor antagonists is mediated centrally in the ferret. *Br. J. Pharmacol.*, **112,** 516P.

GILLIS, R.A., HELKE, C.J., HAMILTON, B.L., NORMAN, W.P. & JACOBOWITZ, D. (1980). Evidence that substance P is a neurotransmitter of baro- and chemoreceptor afferents in the nucleus of tractus solitarius. *Brain Res.*, **181,** 476–481.

GLOWINSKI, J. (1995). The 'septide-sensitive' tachykinin receptor: still an enigma. *TIPS*, **16,** 365–367.

GUARD, S. & WATSON, S.P. (1991). Tachykinin receptor types: classification and membrane signalling mechanisms. *Neurochem. Int.*, **18,** 149–165.

GUO, J.Z., YOSHIOKA, K., YANAGISAWA, M., HOSOKI, R., HAGAN, R.M. & OTSUKA, M. (1993). Depression of primary afferent-evoked responses by GR71251 in the isolated spinal cord of the neonatal rat. *Br. J. Pharmacol.*, **110,** 1142–1148.

HALL, J.M. & MORTON, I.K.M. (1992). Novel selective agonists and antagonists confirm neurokinin NK_1 receptors in guinea-pig vas deferens. *Br. J. Pharmacol.*, **102,** 511–517.

HELKE, C., JACOBOWITZ, D. & THOA, N. (1981). Capsaicin and potassium evoked substance P release from the nucleus tractus solitarius and spinal trigeminal nucleus *in vitro*. *Life Sci.*, **29,** 1779–1785.

HOSOKI, R., YANAGISAW, M., GUO, J.Z., YOSHIOKA, K., MACEHARA, T. & OTSUKA, M. (1994). Effects of RP67580, a tachykinin NK_1 receptor antagonist, on a primary afferent-evoked response of ventral roots in the neonatal rat spinal cord. *Br. J. Pharmacol.*, **113,** 1141 – 1146.

JACQUIN, T., DENAVIT-SAUBIE, M. & CHAMPAGNAT, J. (1989). Substance P and serotonin mutually reverse their excitatory effects in the rat nucleus tractus solitarius. *Brain Res.*, **502,** 214 – 222.

JENSEN, R.T., JONES, S.W., FOLKERS, K. & GARDNER, J.D. (1984). A synthetic peptide that is a bombesin receptor antagonist. *Nature*, **309,** 61 – 63.

KING, M.S., WANG, L. & BRADLEY, R.M. (1993). Substance P excites neurons in the gustatory zone of the rat nucleus tractus solitarius. *Brain Res.*, **619,** 120 – 130.

KNOX, A.P., STROMINGER, N.L., BATTLES, A.H. & CARPENTER, D.O. (1993). Behavioural studies of emetic sensitivity in the ferret. *Brain Res. Bull.*, **31,** 477 – 484.

LEPRE, M., OLPE, H.R. & BRUGGER, F. (1996). The effects of neurokinin-1 receptor agonists on spinal motorneurones of the neonatal rat. *Neuropharmacology*, **35,** 511 – 522.

LESLIE, R.A. (1985). Neuroactive substances in the dorsal vagal complex of the medulla oblongata: nucleus of the tractus solitarius, area postrema, and the dorsal motor nucleus of the vagus. *Neurochem. Int.*, **7,** 191 – 211.

LJUNGDAHL, A., HOKFELT, T. & NILSSON, G. (1978). Distribution of substance P-like immunoreactivity in the central nervous system of the rat. I. Cell bodies and nerve terminals. *Neurosciene*, **3,** 861 – 943.

MCLEAN, S., GANONG, A., SEYMOUR, P.A., SNIDER, R.M., ROSEN, T., BRYCE, D.K., LONGO, K.P., REYNOLDS, L.S., ROBINSON, G., SCHMIDT, A.W., SIOK, C. & HEYM, J. (1992). Pharmacology of CP-99,994; a non-peptide antagonist of the tachykinin NK_1 receptor. *Regul. Peptides*, **1,** S120.

