Spinal Afferent Innervation of the Uterus

Kelsi N. Dodds and Nick J. Spencer

Abstract The extrinsic neural innervation of the uterus plays an important role in modulating uterine functions that are critical to reproductive success. Its sensory division serves dual afferent and efferent roles: relaying information on innocuous and noxious stimuli from the uterus to the central nervous system and regulating uterine smooth muscle activity via local release of neuropeptides. Such sensory innervation is primarily supplied by spinal afferents with nerve cell bodies in thoracolumbar and lumbosacral dorsal root ganglia (DRG). Here, we summarise the neuroanatomy and physiology of spinal afferents innervating the rodent uterus. Findings arising from techniques pioneered in our laboratory are highlighted, which target select DRG for labelling and manipulation of specific spinal afferent populations. Future insights in this field are anticipated to expose new mechanisms related to disorders of uterine sensation, such as dysmenorrhoea.

Keywords Uterus · Female reproductive tract · Pelvic pain · Calcitonin gene-related peptide · Spinal ganglia

1 Introduction

The uterus is a major visceral organ that forms part of the female reproductive tract. It is richly supplied by extrinsic sensory, parasympathetic, and sympathetic nerve fibres that modulate uterine contractions, blood flow, and other regulatory processes critical to reproductive function and behaviour. Intriguingly, nerves of the uterus are highly plastic, undergoing major episodes of reorganisation throughout the lifespan

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to accommodate changes in reproductive need. The primary source of uterine sensory innervation is supplied by spinal afferent neurons, whose cell bodies lie adjacent to the spinal cord in dorsal root ganglia (DRG). These afferents detect a wide range of sensory information in the uterus, including mechanical stimuli, hypoxic conditions, and inflammatory mediators, providing important feedback for neuroendocrine and sensorimotor reflexes related to reproduction, as well as pain (Houdeau et al. 2002; Berkley et al. 1993).

Most, if not all, spinal afferent neurons innervating the uterus are peptidergic, distinguished by their immunoreactivity to calcitonin gene-related peptide (CGRP) (Dodds et al. 2021). Other co-transmitters may be present, such as substance P, neurokinin A, cholecystokinin, galanin, and secretoneurin (Traurig et al. 1991; Collins et al. 2000; Shew et al. 1992). In addition to their afferent role, uterine sensory nerves commonly demonstrate efferent functions by releasing these peptides from their endings into the uterine wall. CGRP, for example, is well known to cause relaxation of spontaneous and evoked contractions of the uterine muscle (Anouar et al. 1998; Naghashpour et al. 1997; Klukovits et al. 2004) and vasculature (Gangula et al. 2003), while substance P potently stimulates uterine contractility (Shew et al. 1991).

This minireview principally draws from results obtained in rodents, where uterine spinal afferents are currently best characterised. We first provide an overview of the central origins of spinal afferent neurons and their anatomical distribution within the uterus. Detailed morphological characteristics are then described, comparing several unique features of uterine spinal afferents to those observed in other visceral organs, such as the colon. Recent findings revealed by techniques developed in our laboratory that allow for selective labelling and manipulation of specific spinal afferent populations are highlighted. We conclude by summarising known uterine afferent adaptations, and their proposed functional contributions, to changes and processes involved in major reproductive stages. For brevity, the term ‘uterus’ herein refers to the main body of the uterine horn.

