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Review

Mammalian tachykinins and uterine smooth muscle: the challenge escalates

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Abstract

We review the actions of mammalian tachykinins on uterine smooth muscle. Derived from sensory neurones and non-neuronal cells within the female reproductive tract, tachykinins are potent uterotonic agents. Three tachykinin receptor genes, and the gene encoding neprilysin, the enzyme that inactivates tachykinins, are present in rat, mouse and human myometrium. In rat and human, the tachykinin NK_2 receptor is important in mediating the uterotonic effects of tachykinins; actions at this receptor remain relatively stable or vary only slightly in the face of changing hormonal and gestational status. In contrast, ovarian steroids and pregnancy regulate expression of the tachykinin NK_3, and to a lesser extent, the tachykinin NK_1 receptor, as well as the activity of neprilysin. In the oestrogen primed mouse uterus, the tachykinin NK_1 receptor primarily mediates tachykinin uterotonic effects, but there is a switch to the tachykinin NK_2 receptor by late pregnancy. The possible physiological and pathological roles of tachykinins, including hemokinins and endokinins, in normal and premature labour, stress-induced abortion and menstrual disorders are briefly discussed.

Keywords: Myometrium; Tachykinin receptors; Neprilysin; Neurokinin A; Neurokinin B; Substance P

Contents

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1. Introduction

In 2000, two of the present authors published an update of the then body of knowledge of the effects of tachykinins and of tachykinin receptors on uterine smooth muscle (Patak et al., 2000a). This update was based on a presentation in a symposium entitled "Tachykinins: The Challenge Continues" held at a meeting of the Australian Physiological and Pharmacological Society in Newcastle, NSW. Since that review was written there have been notable advances in the field of tachykinins and tachykinin-like peptides. In the present review, we present those that have major relevance to the field of uterine smooth muscle physiology and pharmacology. These point to the need for further investigation of the effects and roles of novel members of the mammalian tachykinin family on the uterus.

Mammalian tachykinins comprise a family of peptides now known to contain the products of three genes, whose names in humans are TAC1 (*Tac1* in mouse and rat), *TAC3* (*Tac2* in mouse and rat) and *TAC4* (*Tac4* in mouse and rat). Several reviews on the growing tachykinin family have been published recently (Page, 2004; Patacchini et al., 2004; Pennefather et al., 2004). The family members include substance P (SP), neurokinin A (NKA) and its N-terminally extended analogues, neuropeptide K and neuropeptide γ, all derived from the *TAC1* (*Tac1*) gene, neurokinin B (NKB) derived from the *TAC3* (*Tac2*) gene, and the hemokinins and endokinins derived from the *TAC4* (*Tac4*) gene. The primary sequences and alignments of the mammalian peptides, which all share the amidated C-terminus motif Phe-X-Gly-Leu-Met-NH_2, are shown in Table 1. The sequence of the product of the mouse and rat *Tac4* gene, m-hemokinin (Zhang et al., 2000), differs from human hemokinin, while the products of the other rat *Tac* genes are similar to those in the human. In addition, other substance P-like peptides derived from the *TAC4* gene, but having a distinct C-terminus motif (Phe-Gln-Gly-Leu-Leu-NH_2) have also been described and are designated tachykinin gene-related peptides (Page, 2004; Patacchini et al., 2004). Substance

Table 1
Structures of mammalian tachykinins

Gene	Peptide												Reference
TAC1 *Tac1*	Substance P		R	P	K	P	Q	Q	**F**	F	**G**	**L**	**M-NH_2**	Chang et al. (1971)
TAC1 *Tac1*	Neurokinin A			H	K	T	D	S	**F**	V	**G**	**L**	**M-NH_2**	Nawa et al. (1984)
TAC1 *Tac1*	Neuropeptide K	DADSSIVEKQVAL LKALYGHGQISHK	R	H	K	T	D	S	**F**	V	**G**	**L**	**M-NH_2**	Tatemoto et al. (1985)
TAC1 *Tac1*	Neuropeptide γ	DAGHGQISHK	R	H	K	T	D	S	**F**	V	**G**	**L**	**M-NH_2**	Kage et al. (1988)
TAC3 *Tac2*	Neurokinin B			D	M	H	D	F	**F**	V	**G**	**L**	**M-NH_2**	Kangawa et al. (1983)
TAC4	Hemokinin-1 (human)		T	G	K	A	S	Q	**F**	F	**G**	**L**	**M-NH_2**	Kurtz et al. (2002); Page et al. (2003)
TAC4	Endokinin A	DGGEEQTLSTEAETWVIV ALEEGAGPSIQLQLQEVK	T	G	K	A	S	Q	**F**	F	**G**	**L**	**M-NH2**	Page et al. (2003)
TAC4	Endokinin B	DGGEEQTLSTEA ETWEGAGPSIQLQLQEVK	T	G	K	A	S	Q	**F**	F	**G**	**L**	**M-NH2**	Page et al. (2003)
Tac4	Hemokinin-1 (rat, mouse)		R	S	R	T	R	Q	**F**	Y	**G**	**L**	**M-NH2**	Zhang et al. (2000); Kurtz et al. (2002); Page et al. (2003)

The residues common to all tachykinins are shown in bold.

P, mouse and rat hemokinins, endokinin A and B are tachykinin NK_1 receptor-preferring (Page, 2004; Patacchini et al., 2004; Zhang et al., 2000; Kurtz et al., 2002), while neurokinin A and its N-terminally extended analogues are tachykinin NK_2 receptor-preferring, and neurokinin B is tachykinin NK_3 receptor-preferring (Maggi, 1995; see Pennefather et al., 2004).

