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NK_2 receptors mediate tachykinin-induced contractions of rat uterus during the oestrous cycle

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Abstract

We examined tachykinin-induced contractions of uteri from rats during the oestrous cycle. The potencies of substance P, neurokinin A, neurokinin B and the tachykinin NK_2 receptor-selective agonist, [Lys^5, $MeLeu^9$, Nle^{10}] neurokinin A-(4–10), and of the non-peptide tachykinin NK_1, NK_2 and NK_3 receptor antagonists (*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl}-4-phenyl-1-azonia-bicyclo[2.2.2]octane (SR 140333), (*S*)-*N*-methyl-*N* [4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide (SR 48968) and (*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide (SR 142801), were examined. The relative agonist potencies, i.e., [Lys^5, $MeLeu^9$, Nle^{10}] neurokinin A-(4–10) ≥ neurokinin A > neurokinin B ≥ substance P were similar in preparations from rats in dioestrus/metoestrus and those in proestrus/oestrus. Apparent pK_B values for SR 48968 versus neurokinin A and [Lys^5, $MeLeu^9$, Nle^{10}] neurokinin A-(4–10), were 9.9 and 9.2, respectively, indicating activation of an NK_2 receptor. SR 140333 (10 nM) produced only a small rightward shift of the log concentration–response curve to substance P. SR 48968 (3 nM), but not SR 142801 (100–300 nM) reduced the effect of neurokinin B. These data indicate that in the rat tachykinin-induced contractions of the uteri during the oestrous cycle are mediated primarily by tachykinin NK_2 receptors, and that fluctuations in ovarian hormonal levels during the oestrous cycle have little influence on the uterine response to tachykinins.

Keywords: Tachykinin NK_2 receptors; Non-peptide tachykinin receptor antagonist; Uterine contraction; Oestrous cycle

1. Introduction

Substance P and neurokinin A coexist with calcitonin gene-related peptide in capsaicin-sensitive primary afferent neurones supplying the reproductive tract of the female rat (Papka and Traurig, 1988; Papka et al., 1985; Shew et al., 1991; Traurig et al., 1991; Amira et al., 1995). Capsaicin-sensitive neurones, as well as relaying information to the central nervous system, may release peptide neurotransmitters from their efferent terminals and collaterals to produce effects on peripheral tissues (Lembeck and Holzer, 1979; Maggi and Meli, 1988). It is, therefore, possible that these neurotransmitters may play a role in the regulation of uterine function, for example in inducing myometrial contractility. In fact it is now well-established that substance P and neurokinin A produce contractions of the longitudinally arranged smooth muscle layer of the uterus from oestrogen-primed rats (Pennefather et al., 1993; Fisher and Pennefather, 1997, 1998; Fisher et al., 1993; Magraner et al., 1997, 1998).

There are three major tachykinin receptor subtypes, NK_1, NK_2 and NK_3 (Henry, 1986). The studies cited above all indicate that the tachykinin NK_2 receptor subtype plays a major role in mediating uterotonic responses to tachykinins in the oestrogen-primed rat. Some involvement of the tachykinin NK_1 receptor has also been proposed (Fisher and Pennefather, 1997; Magraner et al., 1998); this receptor subtype is known to be expressed in the uterus of the oestrogen-primed rat (Magraner et al., 1998). Messenger RNA for the tachykinin NK_3 receptor is also expressed in the oestrogen-primed uterus, but in very low levels (Pinto et al., 1997), and studies thus far indicate that this receptor has minimal involvement in mediating contractions of uteri from oestrogen-primed rats (Fisher and Pennefather, 1997; Magraner et al., 1998).

Although there has recently been considerable investigation of the tachykinin receptors mediating uterine con-

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traction in tissues from the oestrogen-primed rat, little information exists about the actions of tachykinins in uterine tissues taken from rats during the oestrous cycle. In a study that predated the studies of tachykinin actions in uterus from oestrogen-primed animals, Barr et al. (1991) inferred the presence of tachykinin NK_3 receptors mediating contractions of uterine tissues taken from rats that had not been treated with oestrogen. They also proposed that the expression of the tachykinin NK_1 receptor might be ovarian cycle stage-dependent. Cycle stage was not, however, determined in their study.