MCLEAN, S., GANONG, A., SEYMOUR, P.A., SNIDER, R.M., DESAI, M.C., ROSEN, T., BRYCE, D.K., LONGO, K.P., REYNOLDS, L.S., ROBINSON, G., SCHMIDT, A.W., SIOK, C. & HEYM, J. (1993). Pharmacology of CP-99,994; a non-peptide antagonist of the tachykinin NK_1 receptor. *J. Pharmacol. Exp. Ther.*, **267,** 472 – 479.

MAGGI, C.A., PATACCHINI, R., MEINI, S. & GUILLIANI, S. (1993). Evidence for the presence of a septide-sensitive tachykinin receptor in the circular muscle of the guinea-pig ileum. *Eur. J. Pharmacol.*, **235,** 309 – 311.

MANTYH, P.W., GATES, T., MANTYH, C.R. & MAGGIO, J.E. (1989). Autoradiographic localisation and characterisation of tachykinin receptor binding sites in the rat brain and peripheral tissues. *J. Neurosci.*, **9,** 258 – 279.

MARTINI-LUCCARINI, F., REYNAUD, J.C. & PUIZILLOUT, J.J. (1996). Effects of tachykinins on identified dorsal vagal neurons: an electrophysiological study *in vitro*. *Neuroscience*, **71,** 119 – 131.

MAUBACH, K., GALE, J.D., BERRETTA, N. & JONES, R.S.G. (1995c). Whole-cell voltage clamp analysis of the effect of neurokinin A on rat nucleus tractus solitarius neurones *in vitro*. *J. Physiol.*, **489,** 18P.

MAUBACH, K., HAGAN, R.M. & JONES, R.S.G. (1994a). Characterisation of tachykinin receptors in the rat dorsal vagal motor nucleus. *Neuropeptides*, **26** (Suppl. 1), 47.

MAUBACH, K., HAGAN, R.M. & JONES, R.S.G. (1994b). Responses of neurones in the nucleus tractus solitarius to NK_1 receptor agonists. *Can. J. Phys. Pharmacol.*, **72,** 460.

MAUBACH, K., HAGAN, R.M. & JONES, R.S.G. (1995b). Electrophysiological characterisation of tachykinin receptors in the rat nucleus tractus solitarius *in vitro*: neurokinin A may act at an NK_1 receptor sub-type. *Br. J. Pharmacol.*, **114,** 88P.

MAUBACH, K.A., JONES, R.S.G., STRATTON, S.C. & GALE, J.D. (1995a). Autoradiographic distribution of substance P binding sites in the brainstem of the rat and ferret. *Br. J. Pharmacol.*, **116,** 249P.

MEINI, S. PATACCHINI, R. & MAGGI, C.A. (1994). Tachykinin NK_1 receptor subtypes in the rat urinary bladder. *Br. J. Pharmacol.*, **111,** 739 – 746.

MEINI, S., PATACCHINI, R., LECCI, A., POULOS, C., ROVERO, P. & MAGGI, C.A. (1995). GR73632 and [Glu(OBzl)^{11}]substance P are selective agonists for the septide-sensitive tachykinin NK_1 receptor in the rat urinary bladder. *Neuropeptides*, **28,** 99 – 106.

MIN, C.K., OWENS, J. & WEILAND, G.A. (1994). Characterisation of the binding of [^{3}H]substance P to the nicotinic acetylcholine receptor of torpedo electroplaque. *Mol. Pharmacol.*, **45,** 221 – 227.

MORILAK, D.A., MORRIS, M. & CHALMERS, J. (1988). Release of substance P in the nucleus tractus solitarius measured by *in vivo* microdialysis: response to stimulation of the aortic depressor nerves in rabbit. *Neurosci. Lett.*, **94,** 131 – 137.

MORIN-SURUN, M.P., JORDAN, D., CHAMPAGNAT, J., SPYER, K.M. & DENAVIT-SAUBIE, M. (1984). Excitatory effects of iontophoretically applied substance P on neurons in the nucleus tractus solitarius of the cat: lack of interaction with opiates and opioids. *Brain Res.*, **307,** 388 – 392.