It was generally believed that the source of tachykinin peptides in the periphery as well as in the central nervous system was primarily neuronal (Otsuka and Yoshioka, 1993). Release of tachykinins and co-localised calcitonin gene-related peptide (CGRP) from the peripheral ends of capsaicin-sensitive afferent fibres was proposed to be key in allowing regulation of effector organ activity (Lembeck and Holzer, 1979; Maggi and Meli, 1988). However, reports of a non-neuronal location of substance P, based on immunoassays and immunohistochemical studies, suggested the presence of substance P in a wide variety of non-neuronal cells (Linnik and Moskowitz, 1989; Maggi, 1997). It now seems likely, however, that hemokinins and endokinins rather than substance P may be the most abundant peptides in such cells (Page, 2004; Patacchini et al., 2004). Since these and other tachykinins are expressed in several regions of the female reproductive tract including the placenta, there is an increasing focus on the need to investigate and understand the roles of the tachykinin peptides in reproductive function in the female.

Traurig and Papka (1993) and Patak et al. (2000a) have published earlier reviews on tachykinins and the reproductive tract. Knowledge of the receptors at which tachykinins act in uterine smooth muscle, of the hormonal and pregnancy induced regulation of tachykinin and tachykinin receptor expression, and of the regulation of tachykinin degrading enzymes is slowly emerging. These aspects will be the major focus of this review.

2. Distribution and regulation of uterine mammalian tachykinin genes and peptides

2.1. Rat

Tachykinin immunoreactivity has been demonstrated in the female reproductive tract of numerous species. The rat, however, has been the most extensively studied (Traurig and Papka, 1993). Fibres expressing substance P-like immunoreactivity were demonstrated supplying myometrium as well as endometrium, but were less dense than in the ovary and the cervix. Neurokinin A-immunoreactive fibres are also present, co-localised with substance P and also CGRP (Traurig and Papka, 1993). Neurokinin B immunoreactivity has yet to be described, but the presence of the *Tac2* gene, which encodes this tachykinin, has been reported (Cintado et al., 2001; Pinto et al., 2001). The cellular location of this expression was not identified, but age and hormonal status affected its level. cDNA for the novel peptide hemokinin-1 is also present in the uterus of this species (Page, 2004) but the cellular location again was not specified.

The effect of hormonal status and pregnancy on the sensory innervation of the rat uterus is controversial (Patak et al., 2000a). Traurig et al. (1984) reported that substance P immunoreactivity in the rat uterus was unchanged by ovariectomy and pregnancy and that hence the afferent innervation of the uterus and cervix represented stable elements in this species. This view is reflected in the recent suggestion (Collins et al., 2002) that tachykinins play a potentially important role in cervical ripening. Papka et al. (2001) have, however, described the presence of oestrogen receptors in the dorsal root ganglia with fibres supplying the uterus and cervix, indicating that the afferent innervation of the rat uterus is a target for this steroid. In separate studies, pregnancy was reported to lead to either hypoinnervation (Schmidt et al., 2003) or hyperinnervation of the rat uterus (Amira et al., 1995).

2.2. Mouse

Surprisingly few studies have investigated the presence and roles of tachykinins on the mouse uterus, despite the fact that this species is very frequently used for producing gene knockouts and, accordingly, is of relevance in determining the roles played by tachykinins in reproductive processes. Tachykinin-immunoreactive nerves were reported to be present in the mouse uterus (Huang et al., 1984). The tachykinin-expressing fibres are quite sparse and are primarily associated with blood vessels and endometrial glands. Fig. 1 illustrates immunostaining of mouse uterus to tachykinins and to CGRP, which, in other species, is colocalised with tachykinins. The distribution of the peptide-containing fibres related to myometrial smooth muscle was between the circular and longitudinal layers.

Pintado et al. (2003) reported the presence, in mouse uterus, of the preprotachykinin (*PPT*) genes, *Tac1*, *Tac2* and *Tac4*, which encode substance P/neurokinin A, neurokinin B and hemokinin-1, respectively. Subsequently, the presence of these genes, and their hormonal/gestational regulation in mouse uterus was further investigated (Candenas et al., 2003). These authors suggested that hemokinin-1 may be the most abundantly expressed tachykinin in mouse uterus. The presence of hemokinin-1 protein in the mouse uterus has, however, yet to be demonstrated.

2.3. Guinea-pig

Substance P-like immunoreactivity is present in the guinea-pig uterus (Huang et al., 1984; Heinrich et al., 1986). Alm and Lundberg (1988) indicated that nerves expressing substance P-like immunoreactivity are sparse and found predominantly in the endometrium associated with glandular crypts and blood vessels, while neurokinin A-like immunoreactivity was even more sparse. They