The main aims of this study were to examine the actions of tachykinins in tissues taken from rats at known stages of the 4-day oestrous cycle, and to determine whether, as in the oestrogen-primed rat, uterine contractions in untreated rats were mediated predominantly by the tachykinin NK_2 receptor subtype. Accordingly, we have (1) determined whether the relative uterotonic potencies of the mammalian tachykinins differ at different stages of the oestrous cycle, and (2) estimated the apparent pK_B values for the non-peptide tachykinin NK_1, NK_2 and NK_3 receptor antagonists versus the tachykinins in tissues taken from rats that had not received oestrogen treatment. The antagonists used were ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl}-4-phenyl-1-azonia-bicyclo[2.2.2]octane, chloride) (SR 140333, NK_1 receptor-selective; Emonds-Alt et al., 1993); ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) (SR 48968; NK_2 receptor-selective; Emonds-Alt et al., 1992), and ((*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide) (SR 142801, NK_3 receptor-selective; Emonds-Alt et al., 1995).

2. Methods

2.1. Animals and tissue preparations

Mature female virgin Sprague–Dawley rats (200–250 g) were housed at 22°C with a photoperiod of 12 h light and 12 h dark. The rats had access to rodent chow and water ad libitum. Prior approval of animal experimentation was obtained from the Standing Committee of Ethics in Animal Experimentation of Monash University. Animal Ethics no.: 94/060.

Rats were killed by stunning and cervical dislocation between 9:00 AM and 12:00 noon. Vaginal smears were then taken to allow subsequent microscopic examination to determine cycle stage. Slides were air-dried and fixed with methanol prior to staining with Giemsa stain diluted in distilled water (1:10). The abdomen was opened and both uterine horns were carefully removed and separated from connective tissue. These were placed in a Petri dish containing modified Krebs' Henseleit solution of the following composition: (mM): NaCl 118, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.1, KH_2PO_4 1.18, $NaHCO_3$ 25, glucose 11.66, $CaCl_2$ 1.9. Each uterine horn was medially transected (approximately 1–1.5 cm) providing four preparations from each rat. Each preparation was mounted in a 5 ml organ bath that contained warm (37°C) aerated modified Krebs' Henseleit solution and bubbled with 5% CO_2 in O_2. The organ baths had previously been coated with silicon ('coatasil', dimethyldichlorosilane, Searle) to prevent adsorption of the peptides to the glass. The preparations were set up under a resting force of 1 g, for measurement of contractile force of the longitudinally arranged muscle layer using a Grass FT03 force transducer. The force developed was recorded via a MACLAB data acquisition system and a LC111 computer.

2.2. Agonist studies

Log concentration–response curves to neurokinin A, neurokinin B, substance P and the tachykinin NK_2 receptor-selective agonist, [Lys^5, $MeLeu^9$, Nle^{10}] neurokinin A-(4–10) (Chaissang et al., 1991) were constructed. In all experiments with neurokinin A, substance P and neurokinin B the neutral endopeptidase inhibitor, *N*-[*N*-[1-(*S*)-carboxyl-3-phenylpropyl]-(*S*)-phenyl-alanyl-(*S*)-isoserine (SCH 39370, 3 μM) (Sybertz et al., 1989) was included prior to constructing concentration–response curves and was re-added to the baths each time the tissues were washed. Since [Lys^5, $MeLeu^9$, Nle^{10}] neurokinin A-(4–10) is not inactivated by this enzyme (Fisher and Pennefather, 1997), the peptidase inhibitor was not included in experiments using this agonist. Captopril (10 μM) was also present in the bathing medium when log concentration–response curves to substance P were constructed. In a subset of experiments amastatin (30 μM), an aminopeptidase inhibitor, was included in the bathing medium when log concentration–response curves to neurokinin A were constructed.

Preparations were allowed to equilibrate for 60 min before initial agonist addition. Each agonist concentration remained in contact with the tissue for 5 min. The tissue was then washed with three times the bath volume and the next concentration added after a further 15 min. Only one discrete concentration–response curve was obtained from each preparation; the concentration progression ratio was half a log unit. Only one agonist was applied to any one preparation from each animal.

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. Tissues were weighed at the end of each experiment so that the force developed (g min) could be normalised (g min/mg tissue weight). Responses to agonists were expressed as a percentage of that to methacholine (0.1 mM; found to be the concentration to cause a maximum response).

