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Potencies of agonists acting at tachykinin receptors in the oestrogen-primed rat uterus: effects of peptidase inhibitors

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Abstract

The uterotonic potencies of the naturally occurring mammalian tachykinins and the synthetic subtype-selective agonist analogues of these agents [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) and [Nle^{10}]neurokinin A-(4–10) (tachykinin NK_2 receptor-selective), [Sar^9,Met(O_2)^{11}]substance P (tachykinin NK_1 receptor-selective) and senktide (tachykinin NK_3 receptor-selective) were determined using preparations from oestradiol-treated rats. The endopeptidase 24.11 inhibitor, *N*-[*N*-[1-(*S*)-carboxyl-3-phenylpropyl]-(*S*)-phenylalanyl-(*S*)-isoserine (SCH 39370), potentiated responses to neurokinin A, neurokinin B and substance P, but not to [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) or senktide. [Nle^{10}]neurokinin A-(4–10) effects were potentiated by SCH 39370 with amastatin and those to [Sar^9,Met(O_2)^{11}]substance P were potentiated by SCH 39370 and captopril in combination. In the presence of optimal concentrations of peptidase inhibitors the relative order of agonist potency was: neurokinin A > substance P > neurokinin B for the naturally occurring mammalian tachykinins and [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) > [Nle^{10}]neurokinin A-(4–10) > [Sar^9,Met(O_2)^{11}]substance P > senktide for the synthetic tachykinin analogues. Thus, while a tachykinin NK_2 receptor predominates in the oestrogen-primed uterus, a tachykinin NK_1 receptor may also be present. The non-peptide tachykinin NK_3 receptor antagonist, SR 142801, did not antagonise the effects of senktide suggesting that tachykinin NK_3 receptors do not mediate its relatively minor effect on the uterus of the oestrogen-primed rat.

Keywords: Tachykinin receptor; Peptidase inhibitor; Oestrogen; Uterus; SR 142801

1. Introduction

We have previously reported experiments which indicate that the tachykinin NK_2 receptor is the predominant receptor mediating tachykinin-induced contraction of the longitudinally-arranged smooth muscle of the uterus of the oestrogen-primed rat (Pennefather et al., 1993; Fisher et al., 1993). However the order of agonist potency in the former study was neurokinin A > substance P > neurokinin B, raising the possibility that a tachykinin NK_1 receptor may also be present. Other investigators have, however, suggested the presence of a post-synaptic non-neuronal tachykinin NK_3 receptor in uterus following experiments in which senktide was used on tissues from untreated rats (Barr et al., 1991). In addition, it has recently been suggested that oestrogen negatively regulates expression of tachykinin NK_3 receptors in rat uterus (Pinto et al., 1997).

In the present study we have used a wider range of agonists at tachykinin receptors to probe further the nature of the tachykinin receptor subtypes present. Comparisons of tachykinin agonist potencies on uterine preparations are, however, confounded by differing susceptibilities of agonists to inactivation by endogenous peptidases, particularly endopeptidase 24.11 (EC 3.4.24.11) (Fisher et al., 1993). Thus we have conducted the present comparison of agonist potencies in the presence of appropriate peptidase inhibitors.

The aims of the present experiments were three-fold. The first was to determine the optimal concentration of the endopeptidase 24.11 inhibitor SCH 39370 (Sybertz et al., 1989) for potentiation of neurokinin A. The second was to determine the effects of this peptidase inhibitor, alone and in combination with the aminopeptidase inhibitor, amastatin, or the angiotensin converting enzyme inhibitor, captopril, on the potencies of the mammalian tachykinins substance P, neurokinin A and neurokinin B, of the tachykinin NK_2 subtype-selective agonists, [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) (Chassaing et al.,

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1991) and [Nle^{10}]neurokinin A-(4–10) (Regoli et al., 1988), the tachykinin NK_1 agonist [Sar^9,Met(O_2)^{11}]substance P (Regoli et al., 1988) and the tachykinin NK_3 agonist, [succinyl-Asp^{6}MePhe^8]substance P(6–11) (senktide) (Laufer et al., 1986). The third aim was to assess, using optimal combinations of peptidase inhibitors the relative potencies of these agonists in mediating contractions of uterus from oestrogen-primed rats. In addition, experiments have been performed using the non-peptide tachykinin NK_3 receptor antagonist SR 142801 ((*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamine) (Emonds-Alt et al., 1995) to determine whether it modified the action of the tachykinin NK_3 receptor agonist senktide in the oestrogen-primed rat uterus.