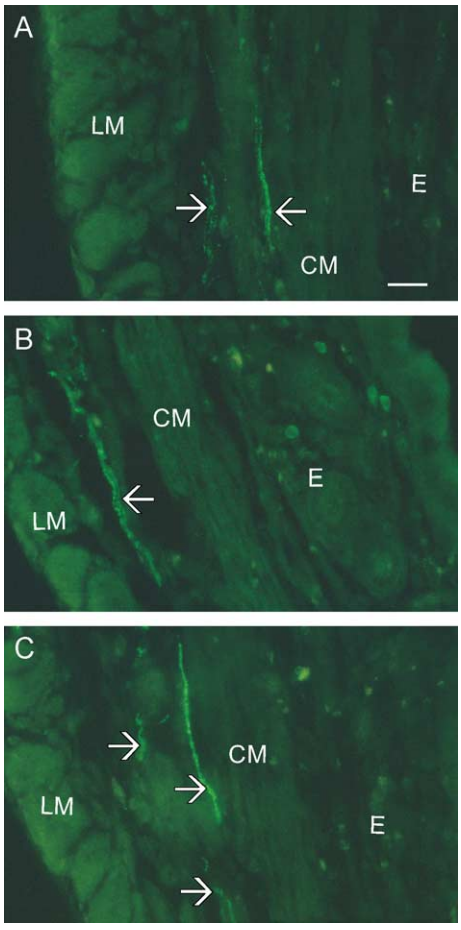

Fig. 1. Photomicrographs showing cross-sections of oestrogen-treated mouse uterus stained with antibodies for (A) substance P (B) neurokinin A and (C) CGRP. Immunostaining to the tachykinins was sparse and ran between the circular and longitudinal myometrial layers. CM, circular layer; E, endometrium; LM, longitudinal layer. Objective ×40. The scale bar represents 6 μm. Data from Patak (2003).

proposed that these tachykinin-immunoreactive nerves, like the sympathetic nerves supplying the uterus in this species, degenerated during pregnancy. While this distribution argues against a role for tachykinins of neuronal origin to affect uterine contractility, nothing is known of the distribution of extraneuronal tachykinins.

2.4. Human

Tachykinin-like immunoreactivity, colocalised with CGRP, has been reported to be found in human myometrial layers, around blood vessels and close to the epithelium (Samuelson et al., 1985; Heinrich et al., 1986; see Patak et al., 2000a). The innervation was reported to be sparse relative to that of the uterine cervix. Indeed, in a recent (unpublished observations) study in our laboratories, we did not observe any significant immunoreactivity in the outer myometrium from either non-pregnant or pregnant women. This absence may reflect differential distribution of the sensory supply to the regions of the human uterus, or pregnancy-induced neuronal degeneration as reported for the guinea-pig (Alm and Lundberg, 1988).

It may be, however, that the main tachykinin peptide present in non-neuronal cells in the human uterus is endokinin B rather than substance P (Page, 2004), since antibodies to substance P exhibit cross-reactivity with endokinins (Page, 2004). While real-time PCR has indicated *TAC4*, the precursor of endokinin B, in the human uterus, unequivocal demonstration of the presence of endokinin protein in human uterus has yet to be confirmed, as have the cells in which *TAC4* is located (Page, 2004). Using real-time PCR, the presence of *TAC3* in the non-pregnant human uterus has also been reported (Patak et al., 2003a). *TAC1*, the gene that encodes substance P and neurokinin A, was clearly detectable only in uteri from non-pregnant women (Patak et al., 2003a).

3. Distribution and regulation of uterine mammalian tachykinin receptor genes

Tachykinins interact with membrane receptors belonging to the family of G protein-coupled receptors. The genes encoding these receptors are named *TACR1*, *TACR2* and *TACR3* in human and *Tacr1*, *Tacr2* and *Tacr3* in mouse and rat. The three tachykinin receptor genes with a similar structural organisation have protein-coding regions divided into five exons, with introns interrupting the coding sequence in identical positions (see Pennefather et al., 2004). It is therefore not surprising that the known mammalian tachykinins are not totally selective.

3.1. Rat

Molecular studies indicate that *Tacr1*, *Tacr2* and *Tacr3*, encoding for tachykinin NK_1, NK_2 and NK_3 receptors, respectively, are expressed in rat uterus (Candenas et al., 2001; Cintado et al., 2001; Magraner, 1998; Pinto et al., 1999, 2001). These workers reported that *Tacr3* is strongly down-regulated by oestrogen in ovariectomized rats and up-regulated in older animals. This is, to our knowledge, the only described example of regulation of *Tacr3* expression. Uterine *Tacr1* mRNA decreased after treatment of ovariectomized rats with progesterone and increased after treatment with oestrogen while the expression of the *Tacr2* gene remained fairly stable (Pinto et al., 1999).

3.2. Mouse

Tacr1, *Tacr2* and *Tacr3* were all expressed, at different levels, in the uterus of superovulated, unfertilized mice (Pintado et al., 2003). Specific transcripts for all were seen in the non-pregnant mouse uterus. In contrast, in the late pregnant mouse uterus the genes encoding the tachykinin receptors were detected only in trace amounts (*Tacr1* and *Tacr2*) or were undetectable (*Tacr3*) (Candenas et al., 2003).

3.3. Guinea-pig

cDNAs encoding the tachykinin NK_1, NK_2 and NK_3 receptors have been cloned in the guinea-pig (see Pennefather et al., 2004). Early studies described the presence of the NK_1 receptor cDNA in the guinea-pig uterus and the reduction of its expression during pregnancy (Gorbulev et al., 1992). Further studies are clearly needed to identify tachykinin receptor expression and function in the uterus of this species.

3.4. Human

The characteristic pattern of expression of tachykinins and their receptors differs in uteri from pregnant and non-pregnant women. Real-time PCR studies indicate that *TACR2*, the gene encoding the tachykinin NK_2 receptor, is more strongly expressed in myometrium from non-pregnant women than in that from pregnant women (Patak et al., 2003a). *TACR1*, which encodes the tachykinin NK_1 receptor, was present in uterine smooth muscle from both non-pregnant and pregnant women, while a low expression of *TACR3*, which encodes the tachykinin NK_3 receptor, was only observed in non-pregnant uteri.