2 Central Origins and Distribution of Spinal Afferents Innervating the Uterus

The cell bodies and central axons of uterine spinal afferents are bimodally distributed along the spinal column, forming peak populations in the thoracolumbar (T12-L2) and lumbosacral (L6-S1) regions. The peripheral axons of thoracolumbar afferents predominantly travel from their uterine targets to the spinal cord via the hypogastric and splanchnic nerves, while lumbosacral axons travel via the pelvic nerves (Berkley et al. 1988; Nance et al. 1988; Herweijer et al. 2014). Sympathetic and parasympathetic efferent fibres accompany spinal afferents within the hypogastric and pelvic pathways, respectively (Nance et al. 1988), and some afferent axon collaterals synapse with cholinergic parasympathetic fibres in uterine-associated

autonomic ganglia (Papka and Traurig 1989; Papka and Mcneill 1993; Houdeau et al. 2002). In addition to spinal afferents, few sensory nerve fibres originate from nodose ganglia, which innervate the uterus via the vagal nerves (Ortega-Villalobos et al. 1990; Collins et al. 1999; Dodds et al. 2021) (Fig. 1).

The distribution of spinal afferent innervation within the uterus follows a topographical arrangement, such that fibres projecting from thoracolumbar DRG largely occupy the cranial (ovarian-to-mid) region of the uterine horn, as well as the adjacent ovaries and oviducts. Conversely, lumbosacral DRG preferentially innervate the caudal (mid-to-cervical) uterine horn, extending distally to include the uterine cervix and vagina (Nance et al. 1988; Berkley et al. 1988). The anatomical area(s) occupied by uterine-projecting vagal afferent fibres is less well established, but may include the entire uterus (Ortega-Villalobos et al. 1990).

Marked regional division of spinal afferent innervation has been demonstrated in the rat (Nance et al. 1988) and mouse (Kyloh et al. 2022) uterus following the unilateral removal of thoracolumbar DRG, in vivo. Subsequent loss of substance P or CGRP immunoreactivity, respectively, due to deafferentation in the uterine horn, revealed the spatial territories occupied by thoracolumbar neurons. Since rodent uteri display a predominantly ipsilateral spinal afferent input to each half of the female reproductive tract, nerve fibre density along the uterine horn of unilateral DRG-removed animals can be compared against an internal control of the unaffected, contralateral side. Using this method in mice, for example, we showed mean CGRP-immunoreactivity depletion of ~60% in the cranial region of the uterine horn following thoracolumbar DRG removal, versus ~45% in the caudal uterus (Kyloh et al. 2022) (Fig. 2).

In addition to the different regional projections of spinal afferent pathways, the uterus displays local variations in the density of nerve fibres. Total innervation (afferent and efferent) is highest in the caudal region of the uterine horn and denser in the cranial region than the mid-uterus (Traurig et al. 1991; Zoubina et al. 1998), while CGRP-containing afferent fibres are most concentrated within the cranial region, compared to the middle and caudal portions (Zoubina et al. 1998; Kyloh et al. 2022).

It remains undetermined whether the dual central distribution, and/or regional innervation of the uterus, simply reflects the anatomical segregation of the ovarian and uterine arteries, along which spinal afferents primarily enter the uterine horn (Nance et al. 1988). Alternatively, these distinct afferent pathways could serve functionally different roles between the cranial and caudal ends of the uterus. Indeed, it has been proposed that afferent activity of the hypogastric nerves primarily plays a role in pregnancy and nociception, whereas pelvic innervation may facilitate processes of mating and conception, with both nerves serving functions during parturition (Berkley et al. 1993). There is also an interesting possibility that the roles of each spinal pathway may shift under pathological conditions or during pregnancy (Temple et al. 1999; Kirby et al. 2010).

Research on other pelvic visceral organs, like the colon, supports the concept that different stimulus modalities can be conducted via the two neural pathways. For instance, colonic spinal afferents appear to relay non-noxious mechanosensory

