

Chapter 10

Beyond the GnRH Axis: Kisspeptin Regulation of the Oxytocin System in Pregnancy and Lactation

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Abstract Circulating oxytocin is critical for normal birth and lactation. Oxytocin is synthesised by hypothalamic supraoptic and paraventricular neurons and is released from the posterior pituitary gland into the circulation. Oxytocin secretion depends on action potentials initiated at the cell body, and we have shown that intravenous (IV) administration of kisspeptin-10 transiently increases the firing rate of supraoptic nucleus oxytocin neurons in anaesthetised, non-pregnant, pregnant and lactating rats. This peripheral effect is likely via vagal afferent input, because disruption of vagal afferents prevented the excitation. In our initial studies, intracerebroventricular (icv) administration of kisspeptin-10 did not alter the firing rate of oxytocin neurons in non-pregnant rats. Remarkably, we have now gathered unpublished observations showing that icv kisspeptin-10 transiently excites oxytocin neurons in late pregnancy and during lactation, suggesting that a central kisspeptin excitation of oxytocin neurons emerges at the end of pregnancy, when increased oxytocin secretion is required for delivery of the fetus and for milk let-down after delivery.

Introduction

This chapter considers a newly emerging area of investigation in the kisspeptin field: the effects of kisspeptin on reproduction outside the hypothalamic-pituitary-gonadal axis. When the endogenous ligand for the orphan GPR54 receptor (now commonly known as Kiss1r in rodents and KISS1R in humans) was identified as kisspeptin, its possible role in modulating secretion of the hormone oxytocin was

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highlighted because intravenous (IV) administration of kisspeptin-10 increased plasma levels of oxytocin in non-pregnant female rats [1]. However, this result was quickly overshadowed by the discovery of the role of Kiss1r/KISS1R and kisspeptin neurons in fertility through actions on GnRH neurons.

In 2011, the possibility of an important role for kisspeptin in the regulation of the oxytocin system was again brought to light by our finding that intravenous kisspeptin-10 increases the firing rate of oxytocin-secreting neurons [2]. Here, we will give an overview of that work and our more recent findings that suggest that central kisspeptin excitation of oxytocin neurons emerges over the course of pregnancy, which might have important implications for successful delivery of the offspring at birth and successful delivery of milk during lactation.

Pregnancy and Lactation

Reproduction is the process by which new offspring are produced and is a fundamental process of life. The hypothalamic-pituitary-gonadal axis, including the kisspeptin neurons, is a critical part of reproduction, but fertility is only the first step in successful reproduction. Reproduction also requires successful pregnancy, parturition and, in mammals, lactation. In all mammals, there are complex interactions of neural, endocrine and behavioural processes that enable a female to successfully meet the challenge of pregnancy and lactation. Elucidating these different mechanisms and pathways is important for understanding the transient physiological states of pregnancy and lactation. The mechanisms that are recruited during these states reflect profound changes in brain neurochemistry and morphology, and this includes the oxytocin system.

The hormone oxytocin has recently come to prominence as an important modulator of various behaviours, including social recognition, pair bonding, anxiety and maternal behaviours [3]. However, oxytocin is best known for its role in parturition and lactation [4]. The word oxytocin comes from the Greek ‘quick birth’, and oxytocin facilitates mammalian reproduction through uterine contractions during labour (parturition) and milk ejection in response to suckling during lactation [5]. Circulating oxytocin concentrations rise progressively over the course of pregnancy [6, 7], but only induce uterine contraction when specific oxytocin receptors (OTRs) are up-regulated in the uterus immediately prior to birth [8, 9]. The contracting uterus provides positive feedback to induce further oxytocin secretion (‘the Ferguson reflex’, Fig. 10.1) [10].