A splice variant of *TACR2* which, if translated, would give rise to a truncated protein has recently been identified in the rat and human uterus (Candenas et al., 2002). A recent report has described the existence of an isoform of the NK_3 receptor that also results from the loss of the second exon (GenBank accession no. CD013878; Jin et al. (1994)). The strong structural similarity between these NK_2 and NK_3 receptor mRNA isoforms suggests that this could be a common mechanism of regulation for all tachykinin receptors.

4. Distribution and regulation of tachykinin degrading peptidases

A number of cell surface peptidases are capable of degrading tachykinins. These include angiotensin-converting enzyme, neprilysin and several aminopeptidases. The active sites of these enzymes project into the intercellular space making them potentially important in inactivating tachykinins. The key enzyme in inactivating substance P, neurokinin A and neurokinin B is neprilysin. Although angiotensin-converting enzyme can also cleave the C-terminal Phe^8–Gly^9 bond of substance P (Erdos and Skidgel, 1988), neprilysin is the more effective enzyme in terminating its actions.

Neprilysin is a membrane-associated glycoprotein with a short intracytoplasmic region, a transmembranous hydrophobic stretch, and a bulky extracellular domain that contains the active site (see Turner et al., 2001 for a review). It is also known as membrane metallo-endopeptidase, neutral endopeptidase, or as enkephalinase. The gene that encodes this enzyme is named *MME* in humans and *Mme* in mouse and rat.

It was proposed that tachykinins are among the best substrates for neprilysin (Katayama et al., 1991). Accordingly its presence in the uterus of several species is of potential importance in inactivation of tachykinin peptides. The Gly^9–Leu^{10} bond in tachykinins is particularly susceptible to this enzyme (Hooper et al., 1985). Since the presence of this bond at the C-terminus of the tachykinins is crucial for receptor activation, its cleavage completely inactivates the tachykinins. A number of N-terminally truncated tachykinins retain biological activity (Rovero et al., 1989) indicating that aminopeptidases, which can cleave amino acids from the N-terminus of neurokinin A and neurokinin B but not substance P (Hooper et al., 1985; Hooper and Turner, 1985), are of less importance in terminating the activity of these peptides.

4.1. Rat

Pinto and colleagues have described the presence of *Mme*, the gene that encodes neprilysin, in the rat uterus and examined its regulation by oestrogen and pregnancy (Pinto et al., 1999; Candenas et al., 2001). In ovariectomized rats, neprilysin mRNA was fourfold lower in uteri from oestrogen-treated than from progesterone-treated rats. Neprilysin protein is expressed in smooth muscle cells in the rat uterus (Ottlecz et al., 1991); its activity peaks during pregnancy and drops just before term. Functional studies, in which log concentration–response curves to tachykinins were constructed, were conducted in the absence and presence either of two inhibitors of this enzyme, phosphoramidon and SCH 39370 (*N*-[*N*-[1-(*S*)-carboxyl-3-phenylpropyl]-(*S*)-phenyl-alanyl]-(*S*)-isoserine; Sybertz et al., 1989). These studies indicated that neprilysin plays a key role in limiting the contractile response of the oestrogen primed uterus in particular to neurokinin A (Fisher et al., 1993; Fisher and Pennefather, 1997; Magraner et al., 1998). The potentiation of neurokinin A by the neprilysin inhibitors ranged from ≈20- to 40-fold. Substance P and neurokinin B (Pennefather et al., 1993; Fisher and Pennefather, 1997) were potentiated to a much lesser extent (3- to 8-fold). In none of these studies, however, was the order of agonist potency of the three peptides altered in the presence of the peptidase inhibitors.

In a study using tissue from rats taken during the oestrous cycle, Shintani et al. (2000) observed no potentiation of the contractile effects of neurokinin A by phosphoramidon. They did, however, observe that phosphoramidon caused an approximately fivefold increase in the potency of neurokinin A in uterus from 18-day pregnant rats. Moreover, Candenas et al. (2001) reported a potentiation of the activity of neurokinin A in the presence of phosphoramidon in uterine preparations taken from rats at days 1, 6, 11, 16 and 21 of pregnancy.

Although angiotensin-converting enzyme is present in rat uterus, its inhibition by captopril did not modify uterine responses to substance P (Pennefather et al., 1993). Neither amastatin nor bestatin altered the responses to neurokinin A, neurokinin B or substance P (Fisher et al., 1993; Pennefather et al., 1993; Fisher and Pennefather, 1997). In the case of neurokinin A, this might reflect the fact that cleavage of bonds between Hys^1–Lys^2, Lys^2–Thr^3 and Thr^3–Asp^4 leave C-terminal 2–, 3– and 4–10 fragments which retain potent agonist activity on the rat uterus (Fisher and Pennefather, 1998).

Taken together, these findings suggest that neprilysin plays an important role in regulating the actions of tachykinin peptides in the rat uterus.

4.2. Mouse

cDNA encoding for neprilysin is expressed in the mouse uterus (Pintado et al., 2003; Candenas et al., 2003). However, the importance of neprilysin and other peptidases in regulating tachykinin-induced uterotonic effects has been little investigated. A combination of thiorphan, captopril and bestatin had a modest influence only on the potencies of mammalian tachykinins on mouse uterus (Patak, 2003; Patak et. al., unpublished observations). Thus in the oestrogen primed mouse responses to neurokinin A, but not to substance P were potentiated approximately 2- to 3-fold in the presence of peptidase inhibitors. In the uterus from the 17-day pregnant mouse, the peptidase inhibitors, used in the same concentrations as those that potentiated neurokinin A effects in the oestrogen primed mouse uterus, were ineffective (Patak, 2003). Clearly, the influence of peptidases on the uterotonic effects of tachykinins in this species merits further investigation, including the use of neprilysin inhibitors other than thiorphan.