2. Materials and methods

2.1. Animals and tissue preparations

Mature virgin Sprague–Dawley rats (160–230 g) were pretreated with oestradiol-17β cypionate (20 μg/kg s.c.) 24 h before use. Rats were killed by stunning and cervical dislocation and four preparations were obtained from each animal by medial transection of uterine horns. These were set up, under a resting force of 1 g, for recording of contractile force of the longitudinally-arranged muscle layer using a Grass FT03 force transducer and a Grass79C polygraph. Preparations were maintained at 37°C, bubbled with 5% CO_2 in O_2, in a solution of the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4$, 1.1; KH_2PO_4, 1.18; $NaHCO_3$, 25; glucose, 11.66; $CaCl_2$, 1.9. The glass organ baths in which tissues were placed were coated with silicon (Coatasil, dimethyldichlorosilane, Searle) to prevent peptides adsorbing to the glass.

2.2. Agonist addition

Preparations were allowed to equilibrate for 60–90 min prior to the first agonist addition and 20 min was allowed between agonist doses. To avoid tachyphylaxis only one log concentration–response curve to a single agonist was constructed on any one tissue. Each concentration remained in contact with the tissue for 5 min. The tissue was then washed with 3–5 times the bath volume. Carbachol (100 μM) was applied at the end of each experiment so that the effects of the tachykinins could be expressed as a percentage of the response to this agonist.

2.3. Enzyme inhibitors

Log concentration–response curves to agonists were constructed in the absence and presence of enzyme inhibitors, alone or in combination. Enzyme inhibitors were allowed to equilibrate with the tissues for 30 min prior to the first addition of agonists and readded after each agonist concentration. Enzyme inhibitors tested include: the aminopeptidase inhibitor, amastatin (10 μM); the angiotensin converting enzyme inhibitor, captopril (10 μM) and the endopeptidase 24.11 inhibitor SCH 39370 (3–30 μM).

2.4. Antagonist addition

Three concentrations of senktide were added noncumulatively to two preparations from each rat. On one SR 142801 (30 nM) was then added and on the other the vehicle control, ethanol (final bath concentration 0.3%) was added. Following equilibration for 140 min, the additions of the three concentrations of senktide were repeated.

2.5. Data analysis

The area under the force–time curve, measured during the 5 min period that each concentration was in contact with the tissue, was used as the index of uterotonic action (Pennefather et al., 1993). Areas were measured from polygraph tracings using a digitising tablet and the SigmaScan software package (Jandel Scientific, Sausalito, CA, USA) and expressed as g · min. Responses were converted to a percentage of the corresponding response to carbachol (100 μM) and presented as mean ± S.E.M. and E_{max} was defined as the maximum response to an agonist, obtained from the curve.

To determine the effects of peptidase inhibitors on agonist potency mean log concentration–response curves were constructed. If regression lines over the linear range of concentration–response curves were parallel to those obtained in the absence of a peptidase inhibitor, a potency ratio with 95% confidence limits were obtained using the analysis outlined in Documenta Geigy (1970) as described previously (Orre et al., 1996). Shifts were considered significant when the 95% confidence limits did not include one. One-way analysis of variance and Student's unpaired and paired *t*-tests were also used ($P < 0.05$) for comparisons of responses obtained in the absence and presence of peptidase inhibitors and in the absence and presence of the antagonist SR 142801. The negative log of the concentration of each agonist giving 50% of the response to carbachol ± S.E.M., or the negative log of the agonist giving 50% of the maximum response to the agonist was determined using nonlinear regression analysis in the program GraphPad Prism Version 2.0.