Oxytocin is the strongest uterotonic substance known and is widely used to induce labour in humans, yet oxytocin-deficient mice successfully complete parturition [11]. This suggests that oxytocin secretion is not essential for birth. However, the importance of oxytocin for the initiation and maintenance of labour, and for delivery, remains controversial. Oxytocin plays a vital role in timing of delivery, because oxytocin-deficient mice give birth at random times following a circadian clock reset [12]. Furthermore, OTR antagonists administered prior to labour delay

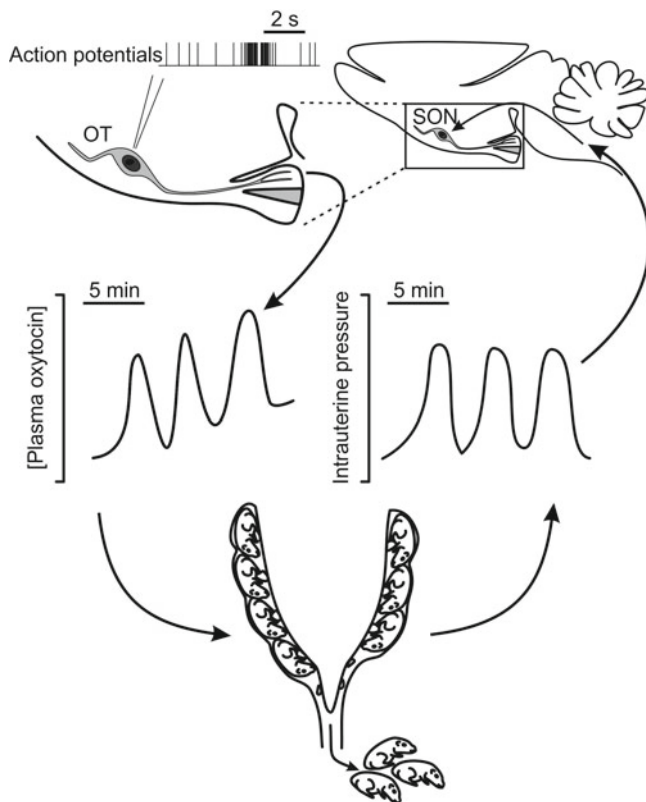


Fig. 10.1 A schematic representation of oxytocin regulation of parturition. Oxytocin cells fire in bursts similar to those seen during lactation but which are superimposed upon a higher baseline activity (*left hand side*). This pattern of activity results in pulsatile secretion of oxytocin from the posterior pituitary gland which acts on myometrial oxytocin receptors to induce uterine contractions and cause fetal expulsion. The uterine contractions feedback to the supraoptic nucleus (SON) via the nucleus tractus solitarii (NTS) in the brainstem (*right hand side*) to further enhance oxytocin cell activity and thus maintain parturition. Each burst of activity precedes the birth of a pup

the onset of delivery, and when given early in delivery, they increase the time between the deliveries of each pup [13]. Additionally, selective oxytocin agonists given centrally accelerate birth, as well as the onset of maternal behaviour [14, 15]. This suggests that, at least in rats, oxytocin is important for the initiation and the maintenance of parturition [13].

While it might be controversial as to whether oxytocin plays an indispensable role in parturition, the critical role that oxytocin plays in milk let-down during lactation is not disputed. The release of milk is mediated by secretion of oxytocin from the posterior pituitary gland, and oxytocin's action at OTR in the mammary gland induces a rise in intra-mammary pressure and release of milk: an oxytocin-mediated reflex upon suckling [16]. The oxytocin knockout mice fail to deliver milk to their

offspring, resulting in the death of the pups. These mice have normal milk production, as well as normal ductal/glandular epithelium in the mammary gland, and the pups latch and suckle the nipples but, unlike wild type animals, this fails to induce milk ejection. Exogenous oxytocin administration in these mice can produce sufficient milk let-down to release milk and keep pups alive [11], highlighting the essential role for oxytocin in the milk-ejection reflex.