4.3. Guinea-pig

Except for work presented in a PhD thesis from our laboratories (Fisher, 1997), there is a dearth of information about the actions, and regulation of the actions of tachykinins on the guinea-pig uterus. Perhaps only negative results have been obtained in other studies?

Fisher's experiments showed that, in the absence of enzyme inhibitors, neither substance P nor neurokinin A contracted preparations of circularly arranged uterine smooth muscle from guinea-pigs that were in dioestrus, or oestrogen-treated or late pregnant. Negative results were also obtained using comparable preparations of longitudinally arranged smooth muscle. This may reflect, in part, the presence of inactivating peptidases. While direct studies in this species of the expression of enzymes degrading these peptides have not been reported, indirect evidence from functional studies described in Section 5.3 indicates that neprilysin inactivates tachykinins in this species.

4.4. Human

Neprilysin mRNA is present in myometrium from non-pregnant women; real-time PCR indicates that this mRNA expression is significantly greater in pregnant than in non-pregnant women (Patak et al., 2003a). Several studies indicate that the major site of neprilysin protein expression in uteri from non-pregnant women is in the endometrium, while in pregnancy neprilysin may be associated with fetal membranes (Casey et al., 1991; Germain et al., 1994; see Patak et al., 2000a for other references). These workers have reported hormonal modulation of this enzyme in human uterus, such that after exposure to oestrogen, progesterone up-regulated its activity.

Indirect evidence for the presence of neprilysin in myometrial strips from pregnant women has come from studies of the effects of thiorphan 10 μM, on the response of these strips to substance P and neurokinin A. In this concentration, thiorphan produced significant enhancement of responses to both peptides (Patak et al., 2000b). In contrast, in preparations from non-pregnant women, thiorphan alone and in combination with bestatin to inhibit aminopeptidases did not affect responses to neurokinin A (Patak et al., 2003a). However, in the latter study a lower concentration (3 μM) of thiorphan was used. Further experiments are required to determine the functional role of this enzyme, which also cleaves oxytocin, in the pregnant and non-pregnant uterus.

5. Tachykinin receptor-mediated contractions and their regulation in isolated uterine preparations

Early studies of the uterotonic actions of tachykinins were with tissue from rats and humans (see Patak et al., 2000a). There was little focus in these studies on the possibility that effects of the mammalian peptides were constrained by the actions of peptidases; nor were selective agonists and non-peptide antagonists readily available. Since uterine smooth muscle often exhibits marked spontaneous activity, which complicates measurements of agonist responses, some workers use bathing solutions with very low concentrations of calcium to suppress this activity (e.g. Hamlin et al., 2000). Given that the effects of tachykinins are due in part to the influx of extracellular calcium into cells (Magraner et al., 1997; Shintani et al., 2000), the use of low calcium solutions may lead to some loss of activity.

In later studies in which the receptors mediating the effects of tachykinins have been examined, peptidase inhibitors, selective agonists and antagonists have generally been employed as pharmacological tools. Area under the force–time curve, rather than height of contraction has been increasingly used as a primary measure of contractile activity (Pennefather et al., 1993; Magraner et al., 1998). The former measure better mimics uterine contractile activity in vivo. In addition, while some have used single agonist concentrations to deduce the nature of the receptors

mediating uterine contractile activity (e.g. Hamlin et al., 2000), the use of a wider range of concentrations can provide more complete quantitative information, such as agonist relative potencies, pD_2 and antagonist pK_b values.

5.1. Rat

Reports beginning in the 1990s indicated that tachykinins, and tachykinin receptor-selective agonists, produce a direct contractile effect on uterine smooth muscle from the rat (Barr et al., 1991; Crane et al., 2002; Fisher and Pennefather, 1997, 1998, 1999; Fisher et al., 1993; Hamlin et al., 2000; Moodley et al., 1999; Pennefather et al., 1993; Magraner et al., 1998; Pinto et al., 1999; Shew et al., 1991; Shintani et al., 2000; Williams et al., 2003). Substance P, neurokinin A and neurokinin B cause concentration-dependent contraction of the longitudinally arranged myometrium from cycling (Moodley et al., 1999) and oestrogen primed virgin rats (Fisher and Pennefather, 1999; Magraner et al., 1998; Pennefather et al., 1993). Neurokinin A(2–10), neurokinin A(3–10) and neurokinin A(4–10) are also uterotonic in this species (Fisher and Pennefather, 1998). The relative potency order of neurokinin A>substance P≥neurokinin B and the potency of neurokinin A in contracting the oestrogen primed rat uterus are shown in Table 2. The order of potency indicates activation of tachykinin NK_2 receptors. In cycling rats, the order of potency (neurokinin A>neurokinin B>substance P) also indicates the presence of NK_2 receptors. That tachykinin NK_2 receptor protein is present in rat uterus has been confirmed in radioligand binding studies (Pennefather et al., 1993).