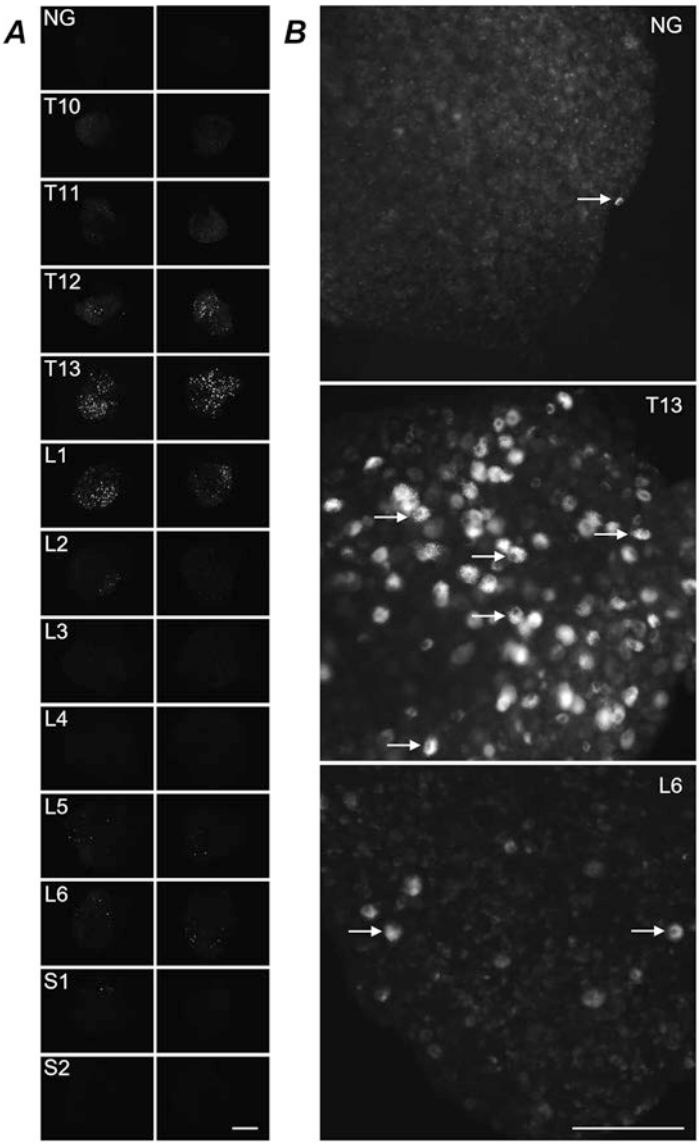

Fig. 1 Central origins of uterine-projecting spinal afferent neurons. (**a**) Pairs of thoracic (T10–13), lumbar (L1–6), and sacral (S1–2) dorsal root ganglia (DRG) and nodose ganglia (NG) retrogradely labelled with DiI neuronal tracer from the mouse uterus, in vivo. The greatest number of positively labelled neuronal cell bodies, indicating the degree of afferent innervation, occurs in thoracolumbar T12-L2 (via the hypogastric nerves), followed by lumbosacral L5-S1 (pelvic nerves) and NG (vagal nerves). (**b**) Higher magnification images of NG, T13, and S1, highlighting the extent of afferent innervation supplied to the uterus by vagal, hypogastric, and pelvic nerves, respectively. Examples of positively labelled neuronal cell bodies are indicated by the arrows. Scale bar in (**a**) = 500 μm for all images in the same panel; scale bar in (**b**) = 200 μm for all images in the same panel. (Some images presented in this figure are adapted from Dodds et al. (2021))