2.6. Materials

The compounds used were: amastatin HCl (Sigma/Auspep); captopril (D-3-mercapto-2-methoxyl oxopropyl-L-proline, Squibb); carbamylcholine chloride (carbachol, Sigma); [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–

10) (a gift from S. Lavielle, Lab De Chimie Organique Biologique); neurokinin A (Auspep); neurokinin B (Auspep); [Nle^{10}]neurokinin A-(4–10) (Auspep); oestradiol-17β cypionate (Hoechst); [Sar^{9},Met(O$_2$)^{11}]substance P (Auspep); SCH 39370 (*N*-[*N*-[1-(*S*)-carboxyl-3-phenylpropyl]-(*S*)-phenyl-alanyl-(*S*)-isoserine) (a gift from E. Sybertz, Schering Plough); SR 142801 ((*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamine) (a gift from X. Emonds-Alt, Sanofi Recherche); substance P (Auspep) and [succinyl-Asp^{6}MePhe^{8}]substance P(6–11) (Senktide, Auspep). All other reagents used were of analytical grade.

Oestradiol-17β cypionate was dissolved in 0.1 ml absolute ethanol, made up to volume with peanut oil and stored in the dark. Captopril and carbachol were dissolved in distilled water. SCH 39370 was dissolved in Na_2CO_3 solution (0.1%) and diluted with distilled water. Senktide and [Nle^{10}]neurokinin A-(4–10) were dissolved in ammonia (0.1 M). SR 142801 was dissolved in absolute ethanol. All remaining compounds were dissolved in dilute hydrochloric acid (0.01 M). Amastatin stock solution (1 mM) was stored at 4°C, while standard solutions of other peptides (100 μM) were aliquoted into plastic vials and stored at −20°C.

3. Results

3.1. Effect of increasing concentrations of SCH 39370 on uterine responses to neurokinin A and [Lys^{5},MeLeu^{9},Nle^{10}]neurokinin A-(4–10)

Fig. 1A shows that log concentration–response curves to neurokinin A were potentiated to a similar extent by all three concentrations of SCH 39370 tested. SCH 39370 3 μM produced a 41.3-fold shift to the left (95% confidence limits = 20.6, 90.8; df = 49). SCH 39370 10 μM caused a 43.4-fold shift (95% confidence limits = 21.4, 96.6; df = 48) and SCH 39370 30 μM, a 44.8-fold shift (95% confidence limits = 23.7, 89.5; df = 44).

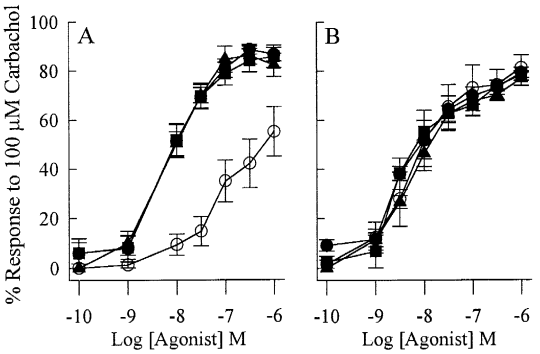

Fig. 1. Log concentration–response curves (A) to neurokinin A and (B) to [Lys^{5},MeLeu^{9},Nle^{10}]neurokinin A-(4–10) on uterine preparations from oestradiol-treated rats in the absence of enzyme inhibitors (○), in the presence of SCH 39370, 3 μM (●), 10 μM (■) and 30 μM (▲). Each point is the mean ± S.E.M., n = 3–9 animals.

In contrast, as can be seen in Fig. 1B, responses to [Lys^{5},MeLeu^{9},Nle^{10}]neurokinin A-(4–10) were unaffected by SCH 39370 3 μM (potency ratio = 1.3, 95% confidence limits = 0.6, 2.9, df = 40), 10 μM (potency ratio = 0.8, 95% confidence limits = 0.4, 1.6; df = 40) and 30 μM (potency ratio = 1.2, 95% confidence limits = 0.6, 2.5; df = 40).

3.2. Effects of peptidase inhibitor combinations

Amastatin 10 μM did not further enhance the response to neurokinin A beyond that obtained in the presence of SCH 39370 3 μM, (potency ratio = 0.9, 95% confidence limits = 0.6, 1.5; df = 33).