Agonists selective for tachykinin NK_1, NK_2 and NK_3 receptors, such as [$Sar^9Met(O_2)^{11}$]substance P, [$Lys^5MeLeu^9Nle^{10}$]neurokinin A(4–10), and senktide, respectively, also contract uterine preparations from ovariectomised, untreated and oestrogen-treated rats (Barr et al., 1991; Crane et al., 2002; Fisher et al., 1993; Hamlin et al., 2000; Magraner et al., 1998; Story et al., 1997; Moodley et al., 1999; Pinto et al., 1999). These agonists are useful pharmacological tools, not only because of their receptor selectivity, but also because of their relative resistance to breakdown by peptidases in rat uterus (Fisher and Pennefather, 1997). Fig. 2 shows log concentration–response curves to the mammalian tachykinins and to tachykinin receptor-selective agonists in cycling rats (Moodley et al., 1999; Story et al., 1997). The data indicating that neurokinin A and [$Lys^5MeLeu^9Nle^{10}$]neurokinin A(4–10) were the most potent agonists show that the NK_2 receptor is

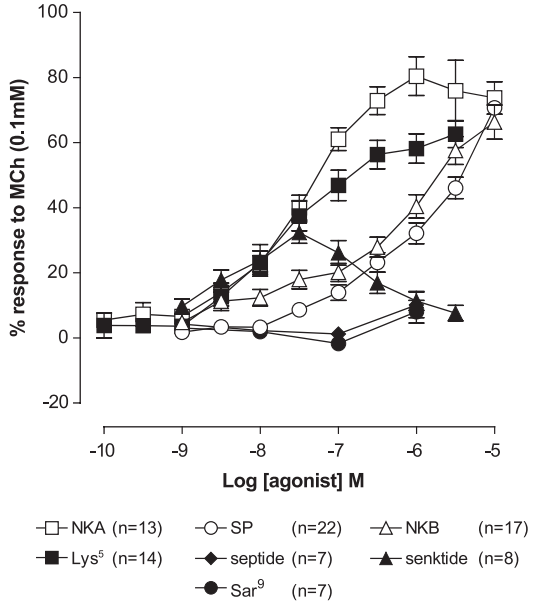

Fig. 2. Log concentration–response curves to mammalian tachykinins and tachykinin receptor-selective agonists on uterine preparations from untreated virgin rats. The relative orders of agonist potency indicate action at NK_2 receptors. SP, substance P; NKA, neurokinin A; NKB, neurokinin B; Sar^9, [$Sar^9Met(O_2)^{11}$]substance P; Lys^5, [$Lys^5MeLeu^9Nle^{10}$] neurokinin A(4–10); MCh, methacholine.

Table 2
Agonist and NK_2 receptor antagonist potencies in uteri from rats, mice (BalbC) and women

Species	Status	Order of agonist potency	pD_2 NKA	SP potency as % NKA	≈pK_b SR 48968 vs. NKA
Rat	Oestrogen primed[a,b]	NKA>NKB≥SP	8.5	1.7	8.8
	Oestrogen primed[c]	NKA>SP≥NKB	8.1	1.2	9.2
	Oestrous cycle[d]	NKA>NKB≥SP	7.6	2.1	9.9
Mouse	Oestrogen primed[e]	SP≥NKA>NKB	7.5	160	8.6
	Pregnant[f]	NKA>>SP≥NKB	7.5	1.5	9.1
Human	Non-pregnant[g]	NKA>SP≥NKB	8.4	1.2	–
	Near-term pregnant[h]	NKA>SP≥NKB	7.7	2.2	–

SP=substance P; NKA=neurokinin A; NKB=neurokinin B.
[a] Pennefather et al. (1993).
[b] Fisher and Pennefather (1999).
[c] Magraner et al. (1998).
[d] Moodley et al. (1999).
[e] Patak et al. (2002a).
[f] Patak (2003).
[g] Patak et al. (2003a).
[h] Patak et al. (2000b).

predominant during the oestrous cycle. Senktide was, as originally reported by Barr et al. (1991), also active, albeit with lower efficacy than the mammalian tachykinins or [Lys^{5}MeLeu^{9}Nle^{10}]neurokinin A(4–10). The inactivity of both [Sar^{9}Met(O$_2$)^{11}]substance P and septide suggests the tachykinin NK$_1$ receptor is of little importance. The contractile responses to substance P and neurokinin B were very similar in tissues from pre- and post-ovulatory rats; neurokinin A had a similar potency but a greater efficacy in the latter group.

Oestrogen treatment of ovariectomised rats down-regulates responses mediated by tachykinin NK$_3$ receptor-selective agonists (Crane et al., 2002; Hamlin et al., 2000; Pinto et al., 1999) while aging increases such responses (Cintado et al., 2001). Oestrogen dominance may cause either down-regulation (Hamlin et al., 2000) or up-regulation (Pinto et al., 1999) of responses to tachykinin NK$_1$ receptor agonists. Candenas et al. (2001) also reported that the tachykinin NK$_1$ receptor-selective agonist [Sar^{9}Met(O$_2$)^{11}]substance P was more potent in late than in early pregnant animals. Shintani et al. (2000) proposed that pregnancy enhanced the response to neurokinin A, but that this increase was offset by an increase in neprilysin activity. In the presence of peptidase inhibitors pD_2 estimates for neurokinin A approaching 8 were obtained in pregnant rats (Shintani et al., 2000; Candenas et al., 2001); these values are similar to those in oestrogen primed rats (Table 2).