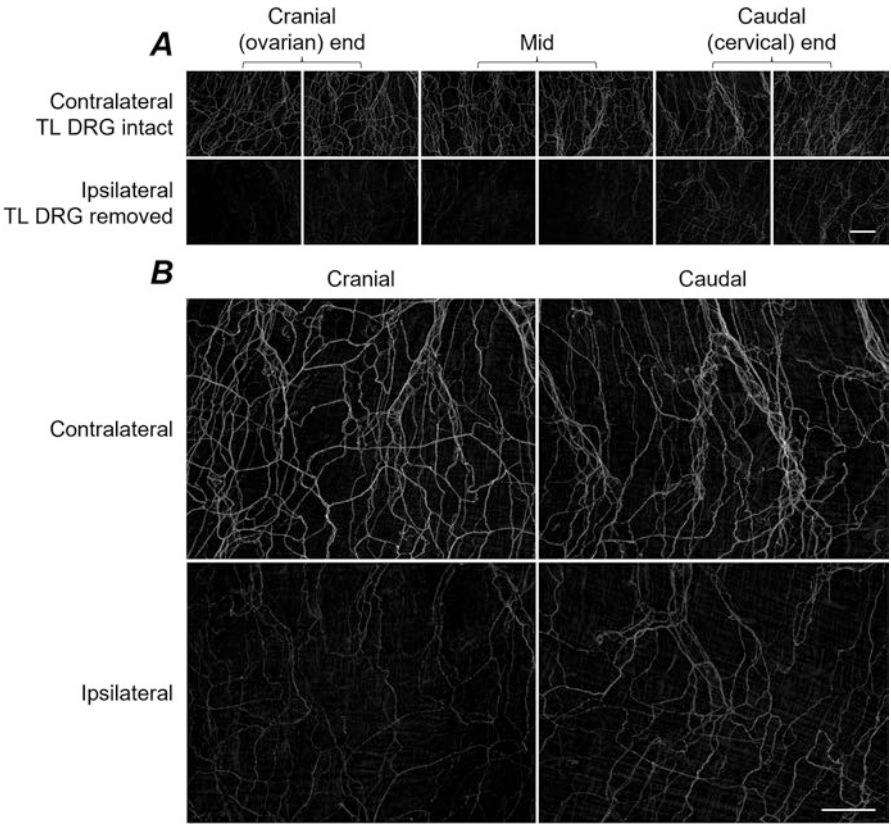

Fig. 2 Distribution of thoracolumbar spinal afferents in the uterus. (**a**) Representative images from the mouse uterine horn following in vivo unilateral removal of thoracolumbar (TL) T13-L2 dorsal root ganglia (DRG). The contralateral uterine horn remains densely innervated across all regions with calcitonin gene-related peptide (CGRP)-containing spinal afferent nerve fibres. However, there is a marked reduction in CGRP immunoreactivity of the ipsilateral uterine horn, which is most pronounced at the cranial (ovarian) end. This indicates that thoracolumbar spinal afferents (via the hypogastric nerves) provide major sensory innervation to the mouse uterus that distribute primarily within the cranial region. (**b**) Enlarged images from cranial and caudal regions of the uterine horn highlighting the extent of CGRP-immunoreactivity depletion on the ipsilateral side. Scale bar in (**a**) = 200 μm for all images in the same panel; scale bar in (**b**) = 200 μm for all images in the same panel. (Some images presented in this figure are adapted from Kyloh et al. (2022))

information through the lumbosacral spinal cord alone, whereas both thoracolumbar and lumbosacral spinal pathways become active following noxious mechanosensitive stimulation (Kyloh et al. 2022; Harrington et al. 2019). Selective DRG removal may be particularly useful in future studies to further distinguish these roles, in both the uterus and adjacent viscera (Kyloh et al. 2022). Subsequent loss of function associated with the targeted deafferentation would therefore indicate processes normally regulated by certain populations of spinal afferent neurons.

3 Morphological Characteristics of Uterine-Projecting Spinal Afferent Neurons

Spinal afferents enter the uterus via the mesometrium, with most axons traversing alongside terminal branches of the ovarian and uterine arteries. Few fibres have also been observed independent of the uterine vasculature, travelling free within the mesometrial space (Dodds et al. 2021; Shew et al. 1991). Upon entry into the uterus, spinal afferent axons distribute within all layers of the uterine wall: the longitudinal and circular smooth muscle (myometrium), the intermuscular vascular plexus, and the inner mucosal lining (endometrium) (Zoubina et al. 1998; Gnanamanickam and Llewellyn-Smith 2011; Dodds et al. 2021). The greatest density of CGRP-immunoreactive neurons occurs within the vascular plexus, the blood vessels of which are thought to serve as a conduit for these nerves travelling to other uterine layers (Zoubina et al. 1998; Haase et al. 1997).