2.3. Antagonist studies

Log concentration–response curves were constructed in the absence or presence of antagonists. The experimental design was such that at least one preparation from each rat acted as a vehicle control for each agonist/antagonist combination used and the other received the related agonist/antagonist combination. Each agonist/antagonist combination was tested on only one of the four preparations obtained from each animal. Peptidase inhibitors were present as outlined above.

Tissues were preincubated with the tachykinin antagonists: SR48968 (3 nM), SR140333 (10 nM) or SR142801 (100 or 300 nM), or vehicle (0.0003–0.03% ethanol), for 120 min before commencing the agonist concentration–response curve. The prolonged incubation time was used as the inhibitory effects of these antagonists were reported to be slow to reach equilibrium (Emonds-Alt et al., 1992, 1993, 1995; Croci et al., 1995).

2.4. Data analysis

2.4.1. Determination of potencies of agonists

Nonlinear regression analysis was undertaken using Graphpad PRISM software. When maximal responses to agonists were obtained agonist potencies were expressed as pD_2 values. Since maximum contractions to substance P and neurokinin B were not reached with the highest concentrations of peptide available, agonist potencies were expressed as the negative log of the concentration producing 50% of the response to methacholine 0.1 mM (pEC_{50}).

2.4.2. Determination of apparent pK_B values for antagonists

To determine whether each pair of log concentration–response curves constructed in tissues from each animal in the absence or presence of vehicle or antagonist was parallel, Hill slopes of each curve were estimated using non-linear regression analysis. When these slopes did not differ significantly as assessed by Student's paired *t*-tests, it was assumed that the curves did not significantly deviate from parallelism, and estimates of EC_{50} values, assuming a similar maximum response, were obtained to determine concentration ratios. The significances of the differences in the EC_{50} values obtained in the presence and absence of antagonist were also determined by Student's paired *t*-tests. The level of significance accepted was $P < 0.05$. The mean estimates of apparent dissociation constants (K_B) for each concentration of antagonist were calculated from these estimates using the equation: K_B = antagonist concentration/(concentration ratio − 1) (Furchgott, 1972). K_B values were converted to negative logarithms and expressed as apparent pK_B values.

2.5. Drugs

The drugs used were: amastatin HCl (Sigma/Auspep); captopril (D-3-mercapto-2-methoxyl oxopropyl-L-proline, Squibb); acetyl-β-methacholine chloride (methacholine, 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) SCH 39370 (*N*-[*N*-[1-(*S*)-carboxyl-3-phenylpropyl]-(*S*)-phenyl-alanyl-(*S*)-isoserine; a gift from Schering) and substance P (Auspep).

SR 140333 ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl]ethyl}-4-phenyl-1-azonia-bicyclo[2.2.2]octane, chloride), SR 48968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) and SR 142801 ((*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide), all gifts from Sanofi Recherche, were used. These drugs were dissolved in ethanol at 1 mM, and diluted in Krebs' solution.

Stock solutions of methacholine (10 mM) and captopril (10 mM) were dissolved and diluted in distilled water. Stock solutions of SCH 39370 (1 mM) were dissolved in 1% Na_2CO_3. Neurokinin A and [Lys^5, MeLeu^9, Nle^{10}] neurokinin A-(4–10) were dissolved in 0.01 M HCl and diluted in Krebs' solution; substance P was dissolved in 0.1 M HCl and neurokinin B in 0.1 M ammonia and then diluted in distilled water.

All other reagents used were of analytical grade.

3. Results

3.1. Effects of methacholine

There was no regional variation in the response (g min/mg tissue weight) to the reference agonist methacholine 0.1 mM in the four segments of rat uterus used (one-way analysis of variance, $P > 0.05$, $n = 10$). Nor was there any significant difference in the mean response of preparations taken from rats in proestrus/oestrus on the one hand ($n = 21$) and those from rats in metoestrus/dioestrus ($n = 12$); (Student's unpaired *t*-test, $P > 0.05$).