In the presence of SCH 39370 3 μM the log concentration–response curve to [Nle^{10}]neurokinin A-(4–10), was shifted 4.8-fold to the left (95% confidence limits = 2.9, 8.5; df = 55). While amastatin 10 μM was without effect on the log concentration–response curve to this agonist (potency ratio = 0.6, 95% confidence limits = 0.3, 1.0; df = 42), in the combined presence of the two peptidase inhibitors there was a 9.6-fold shift (95% confidence limits = 5.5, 19.4; df = 57).

Substance P was potentiated by SCH 39370 3 μM (potency ratio = 4.0, 95% confidence limits = 2.4, 6.8; df = 45) but not by captopril (potency ratio = 1.0, 95% confidence limits = 0.5, 1.8; df = 36). It was not possible to calculate a potency ratio for substance P in the presence of SCH 39370 (3 μM) and captopril (10 μM) in combination in comparison to that in the absence of enzyme inhibitors as the shift was not parallel (Fig. 2A). Student's unpaired *t*-tests showed no significant difference in the positions of curves to substance P in the presence of SCH 39370 (3 μM) alone compared with the response in the presence of both SCH 39370 (3 μM) and captopril (10 μM) ($P < 0.05$; df = 10) at all concentrations of substance P tested.

[Sar^{9},Met(O$_2$)^{11}]substance P, which acted as a partial agonist in these experiments (Fig. 2B, $E_{max} = 43.80 \pm 9.35$), was not significantly potentiated by SCH 39370 3 μM (potency ratio = 2.6, 95% confidence limits = 0.8, 9.9; df = 51) or by captopril (potency ratio = 1.0, 95% confidence limits = 0.4, 2.2, df = 56) alone. In combination, these inhibitors produced a 3.8-fold shift in the response to this agonist (95% confidence limits = 1.3, 9.9; df = 51).

The effects of neurokinin B were biphasic (Fig. 2C) and the potency ratios in the presence of SCH 39370 3 μM, amastatin 10 μM and the combination of the two inhibitors were 2.8, 0.4 and 4.1 (with 95% confidence limits of 1.1, 9.2, 0.1, 1.3 and 0.6, 11.2), (df = 63, 63 and 75), respectively. Like [Sar^{9},Met(O$_2$)^{11}]substance P, senktide

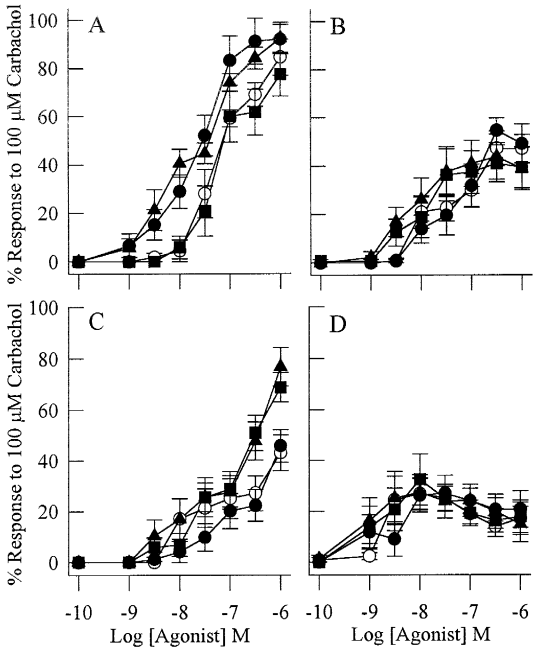

Fig. 2. Log concentration–response curves (A) to substance P, (B) to [Sar^9,Met(O$_2$)^{11}]substance P, (C) to neurokinin B and (D) to senktide on uterine preparations from oestradiol-treated rats in the absence of enzyme inhibitors (○), in the presence of SCH 39370, 3 μM (●), in the presence of captopril, 10 μM (A) and (B) or amastatin, 10 μM (C) and (D) (■) and in the presence of SCH 39370, 3 μM and captopril, 10 μM (A and B) or amastatin, 10 μM (C and D) (▲). Each point is the mean ± S.E.M., $n = 5$–6 animals.

acted as a partial agonist (Fig. 2D, $E_{max} = 26.89 \pm 6.21$) and one-way analysis of variance showed that SCH 39370 and captopril alone and in combination were without effect on its potency.