Non-peptide tachykinin receptor antagonists used to investigate receptors in rat uterus have included SR 48968, ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide), NK$_2$ receptor-selective, Emonds-Alt et al., 1992), SR 140333 ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]octane chloride), NK$_1$ receptor-selective, Emonds-Alt et al., 1993) and SR 142801 ((*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide), NK$_3$ receptor-selective, Emonds-Alt et al., 1995). SR 48968 inhibits the responses of the oestrogen primed uterus to neurokinin A and to NK$_2$ receptor-selective agonists (Fisher and Pennefather, 1999; Magraner et al., 1998; Moodley et al., 1999). In cycling rats, SR 48968 antagonised the effects of both neurokinin A and neurokinin B, and to a lesser extent, substance P (Moodley et al., 1999), indicating actions at NK$_2$ receptors.

SR 140333, although effective in inhibiting the responses to a selective NK$_1$ receptor agonist [Sar^{9}Met(O$_2$)^{11}]substance P, produced only slight antagonism of the effects of substance P (Fisher and Pennefather, 1999; Magraner et al., 1998; Moodley et al., 1999). This finding further indicates that substance P may also act at an NK$_2$ as well as an NK$_1$ receptor in rat uterus.

SR 142801 did not inhibit responses to neurokinin B in cycling rats (Moodley et al., 1999), nor those to all three mammalian tachykinins and selective agonists in oestrogen primed rats (Magraner et al., 1998). In micromolar concentrations, however, it reduced responses to neurokinin B in oestrogen primed rats, and to a lesser extent those to neurokinin A and [Nle^{10}]neurokinin A(4–10) (Fisher and Pennefather, 1999). Especially given that oestrogen treatment down-regulates the tachykinin NK$_3$ receptor in this species (Pinto et al., 1997), these findings are consistent with reports of some non-selectivity of this antagonist (Beaujouan et al., 1997).

There have been few studies of the mechanisms through which tachykinins cause uterine contraction. In tissue from oestrogen primed rats (Magraner et al., 1997), neurokinin A, in common with oxytocin, causes Ca^{2+} release from an intracellular pool and calcium entry mainly through L-type voltage-operated Ca^{2+} channels. Shintani et al. (2000) proposed that neurokinin A induced contractions of the myometrium by increasing both [Ca^{2+}](i) and the myofilament Ca^{2+} sensitivity with the former effect due partly to intracellular Ca $^{2+}$ release but primarily to the Ca^{2+} influx through both voltage-dependent calcium channels and non-specific channels.

5.2. Mouse

Substance P, neurokinin A and neurokinin B all contract mouse uterus by a direct action on myometrium (Patak et al., 2002a). Functional studies reflect pregnancy-induced modulation of tachykinin receptors, or tachykinin receptor-effector coupling, in this species (Table 2). Thus myometrial preparations from oestrogen primed mice were more sensitive to substance P than to neurokinin A, and more sensitive to the tachykinin NK$_1$ receptor-selective agonist, [Sar^{9}Met(O$_2$)^{11}]substance P, than to the tachykinin NK$_2$ receptor receptor agonist, [Lys^{5}MeLeu^{9}Nle^{10}]neurokinin A(4–10). This finding differs from those with the uterus of the oestrogen primed rat (Table 2). Together with the finding that neurokinin A as well as substance P and [Sar^{9}Met(O$_2$)^{11}]substance P were susceptible to blockade by the tachykinin NK$_1$ receptor antagonist, SR 140333, this indicates prominence of the tachykinin NK$_1$ receptor in oestrogen-dominated mouse uterus (Patak et al., 2002a). Neurokinin A, neurokinin B and SR 140333 have all been reported to have significant affinity for the putative "septide" receptor (Beaujouan et al., 2000). Any possible involvement of this receptor in mediating the effects was not, however, further explored.

In stark contrast, in the 17-day pregnant mouse, there is marked amplification of the uterotonic action of the tachykinin NK$_2$ receptor-selective agonist [Lys^{5}MeLeu^{9}Nle^{10}]neurokinin A(4–10) and a decrease in the potency of substance P relative to that of neurokinin A (Patak et al., 2003b; Table 2). Furthermore, the effects of neurokinin A, like those of [Lys^{5}MeLeu^{9}Nle^{10}]neurokinin A(4–10), were blocked by the tachykinin NK$_2$ receptor-selective antagonist, SR 48968, but not by the tachykinin NK$_1$ receptor-selective antagonist SR 140333, although the latter antagonist did have some inhibitory effect on the smaller

responses to substance P. In neither oestrogen primed nor pregnant mice did the tachykinin NK_3 antagonist, SR 142801, inhibit effects of the mammalian peptides.

Taken together, the data so far available indicate that in the mouse, pregnancy may produce a switch in the nature of the predominant uterine tachykinin receptor from NK_1 to NK_2. It remains to be established, however, whether the effects of the tachykinins in oestrogen primed mice reflect those that occur during the oestrous cycle, and indeed at which stage of pregnancy the marked change in the predominant receptor coupled to myometrial contraction occurs.

5.3. Guinea-pig

Substance P, neurokinin A and neurokinin B, in the presence of the neprilysin inhibitor SCH 39370 and/or amastatin and/or captopril, were moderately effective in contracting uterus from dioestrous, oestrogen primed and late pregnant guinea-pigs (Table 3). On circular myometrium from oestrogen-treated animals neurokinin B was somewhat more potent than substance P and neurokinin A, but the magnitudes of the effects produced were smaller than in corresponding preparations from dioestrous guinea-pigs (Fisher, 1997; Table 3). In late pregnant animals, neurokinin B was more potent than neurokinin A and substance P and more effective than in corresponding tissue from oestrogen primed animals. Basically similar data were seen in preparations of longitudinally arranged myometrium, with neurokinin B more effective and potent than substance P and neurokinin A in both dioestrous and late pregnant animals (Table 3). Indeed in preparations from late pregnant animals, neurokinin B was the only effective peptide among those tested; these also included neurokinin A, substance P, [$Lys^5MeLeu^9Nle^{10}$]neurokinin A(4–10) and senktide. These data, albeit preliminary, provide *prima facie* evidence for a role of the tachykinin NK_3 receptor in modulating smooth muscle in the guinea-pig uterus.