3.2. Effects of amastatin on responses to neurokinin A

The aminopeptidase inhibitor, amastatin 30 μM, was without effect on responses to neurokinin A. Thus the pD_2 value for neurokinin A in the absence of amastatin was 7.34 ± 0.18 ($n = 3$) and in its presence 7.23 ± 0.11 ($n = 3$). These experiments were conducted in the presence of the endopeptidase 24.11 inhibitor, SCH 39370 (3 μM).

3.3. Comparison of the effects of agonists on preparations from rats in proestrus / oestrus with those on preparations from rats in metoestrus / dioestrus

The potencies of agonists were not significantly different in the tissues from the two groups of animals (Fig. 1

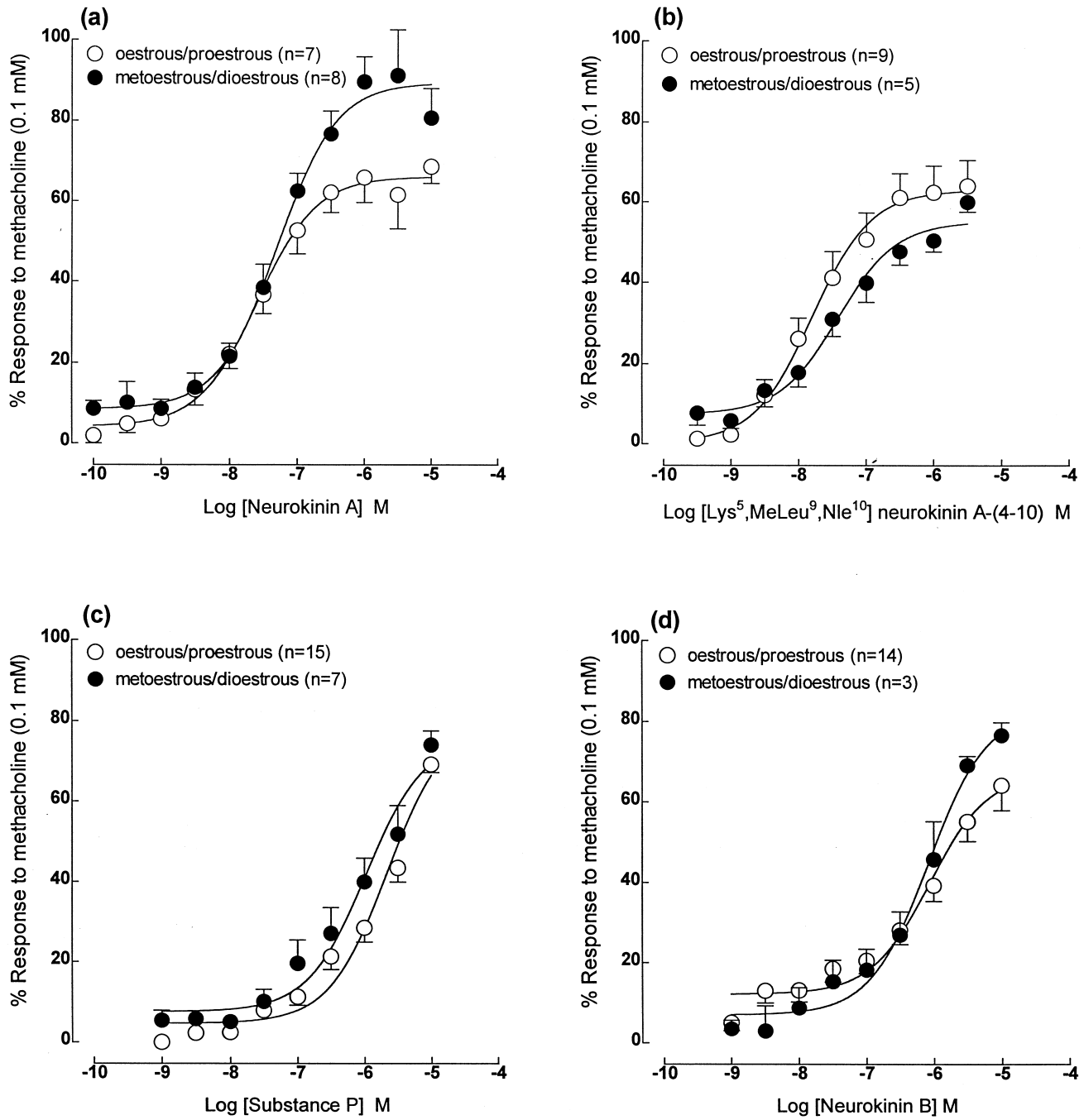


Fig. 1. Log concentration–response curves to (a) neurokinin A, (b) [Lys^5, MeLeu^9, Nle^{10}] neurokinin A-(4–10), (c) substance P and (d) neurokinin B, constructed in tissues from rats in proestrus/oestrus (open symbols) and in those from rats in dioestrus/metoestrus (closed symbols). The results are expressed as percentages of the response to methacholine 0.1 mM; each point represents mean ± S.E.M, n = the number of animals in which each agonist was tested.

and Table 1). pD_2 values and pEC$_{50}$ values are shown in Table 1. The maximum response to neurokinin was, however, significantly greater in preparations from rats in metoestrus/diestrus than in those in proestrus/oestrus (Table 1). The rank order of agonist potency in causing concentration-related contractile responses was [Lys^5, MeLeu^9, Nle^{10}] neurokinin A-(4–10) ≥ neurokinin A > neurokinin B ≥ substance P in tissues from both groups of animals (Table 1).

3.4. Effects of antagonists

In a subset of experiments the antagonists were found to have no significant effects on the responses of the uterine

Table 1
Potencies of tachykinin agonists on preparations from rats during the oestrous cycle