MUSSAP, C.J., GERAGHTY, D.P. & BURCHER, E. (1993). Tachykinin receptors: a radioligand binding perspective. *J. Neurochem.*, **60,** 6.

NAKAYA, Y., KANEKO, T., SHIGEMOTO, R., NAKANISHI, S. & MIZUNO, N. (1994). Immunohistochemical localization of substance P receptor in central nervous system of the adult rat. *J. Comp. Neurol.*, **347,** 249 – 274.

OTSUKA, M., YOSHIOKA, K., YANAGISAWA, M., SUZUKI, H., ZHAO, F.Y., GUO, J.Z., HOSOKI, R. & KURIHARA, T. (1994). Use of NK_1 receptor antagonists in the exploration of physiological functions of substance P and neurokinin A. *Can. J. Phys. Pharm*, **73,** 903 – 907.

PETITET, F., SAFFROY, M., TORRENS, Y., LAVIELLE, S., CHASSAING, G., LOEUILLET, D., GLOWINSKI, J. & BEAUJOUAN, J.C. (1992). Possible existence of a new tachykinin receptor subtype in the guinea-pig ileum. *Peptides*, **13,** 383 – 388.

PLATA-SALAMAN, C.R., FUKADA, A., MINAMI, T. & OOMURA, Y. (1988). Substance P effects on the dorsal motor nucleus of the vagus. *Brain Res. Bull.*, **23,** 149 – 153.

REGOLI, D., BOUDON, A. & FAUCHERE, J.-L. (1994). Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.*, **46,** 4.

REGOLI, D., DRAPEAU, G., DION, S. & COUTURE, R. (1988). New selective agonists for neurokinin receptors: pharmacological tools for receptor characterisation. *Trends Pharmacol. Sci.*, **9,** 290 – 295.

REGOLI, D., DRAPEUA, G., DION, S. & D'ORLEANS-JUSTE, P. (1989). Receptors for substance P and related neurokinins. *Pharmacology*, **38,** 1 – 15.

SNIDER, R.M., CONSTANTINE, J.W., LOWE, J.A. III, LONGO, K.P., LEBEL, W.S., WOODY, H.A., DROZDA, S.E., DESAI, M.C., VINICK, F.J., SPENCER, R.W. & HESS, H.J. (1991). A potent non-peptide antagonist of the substance P (NK_1) receptor. *Science*, **251,** 435 – 437.

STROMINGER, N.L., KNOX, A.P. & CARPENTER, D.O. (1994). The connectivity of the area postrema in the ferret. *Brain Res. Bull.*, **33,** 33 – 47.

TATTERSALL, F.D., RYCROFT, W., HARGREAVES, R.J. & HILL, R.G. (1993). The tachykinin NK_1 receptor antagonist CP-99,994 attenuates cisplatin induced emesis in the ferret. *Eur. J. Pharmacol.*, **250,** R5 – R6.

TELL, F. & BRADLEY, R.M. (1994). Whole-cell analysis of ionic currents underlying the firing pattern of neurones in the gustatory zone of the nucleus tractus solitarii. *J. Neurophysiol.*, **71.** 479 – 492.

TER HORST, G.J. & STREEFLAND, C. (1994). Ascending projections of the solitary tract nucleus. In *Nucleus of the Solitary Tract*. ed. Robin, I. & Barraco, A., pp. 93 – 103. Boca Raton: CRC Press.

WATSON, J.W., GONSALVES, S.F., FOSSA, A.A., MCLEAN, S., SEEGER, T., OBACH, S. & ANDREWS, P.L.R. (1995). The antiemetic effects of CP-99,994 in the ferret and the dog: role of the NK_1 receptor. *Br. J. Pharmacol.*, **115,** 84 – 94.

ZHAO, F.Y., SAITO, K., YOSHIOKA, K., GUO, J.Z., MURAKOSHI, T., KONISHI, S. & OTSUKA, M. (1995). Subtypes of tachykinin receptors on tonic and phasic neurones in coeliac ganglion of the guinea pig. *Br. J. Pharmacol.*, **115,** 25 – 30.

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