5.4. Human

Early studies with human uterus (see Patak et al., 2000a) indicated that substance P and eledoisin elicited contractions of non-pregnant human uterus. In contrast, Barr et al. (1991) reported that tachykinins were ineffective in contracting human myometrium. More recent experiments nevertheless indicated that neurokinin A and [$Lys^5MeLeu^9Nle^{10}$]neurokinin A(4–10) were potent uterotonic agents in myometrium from pregnant and non-pregnant women (Patak et al., 2000a,b, 2003a). The responses to neurokinin A in myometrium in pregnant women were susceptible to breakdown by neprilysin, while those from non-pregnant women were not (Patak et al., 2000b, 2003a). The effects of [$Lys^5MeLeu^9Nle^{10}$]neurokinin A(4–10) (Patak et al., 2000b, 2003b), and also of eledoisin (Patak et al., 2002b) were markedly reduced by SR 48968 indicating activation of a tachykinin NK_2 receptor.

The potency of neurokinin A was significantly greater in myometrium from non-pregnant women than from pregnant women (Patak et al., 2003a). Substance P and neurokinin B were much less potent than neurokinin A (Table 2). In addition, the NK_1 receptor-selective agonist [$Sar^9Met(O_2)^{11}$]substance P and the NK_3 receptor-selective agonist [$MePhe^7$] neurokinin B were inactive in tissues from both pregnant and non-pregnant women (Patak et al., 2000b, 2003b). These data provide further evidence for the predominance of the NK_2 receptor in mediating contraction in human myometrium. The nature of the endogenous agonist for this receptor has yet to be determined.

6. Concluding remarks

Tachykinins have been implicated in menstrual and pregnancy-related disorders (Marx et al., 1999; Page, 2004; Page et al., 2000, Patak et al., 2000a,b, 2003a).

There is persuasive evidence that neurokinin B of placental origin may be involved in pre-eclampsia (Page, 2004; Page et al., 2000). Since actions of neurokinin B on the vasculature (Brownbill et al., 2003), rather than on the myometrium, have been implicated, discussion of this possibility is beyond the scope of the present review.

Marx et al. (1999) have implicated substance P in stress-induced early abortion (see Patak et al., 2000a for earlier references). They suggested that substance P-immunoreactive nerve fibres supplying the decidua were

Table 3
Effects of tachykinins on guinea-pig myometrium in the presence of peptidase inhibitors[a,b]

	Circularly oriented myometrium			Longitudinally oriented myometrium		
Status	Oestrogen primed[c]	Dioestrus[d]	Pregnant[e]	Oestrogen primed[c]	Dioestrus[d]	Pregnant[c]
Efficacy of most potent agonist	least	intermediate	most	least	intermediate	most
Most potent agonist	NKB	NKA ≈ SP ≈ NKB	NKB	NKA ≈ SP ≈ NKB	NKB	NKB

SP=substance P; NKA=neurokinin A; NKB=neurokinin B.

[a] Data from Fisher (1997).

[b] Data for all groups are from n=5 animals, the myometrial layers were not separated, but set up to record from either longitudinally arranged or circularly arranged muscle, respectively.

[c] Animals as in[d] were treated for 14 days with oestradiol-17β cypionate 20 μg/kg every 2nd day s.c. beginning on days 6–10 of the oestrous cycle.

[d] Virgin Dunkin–Hartley guinea-pigs were used on days 6–10 of the 16-day oestrous cycle.

[e] Animals were used on days 65–68 of the 68- to 70-day pregnancy.

involved, acting on mast and other inflammatory cells. However, it seems equally possible, and indeed more likely, that a hemokinin/endokinin might be involved, particularly as antibodies for substance P raised for the C-terminus of the peptide can exhibit cross reactivity with hemokinins (Page, 2004). This observation casts doubt on the possibility that substance P alone is involved. Indeed, Marx et al. (1999) did not include a neuronal marker in their studies, so it is possible that their antibody detected hemokinins/endokinins in non-neuronal cells. This possibility represents a challenge for future work.

The possible participation of a tachykinin in premature labour also merits further investigation. Here neurokinin B and endokinin B may be indicated as primary candidates, as these peptides, rather than products of the *TAC1* gene are more strongly expressed in the human uterus and feto-placental unit (Page et al., 2000; Page, 2004; Patak et al., 2003a). These possibilities represent a further challenge for future work, as do investigations of regulation of the tachykinin inactivating mechanisms in premature labour.

The apparent resistance of tachykinins to peptidase degradation and the relatively high potencies of agonists acting at NK_2 receptors in myometrium from women having hysterectomies for menstrual and menopausal disorders implicate tachykinins in these conditions (Patak et al., 2003a).

Of less obvious clinical relevance, but nevertheless interesting, is the switch that occurs in the predominant receptor mediating tachykinin uterotonic actions in the pregnant mouse. Whether this occurrence reflects down-regulation of the NK_1 receptor, or rapid desensitisation to the actions of substance P or a substance P-like peptide in pregnancy, and whether any similar phenomena occur in other species, represents a challenge for future investigations. The challenge escalates.

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