P/O = proestrus/oestrus; M/D = metoestrus/dioestrus.

Agonist	pD_2/pEC^a_{50} ± S.E.M. (*n*)		Maximum response (% methacholine response)[a] ± S.E.M.	
	P/O	M/D	P/O	M/D
Neurokinin A	7.59 ± 0.13 (7)	7.29 ± 0.10 (8)	66.89 ± 3.28	88.86[b] ± 3.95
Neurokinin B	5.47[a] ± 0.32 (14)	5.86[a] ± 0.26 (3)		
Substance P	5.41[a] ± 0.47 (15)	5.62[a] ± 0.10 (7)		
[Lys^5, MeLeu^9, Nle^{10}] neurokinin A-(4–10)	7.60 ± 0.19 (9)	7.29 ± 0.20 (5)	66.12 ± 4.87	61.62 ± 6.55

[a]Negative log molar concentration of agonist producing 50% of the response to methacholine 0.1 mM.
[b]Significantly different from corresponding value for proestrus/oestrus, Student's unpaired *t*-test, $P < 0.05$. Numbers in brackets represent the numbers of animals used.

preparations to methacholine 0.1 mM (Student's paired *t*-tests, $P > 0.05$; ($n = 17$, 6 and 4, for SR 48968, SR 140333 and SR 142801, respectively). Since there were no significant differences in the positions of agonist log concentration–response curves in uterine preparations from the two groups of rats used, data from the two groups were pooled.

The log concentration–response curve to substance P in the presence of vehicle lay to the left of that in the presence of SR 140333 10 nM (Fig. 2); the rightward shift in the presence of the antagonist was approximately threefold. However, since a maximum response to substance P was not obtained in the absence of an antagonist and since it is now apparent that this antagonist can produce noncompetitive antagonism (Croci et al., 1995), an apparent antagonist pK_B has not been calculated.

Fig. 3 shows log concentration–response curves to neurokinin A and [Lys^5, MeLeu^9, Nle^{10}] neurokinin A-(4–10) in the presence of vehicle and in the presence of SR 48968 (3 nM). In this concentration the antagonist produced parallel rightward shifts of the log concentration–response curves to neurokinin A and [Lys^5, MeLeu^9, Nle^{10}] neu-