3.1 Spinal Afferent Axons

To determine the projections of single spinal afferent neurons within the uterine wall, we recently employed an in vivo anterograde tracing technique in mice, where neuronal tracer was injected into thoracolumbar DRG (Dodds et al. 2021). In this procedure, the injected tracer is taken up by nerve cell bodies and axons in DRG and transported to the peripheral terminals of spinal afferents, allowing detailed morphological analysis of their axons and endings (Kyloh and Spencer 2014; Spencer et al. 2014). The specific uptake and labelling of spinal afferent neurons represents a major advantage of this approach for identification of afferents, over neuroanatomical tracing from visceral nerve trunks that contain multiple populations of afferent and efferent nerve fibres. Previously, this has been a key technical challenge, as the major known neurochemical markers expressed in DRG, including CGRP and vanilloid receptor 1 (TRPV1), are also expressed in vagal afferents (Zhang et al. 2004; Zhong et al. 2008). Mass labelling of axons using alternative approaches, such as transgenic reporter mice, has also precluded the identification and discrimination of individual spinal afferent neurons.

Anterograde tracing from DRG revealed no consistent trajectories of spinal afferent axons in the uterus. Groups of uterine spinal afferent axons were found to branch similar distances in the circumferential and longitudinal axes and with little cranial-caudal polarisation from their entry points into the uterine wall. These ramifications spanned approximately 10% of the length and 50% of the width of the uterine horn. Such features differ from other viscera, such as the colon, where subsets of spinal afferent axons can preferentially ramify along circumferential or longitudinal trajectories (Kyloh and Spencer 2014; Spencer et al. 2014).

Spinal afferent axons typically branched soon after entering the uterine wall, approximately one-third of which displayed varicosities prior to the first

bifurcation. Another unique feature of uterine spinal afferents was that, at each bifurcation, axon diameter (~1 μm) remained constant. This contrasts with colonic and bladder spinal afferents whose axon diameters typically decrease at each branching point (Spencer et al. 2018, 2020b). The implications of this are currently unclear but are likely to influence the conduction velocities of sensory information across different visceral organs.

3.2 Spinal Afferent Endings

In the same study, we reported the first thorough characterisation of the major morphological types of uterine spinal afferent endings and their sites of innervation (Dodds et al. 2021). Anterogradely labelled endings were mostly varicose, colocalised with CGRP immunoreactivity, and only occurred from axonal branches, where multiple endings arose from single axons. Three primary morphological classes of uterine spinal afferent endings were identified – simple, branching, and complex type (Herweijer et al. 2014) – which were further subclassified based on finer terminal details (Fig. 3). Most endings were of simple-type morphology, displaying considerably less structural diversity and complexity compared to those observed in the colon and bladder (Spencer et al. 2018). The reasons for this are not yet known but are likely to underlie inter-organ differences in the sensitivities and responses of spinal afferents to sensory stimuli. Regardless, some or all morphological types of spinal afferent endings identified must contribute to visceral pain arising from the uterus, such as that which occurs in dysmenorrhoea. The next major challenge will be therefore to determine exactly which classes (or subclasses) of uterine spinal afferent endings specifically underlie the detection of innocuous and noxious stimuli, and to compare those with findings from adjacent viscera.

Further, we found that spinal afferent endings terminated within multiple layers of the uterine wall. Overall, most endings occurred in the circular muscle, although there appeared to be some layer specificity for each morphological class: branching-type endings in the vascular plexus, simple-type in the circular muscle, and complex-type evenly divided between these two layers. While endometrial spinal afferents were not directly studied, CGRP expression has been described in the rat endometrium (Gnanamanickam and Llewellyn-Smith 2011; Zoubina et al. 1998; Shew et al. 1990), warranting further investigation. In addition, it is unknown whether the individual spinal afferent axons that have multiple endings in the uterus terminate across several layers of the uterine wall. In the colon, different types of morphological endings arising from single spinal afferent axons terminate across various wall layers, indicating that such neurons can detect and integrate a variety of sensory information (Spencer et al. 2020a, b).