3.3. Order of agonist potency

The mean (± S.E.M.) negative log concentrations of agonists giving 50% of the response to carbachol (100 μM) in the presence of an optimal enzyme concentration were: neurokinin A, 7.84 ± 0.10; [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10), 7.39 ± 0.04; [Nle^{10}]neurokinin A-(4–10), 6.98 ± 0.05; substance P, 7.62 ± 0.04; [Sar^9,Met(O$_2$)^{11}]substance P, 6.12 ± 0.05 and neurokinin B, 6.40 ± 0.05. A value could not be calculated for senktide as the maximum response observed for senktide was less than 50% of the response to carbachol. The negative log concentration giving 50% of the maximum response for [Sar^9,Met(O$_2$)^{11}]substance P was 8.01 ± 0.03 and for senktide was 9.10 ± 0.52. The overall order of agonist potency in the presence of optimal enzyme inhibitor combinations was: neurokinin A > substance P > neurokinin B for the naturally occurring mammalian tachykinins; and [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) > [Nle^{10}]neurokinin A-(4–10) > [Sar^9,Met(O$_2$)^{11}]substance P > senktide for the synthetic tachykinin analogues.

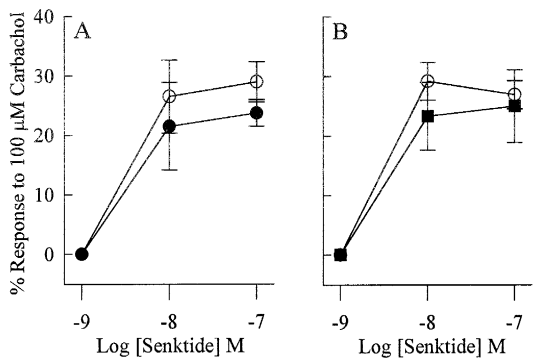

Fig. 3. Log concentration–response curves to senktide on uterine preparations from oestradiol-treated rats. Responses were obtained (A) in the absence (○) and presence (●) of ethanol (0.3%) and (B) in the absence (○) and presence (■) of SR 142801 (30 nM). Each point is the mean ± S.E.M., $n = 4$ animals.

3.4. Effects of SR 142801 on responses to senktide

The responses to the three concentrations of senktide tested were unaffected by either the vehicle control (ethanol, final bath concentration 0.3%), as seen in Fig. 3A, or by SR 142801 (30 nM), as seen in Fig. 3B. This lack of effect was confirmed by Student's paired *t*-tests ($P < 0.05$; df = 3).

4. Discussion

In the present study, maximum potentiation of the effects of neurokinin A occurred with SCH 39370 3 μM, and the extent of this potentiation was approximately twice that we observed previously using phosphoramidon 10 μM (Fisher et al., 1993). The tachykinin NK$_2$-selective agonist [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) was unaffected by the addition of SCH 39370 in concentrations up to 30 μM, a finding consistent with the results previously reported using phosphoramidon (Fisher et al., 1993). Thus, we have confirmed that this tachykinin NK$_2$-selective agonist is stable and that while the inclusion of endopeptidase 24.11 inhibitors in the bathing medium is crucial for accurate estimation of the potency of neurokinin A in the rat uterus such inhibitors need not be present when [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) is used as the agonist. Responses to the other tachykinin NK$_2$-selective agonist used in this study ie. [Nle^{10}]neurokinin A-(4–10), were potentiated in the presence of SCH 39370 (3 μM), indicating that this agonist is a substrate for endopeptidase 24.11. The potentiation observed (≈ 5-fold) was less than that observed for neurokinin A (≈ 40-fold) suggesting that it is less susceptible to degradation than neurokinin A. This is consistent with reports of investigators using other tissues (Brokaw and White, 1994). There was an additional potentiation of the response to [Nle^{10}]neurokinin A-(4–10)