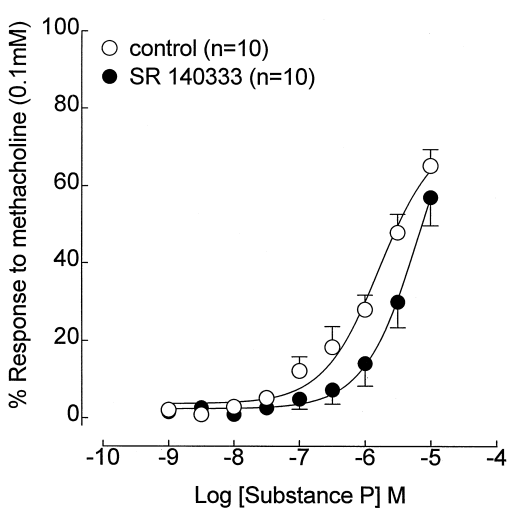
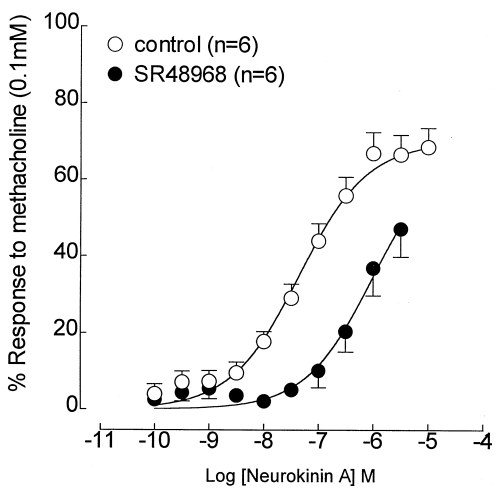


Fig. 2. Log concentration response curves to substance P in the presence of vehicle (0.01% ethanol; open symbols) and SR 140333 (10 nM; closed symbols). The results are expressed as percentages of the response to methacholine 0.1 mM; each point represents the mean ± S.E.M. of experiments in tissues from 10 animals.



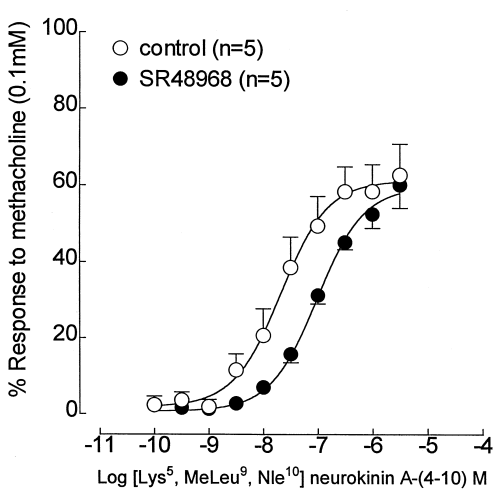


Fig. 3. Log concentration response curves to neurokinin A (upper panel) and to [Lys^5, MeLeu^9, Nle^{10}] neurokinin A-(4–10), (lower panel) in the presence of 0.01% ethanol; (open symbols) and SR 48968 (3 nM; closed symbols). The results are expressed as percentages of the response to methacholine 0.1 mM; each point represents the mean ± S.E.M.; n = number of animals from which tissues were taken.

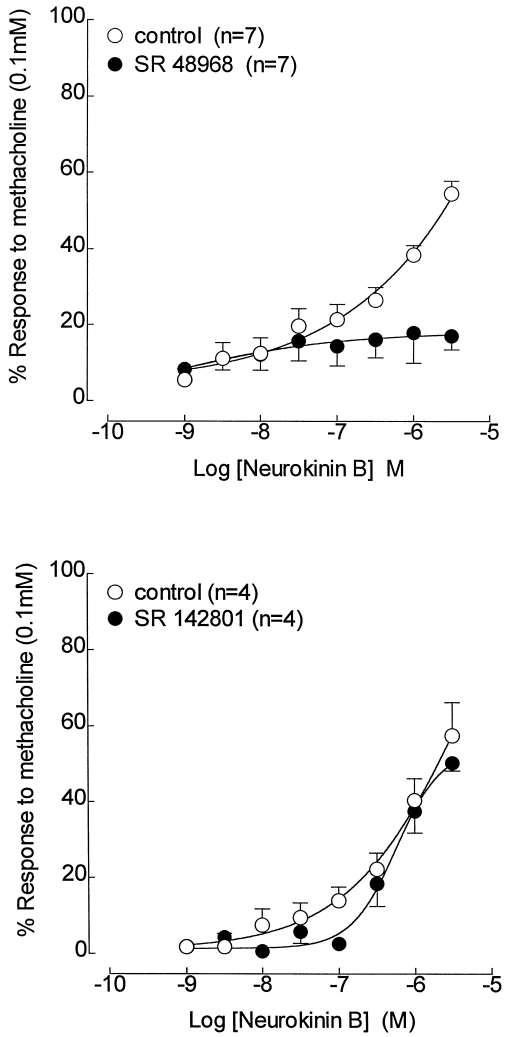


Fig. 4. Log concentration response curves to neurokinin B in the presence of ethanol 0.01% (open symbols) and SR 48968 (3 nM; closed symbols, upper panel) and SR 142801 (300 nM; closed symbols, lower panel) The results are expressed as percentages of the response to methacholine 0.1 mM; each point represents the mean ± S.E.M.; n = the number of animals from which tissues were taken.