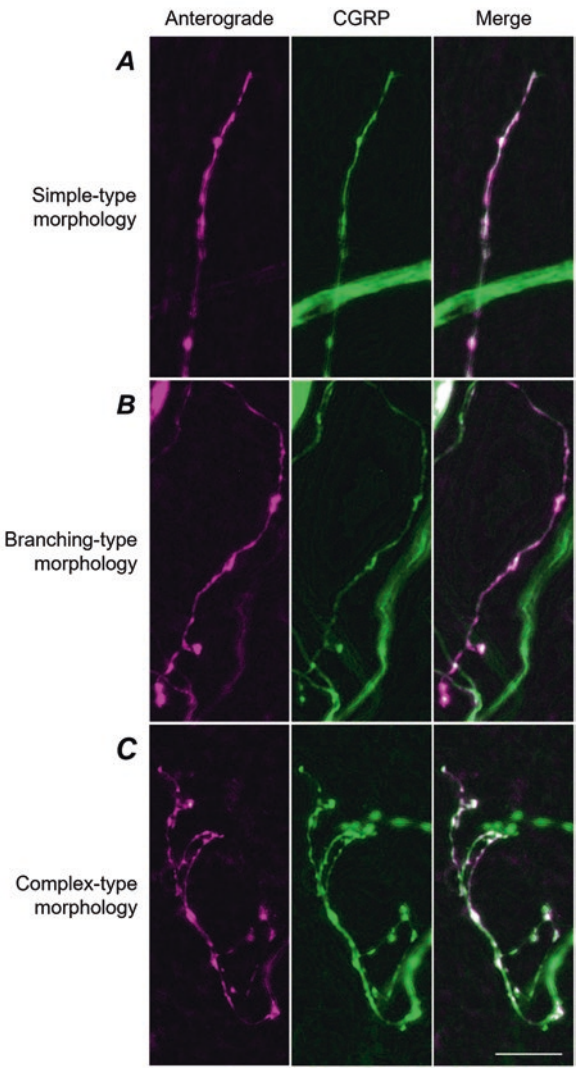

Fig. 3 Major morphological classes of uterine spinal afferent endings. (**a**) Example of a 'simple-type' spinal afferent nerve ending in the mouse uterus. This morphological class was most commonly identified by in vivo anterograde tracing from thoracolumbar (T13-L3) dorsal root ganglia (DRG), often terminating within circular smooth muscle. (**b**) Example of a 'branching-type' spinal afferent nerve ending, typically found within the uterine vascular plexus. (**c**) Example of a claw-like 'complex-type' uterine spinal afferent nerve ending. These endings displayed a wide range of sub-morphologies that also include looped and entangled variations. All morphological types of spinal afferent nerve endings colocalised with calcitonin gene-related peptide (CGRP) immunoreactivity. Scale bar in (**c**) = 20 μm applies across all images. (Some images presented in this figure are adapted from Dodds et al. (2021))

4 Adaptations of Uterine Afferents to Changes in Reproductive Status

The innervation of the female reproductive tract is highly sensitive to changes in sex hormones, undergoing profound metabolic, functional, and structural changes associated with maturation, the reproductive cycle, pregnancy, parturition, and the postpartum period (Brauer and Smith 2015). Sympathetic nerve fibres in the uterine myometrium appear to be most susceptible, displaying marked axonal degeneration under oestrogen-dominant conditions, such as the periovulatory phase and during pregnancy (Zoubina et al. 1998; Haase et al. 1997; Latini et al. 2008). The extent of

such alterations in uterine sensory fibres is less well established, although they are of key interest given their increasingly recognised importance in various reproductive processes. Specific adaptations in uterine spinal afferent neurons are yet to be determined, and, as such, general findings for sensory fibres are presented below.