(100 nM) in the presence of SCH 39370 when amastatin (10 μM) was also added, indicating that there may be some aminopeptidase cleavage of the N-terminal Asp group in this analogue to a less potent hexapeptide. In contrast, no additional potentiation of the effects of neurokinin A occurred in the presence of the aminopeptidase inhibitor amastatin, suggesting that responses to neurokinin A are not affected by amastatin-sensitive aminopeptidases. This may be because aminopeptidase-M hydrolyses the N-terminal of neurokinin A, between His^1–Lys^2, Lys^2–Thr^3 and Thr^3–Asp^4 (Hooper et al., 1985; Hooper and Turner, 1985, 1986), leaving neurokinin A (4–10). This structure has previously been shown to be sufficient for tachykinin NK_2 receptor activation (Rovero et al., 1989; Regoli et al., 1990).

Responses to neurokinin B were also potentiated by SCH 39370, 3 μM. This is consistent with reports this tachykinin is a substrate for endopeptidase 24.11 (Hooper and Turner, 1985). There was no additional potentiation of its effects by amastatin, 10 μM. Our finding that the potency of the tachykinin NK_3-selective agonist, senktide, was unaffected by the presence of enzyme inhibitors at all concentrations tested confirms reports of previous workers that senktide is metabolically stable (Laufer et al., 1986; Wormser et al., 1986).

Responses to substance P were potentiated by SCH 39370 (3 μM), but captopril (10 μM), was without effect on the response to substance P. It has been suggested that in the presence of endopeptidase 24.11, angiotensin converting enzyme cannot compete successfully for available substance P because the K_{cat} for degradation of substance P by angiotensin converting enzyme is considerably less than the K_{cat} for degradation of substance P by endopeptidase 24.11 (Nau et al., 1986). In contrast to substance P, responses to [Sar^9,$Met(O_2)^{11}$]substance P while not significantly affected by captopril (10 μM) or SCH 39370 (3 μM) alone, was potentiated 4-fold in the presence of both enzyme inhibitors. It has previously been suggested that the Sar^9 substitution may possibly protect substance P from the action of some endopeptidases (Regoli et al., 1988).

The results of these experiments indicate that endopeptidase 24.11 plays a key role in inactivating tachykinins in rat uterus and emphasise the need to include peptidase inhibitors in functional studies of tachykinin receptor subtypes in this tissue when using substrates for this enzyme.

The order of agonist potencies in the presence of optimal enzyme inhibitor combinations was: neurokinin A > substance P > neurokinin B for the naturally occurring mammalian tachykinins and [Lys^5,$MeLeu^9$,Nle^{10}]neurokinin A-(4–10) > [Nle^{10}]neurokinin A-(4–10) > [Sar^9,$Met(O_2)^{11}$]substance P > senktide for the synthetic tachykinin analogues. This is consistent with our previous reports that receptors mediating contraction of the oestrogen-primed rat uterus are predominantly of the tachykinin NK_2 subtype (Pennefather et al., 1993; Fisher et al., 1993) and, in addition, indicates that there may be a subpopulation of tachykinin NK_1 receptors present, as we have recently suggested on the basis of an investigation using subtype-selective non-peptide neurokinin antagonists (Fisher and Pennefather, 1996).

Other investigators have reported the presence of a non-neuronal tachykinin NK_3 receptor in rat uterus (Barr et al., 1991). The results obtained in this study in which the non-peptide tachykinin NK_3 antagonist, SR 142801 was without effect on the response to senktide suggest that the tachykinin NK_3 receptors blocked by SR 142801 do not have a significant role in mediating contractions of the uterus of oestrogen-primed rats.

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References

Barr, A.J., Watson, S.P., Lopez Bernal, A., Nimmo, A.J., 1991. The presence of NK_3 tachykinin receptors on rat uterus. Eur. J. Pharmacol. 203, 287–290.

Brokaw, J.J., White, G.W., 1994. Differential effects of phosphoramidon and captopril on NK_1 receptor-mediated plasma extravasation in the rat trachea. Agents Actions 42, 34–39.