rokinin A-(4–10). The apparent pK_B values for SR 48968 versus neurokinin A and [Lys^5, MeLeu^9, Nle^{10}] neurokinin A-(4–10) were 9.9 ± 0.36 ($n = 6$) and 9.2 ± 0.22 ($n = 5$), respectively. The antagonist was less effective against substance P, with an apparent pK_B value of 8.31 ± 0.21 ($n = 6$). The effect of neurokinin B was markedly depressed by SR 48968 (3 nM) (Fig. 4).

SR 142801 (100 nM and 300 nM) did not affect responses to neurokinin B. Log concentration–response curves to neurokinin B in the presence of SR 142801 (300 nM) are shown in Fig. 4.

4. Discussion

In this study we have shown that the tachykinins substance P, neurokinin A and neurokinin B and the stable tachykinin NK_2 receptor-selective analogue, [Lys^5, MeLeu^9, Nle^{10}] neurokinin A-(4–10) (Chaissang et al., 1991) produce concentration-related contractions of the longitudinally arranged smooth muscle layer of the uterus of the virgin rat during the oestrous cycle. The rank order of potency of these agonists, namely [Lys^5, MeLeu^9, Nle^{10}] neurokinin A-(4–10) ≥ neurokinin A > neurokinin B ≥ substance P, was similar in preparations from rats taken in proestrus/oestrus and in those taken in metoestrus/dioestrus. This observation suggests (1) that the changes in ovarian hormonal levels during the oestrous cycle do not influence the potencies of these agonists on this uterine smooth muscle layer, and (2) that the predominant receptor subtype involved in mediating these contractions is the tachykinin NK_2 receptor. The latter conclusion is supported by our finding that the tachykinin NK_2 receptor-selective antagonist, SR 48968, effectively inhibited responses to neurokinin A, neurokinin B and [Lys^5, MeLeu^9, Nle^{10}] neurokinin A-(4–10).

In designing the present set of experiments it was important to ensure that enzymatic degradation of the mammalian tachykinins was minimised. This was achieved through the inclusion of the endopeptidase 24.11 inhibitor, SCH 39370 (Sybertz et al., 1989; Fisher and Pennefather, 1997) in the bathing medium when these peptides were used, since the tachykinins are excellent substrates for this enzyme (Hooper et al., 1985). In addition the angiotensin converting enzyme inhibitor, captopril, was included when substance P was examined. The effects of the aminopeptidase inhibitor, amastatin (Tieku and Hooper, 1992), on the potency of neurokinin A in the presence of SCH 39370, was directly examined to determine whether, as in the uterus of the oestrogen-primed rat (Fisher and Pennefather, 1997), truncation of the N-terminal region was without effect on its potency. Since this was found to be the case, aminopeptidase inhibitors were not included in the majority of the experiments with neurokinin A and neurokinin B.

In a number of experiments maximum responses to agonists were not obtained with the largest concentrations of peptides that could be used. For this reason, the effects of the agonists were expressed as percentages of the responses to methacholine (0.1 mM). As in the case of the oestrogen-primed uterus using carbachol (Munns and Pennefather, 1998), there were no regional variations in response to this muscarinic receptor agonist. Additionally the responses to the reference agonist were similar in the two groups of preparation used, indicating an absence of an influence of cycle stage on responsiveness to the muscarinic agonist. We have previously reported that the potency of carbachol on the rat uterus is not influenced by the hormonal changes that occur during pregnancy in this species (Munns and Pennefather, 1998). It was therefore of interest that the maximum response to neurokinin A was greater in tissues from the metoestrous/dioestrous animals than in those from the proestrous/dioestrous animals. This

raises the possibility that progesterone of luteal origin may have a minor influence on the efficacy of this agonist.