4.1 Puberty and the Reproductive Cycle

During puberty, there is a significant increase in the weight of the uterine horn. Afferent nerve fibres in the uterus containing substance P and CGRP increase relative to this growth, resulting in the maintenance of nerve density and peptide concentration (Brauer et al. 1994). CGRP immunoreactivity remains comparatively unchanged across the ensuing reproductive cycle, despite an overall decrease in total nerve fibres throughout all layers of the rat uterine horn around ovulation (Zoubina et al. 1998). Since myometrial thickness fluctuates in response to sex hormone levels that define the different cycle stages, this finding indicates that afferent nerves are highly dynamic and, akin to puberty, continually adjust their density in parallel to uterine size.

Both the afferent and efferent functions of uterine sensory nerves are also modulated by cycle stage. Heightened sensitivity of the rat hypogastric nerves to uterine distension has been observed during proestrus (pre-ovulation) and oestrus (peri-ovulation) as opposed to metestrus and diestrus (post-ovulation) (Robbins et al. 1990, 1992), suggesting that these afferents are more likely to detect potentially damaging stimuli at a time of reproductive benefit (i.e. during conception). Yet, behavioural outcomes appear to contradict this idea, where a higher percentage of escape responses to noxious uterine stimulation have been reported during the post-ovulatory phases (Bradshaw et al. 1999), perhaps indicating differential central processing of such stimuli across the reproductive cycle phases. Regardless, this still confers reproductive advantage, in that there would be enhanced receptivity to uterine stimulation during the fertile period. The efferent function of uterine sensory nerves is also subject to cyclic variation. CGRP-mediated inhibition of uterine contractions is lowest at oestrus compared to metestrus and diestrus, likely due to changes in CGRP receptor expression and signalling (Naghashpour and Dahl 2000). This may explain why propagating uterine contractions are strongest during oestrus (Dodds et al. 2015), a mechanism that may facilitate gamete transport.

4.2 Pregnancy and Parturition

Throughout pregnancy, there is a gradual decline in the density of CGRP-containing uterine nerves until term, where profound denervation occurs in all layers of the uterine wall (Haase et al. 1997; Anouar et al. 1998). Corresponding levels of myometrial CGRP receptor expression and binding are elevated in pregnancy, followed by a sharp decline at the onset of labour (Yallampalli et al. 1999). Due to the

relaxant effect of CGRP on myometrial contractility and vascular tone, it is thought that this upregulated activity during gestation may be important for maintaining uterine quiescence and regulating uteroplacental blood flow to sustain normal foetal development (Gangula et al. 2002). At term, there is a dramatic loss of CGRP-mediated relaxation (Anouar et al. 1998; Naghashpour et al. 1997) accompanied by increased uterine content of substance P (Amira et al. 1995) and other myometrial stimulatory factors, such as oxytocin. Thus, the concentration of different afferent-associated peptides during labour may collectively assist in generating strong, rhythmic uterine contractions to help bring about normal delivery.

5 Concluding Remarks

Sensory innervation to the rodent uterus is predominantly supplied by CGRP-containing spinal afferent nerves that arise from thoracolumbar and lumbosacral DRG. Using techniques that selectively target these DRG, such as in vivo anterograde tracing and ganglion removal, has allowed significant progress to be made in defining their morphological characteristics and distribution within the uterus, as well as adjacent visceral organs. While we now have a greater understanding of the anatomy of uterine spinal afferents, there is still much to discover about their functional properties: both afferent, in their specificity and sensitivity to various sensory stimuli, and efferent, in their ability to modulate autonomic input to the uterus and directly alter uterine muscular and vascular tone. Taken together, uterine spinal afferent neurons are considered to have a promising role in many different regulatory and reproductive processes and, with further research, may reveal important mechanisms contributing to uterine-associated sensory disorders, such as dysmenorrhoea.

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