Chassaing, G., Lavielle, S., Loeuillet, D., Robilliard, P., Carruette, A., Garret, C., Beaujouan, J.C., Saffroy, M., Petitet, F., Torrens, Y., Glowinski, J., 1991. Selective agonists of NK_2 binding sites highly active on rat portal vein (NK_3 bioassay). Neuropeptides 19, 91–95.

Emonds-Alt, X., Bichon, D., Ducoux, J.P., Heaulme, M., Miloux, B., Poncelet, M., Proietto, V., Ven Broeck, D., Vilian, P., Neliat, G., Soubrie, P., Le Fur, G., Breliere, J.-C., 1995. SR 142801, the first potent non-peptide antagonist of the tachykinin NK_3 receptor. Life Sci. 56, PL27–32.

Fisher, L., Pennefather, J.N., 1996. NK_1 and NK_2 receptors mediate contractile responses to tachykinins in the uterus of the oestrogen-primed rat. Proc. Aust. Physiol. Pharmacol. Soc. 27, 205P.

Fisher, L., Pennefather, J.N., Hall, S., 1993. Tachykinin receptors in the rat isolated uterus. Regul. Pept. 46, 396–398.

Hooper, N.M., Turner, A.J., 1985. Neurokinin B is hydrolysed by synaptic membranes and by endopeptidase 24.11 ('enkephalinase') but not by angiotensin converting enzyme. FEBS Lett. 190, 133–136.

Hooper, N.M., Turner, A.J., 1986. The hydrolysis of neurokinins A and B by cell-surface peptidases. Biochem. Soc. Trans. 14, 348–349.

Hooper, N.M., Kenny, J., Turner, A.J., 1985. Neurokinin A (substance K) is a substrate for endopeptidase 24.11 but not for peptidyl dipeptidase A (angiotensin converting enzyme). Biochem. J. 231, 357–361.

Laufer, R., Gilon, C., Chorev, M., Selinger, Z., 1986. Characterisation of a neurokinin B receptor site in rat brain using a highly selective radioligand. J. Biol. Chem. 261, 10257–10263.

Nau, R., Schafer, G., Deacon, C., Cole, T., Agoston, D.V., Conlon, J.M., 1986. Proteolytic inactivation of substance P and neurokinin A in the longitudinal muscle layer of guinea-pig small intestine. J. Neurochem. 47, 856–864.

Orre, M., Pennefather, J.N., Story, M.E., Haynes, J.M., 1996. The effects of P_2 purinoceptor agonists on the isolated portal vein of the guinea pig. Eur. J. Pharmacol. 316, 229–236.

Pennefather, J.N., Zeng, X.-P., Gould, D., Hall, S., Burcher, E., 1993. Mammalian tachykinins stimulate rat uterus by activating NK_2 receptors. Peptides 14, 169–174.

Pinto, F.M., Magraner, J., Ausina, P., Anselmi, E., Martin, J.D., Candenas, M.L., 1997. Regulation by oestrogens of tachykinin NK_3 receptor expression in the rat uterus. Eur. J. Pharmacol. 324, 125–127.

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., Rhaleb, N.-E., Dion, S., Tousignant, C., Rouissi, N., Jukic, D., Drapeau, G., 1990. Neurokinin A. A pharmacological study. Pharmacol. Res. 22, 1–14.

Rovero, P., Pestellini, V., Rhaleb, N.-E., Dion, D., Rouissi, N., Tousignant, C., Telemaque, S., Drapeau, G., Regoli, D., 1989. Structure-activity studies of neurokinin A. Neuropeptides 13, 263–270.

Sybertz, E.J., Chui, P.J.S., Vemulapalli, S., Pitts, B., Foster, C.J., Watkins, R.W., Barnett, A., Haslanger, M.F., 1989. SCH 39370, a neutral metalloendopeptidase inhibitor, potentiates biological responses to atrial natriuretic factor and lowers blood pressure in desoxycorticosterone acetate-sodium hypertensive rats. J. Pharmacol. Exp. Ther. 250, 624–631.

Wormser, U., Laufer, R., Hart, Y., Chorev, M., Gilon, C., Selinger, Z., 1986. Highly selective agonists for substance P receptor subtypes. EMBO J. 5, 2805–2808.