The order of potency of the three mammalian tachykinins in the present study, i.e., neurokinin A > neurokinin B > substance P, was similar to that described for the uterus of the oestrogen-primed rat by Magraner et al. (1998). It differed from that we reported previously, namely neurokinin A > substance P > neurokinin B (Pennefather et al., 1993) for both functional and binding studies using the uterus of the oestrogen-primed rat. We are unable to account for this difference, but note that both potency orders are broadly consistent with the predominance of an NK_2 receptor (Henry, 1986). The participation of this receptor was confirmed by the studies using the antagonists. In those experiments the tachykinin NK_2 receptor-selective antagonist SR 48968 produced a parallel rightward shift in log concentration–response curves to both neurokinin A and [Lys^5, $MeLeu^9$, Nle^{10}] neurokinin A-(4–10). The apparent pK_B estimates we obtained for SR 48968 versus these two agonists, namely 9.9 and 9.2, respectively, are similar to those obtained on endothelium-denuded rabbit pulmonary artery (Emonds-Alt et al., 1992; Maggi et al., 1993), and human bronchus (Emonds-Alt et al., 1993). They are also substantially similar to those reported by Magraner et al. (1998) for the antagonism of neurokinin A by this antagonist on the uterus of the oestrogen-primed rat. As in the case of the oestrogen-primed uterus (Magraner et al., 1998), SR 48968 was less effective in antagonising the effects of substance P in our experiments than it was against the tachykinin NK_2 receptor-preferring agonists.

The possible participation of an NK_1 receptor in mediating the responses to substance P in these experiments was of particular interest in this study since there had been a report that responses to the tachykinin NK_1 receptor-selective agonist, [$Met(O_2)^{11}$] substance P produced variable effects on the rat uterus (Barr et al., 1991). Barr et al. attributed this variability to cyclic changes in ovarian hormone levels, but did not in fact determine cycle stage during their experiments. No cycle stage related differences in the potency of substance P, however, were discerned in the present study. It was of interest that neither SR140333 nor SR 48968 was particularly effective in antagonising the effects of substance P in the present experiments. It may be that this tachykinin can act on either tachykinin NK_1 or NK_2 receptors to produce its uterotonic effect. Clearly further experiments to determine the effect of a combination of these antagonists on responses to substance P would be of interest.

Several recent studies have indicated that messenger RNA for the tachykinin NK_3 receptor is present in rat uterus (Pinto et al., 1997; Magraner et al., 1998). It has been proposed that the expression of this receptor is negatively regulated by oestrogen (Barr et al., 1991; Pinto et al., 1997). Clearly in the uterus of the oestrogen-primed rat, the effects of neurokinin B (Magraner et al., 1998) as well as those of the tachykinin NK_3 receptor-selective agonist, senktide (Fisher and Pennefather, 1997), may be mediated by the tachykinin NK_2 receptor rather than an NK_3 receptor. In the present study, the tachykinin NK_2 receptor antagonist SR 48968 was effective in antagonizing the effects of neurokinin B, indicating that this agonist acts at tachykinin NK_2 receptors in this tissue. Even in concentrations approximately 10 times higher than its pA_2 at the tachykinin NK_3 receptor in rat portal vein (pA_2 = 7.5; Patacchini et al., 1995); SR 142801 was without effect on responses of the rat uterus to neurokinin B. This suggests that in uterine tissues from the rat taken during the oestrous cycle, as in tissues from oestrogen-primed rats (Fisher and Pennefather, 1997; Magraner et al., 1998) the tachykinin NK_3 receptor plays little if any role in mediating contractility.

In conclusion, the results of the present experiments indicate that, as in the human uterus (Patak et al., 1998), the contractile effects of tachykinins on the rat uterus are mediated primarily by tachykinin NK_2 receptors. It is possible that the release of peptides such as the tachykinins and calcitonin gene-related peptide from the peripheral endings of these afferent fibres may modulate uterine contractility in vivo. Calcitonin gene-related peptide is, however, more potent as an inhibitor of uterine contractions (Pennefather et al., 1990) than are either neurokinin A or substance P as stimulants. Thus uterine quiescence is likely to be the predominant effect resulting from the combined release of these peptides.

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