The Magnocellular Neurosecretory System

Oxytocin is principally synthesised in magnocellular neurons of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus and released from the posterior pituitary gland to act in the periphery. The PVN contains magnocellular neurons that synthesise either oxytocin, or the closely related peptide vasopressin, and parvocellular neurons that contain a range of other peptide hormones and project to the median eminence to control anterior pituitary hormone secretion, as well as to other brain regions. By contrast to the PVN, the SON contains only magnocellular oxytocin and vasopressin neurons, which project a single axon caudally and medially to collect in the hypothalamo-neurohypophysial tract. These axons travel through the internal zone of the median eminence to the posterior pituitary gland (the neurohypophysis), where they end in several thousand neurosecretory axon swellings and terminals, filled with dense-core granules (neurosecretory vesicles) containing oxytocin (or vasopressin) [17]. Exocytosis of the neurosecretory vesicles occurs in response to invasion from action potentials, and once released into the extracellular space, oxytocin enters the general circulation by diffusion through fenestrated capillaries in the posterior pituitary gland [18]. The axon terminals in the posterior pituitary gland cannot maintain intrinsic repetitive firing [19], and so hormone secretion is principally determined by the frequency and pattern of action potentials initiated at the cell bodies. Nevertheless, various factors can modulate the release from magnocellular neuron terminals, including ionic conditions [20], purines [21] and neuropeptides [22].

Firing Patterns of Oxytocin Neurons

The profile of oxytocin secretion from the posterior pituitary gland is co-ordinated by the pattern of action potential discharge at the oxytocin cell bodies [23]. Nevertheless, the axon terminals in the posterior pituitary gland actively modulate the secretory response to action potential invasion by increasing the efficiency of stimulus-secretion coupling at higher firing rates so that each action potential releases more oxytocin per action potential at high action potential frequencies than at low frequencies [24]. As a result, when oxytocin neurons respond to stimulation with a linear increase in firing rate, the hormone output from the posterior pituitary gland is facilitated as the frequency of stimulation increases [24]. This frequency-facilitation

results, in part, from increased calcium entry into the axon terminals through voltage-gated channels to enhance exocytosis. Frequency-facilitation of oxytocin release is most marked between ~5 and 25 Hz, but continues to increase (albeit at a slower rate) beyond ~50 Hz. By contrast, as a consequence of frequency-facilitation, oxytocin neurons firing at less than ~4 Hz release little or no oxytocin to the posterior pituitary gland *in vivo* [25]. Hormone secretion is not sustained upon continuous stimulation and there is 'fatigue' in facilitation over time (although this is more pronounced in vasopressin release), which is rapidly reversed when stimulation is stopped for a few tens of seconds [26]. This frequency-facilitation of oxytocin release is of particular importance during parturition and lactation.

Under normal physiological conditions, oxytocin neuron firing rate is highly variable between neurons, and the neurons fire action potentials in a slow continuous or irregular pattern. Generally, the mean firing rate of oxytocin neurons is approximately 3–5 Hz, with intervals of at least 30 ms between consecutive action potentials [27, 28]. However, during parturition and suckling, co-ordinated intermittent high-frequency bursts are superimposed upon this slow/irregular firing (Fig. 10.1) [29, 30]. Each burst lasts just 1 or 2 s but the action potential activity is intense, often with 100 action potentials per burst in each neuron [31–33]. During one of these milk-ejection bursts, the intervals between consecutive action potentials are between 6 and 10 ms [34]. After a burst, each oxytocin neuron typically falls silent for a few tens of seconds (allowing the recovery from frequency-facilitation fatigue).

Remarkably, these milk-ejection bursts are co-ordinated across the population of oxytocin neurons [30]. Because of the co-ordination of bursts between oxytocin neurons and frequency-facilitation of secretion in each oxytocin neuron, oxytocin is released into the circulation in high concentrations for short periods. This pattern of secretion underpins the episodic contraction of the milk ducts for milk ejection, and similar pattern of activity causes rhythmic contraction of the uterus during parturition [35, 36]. Because these bursts occur every few minutes and last for only a couple of seconds, the net increase in firing rate only averages about one to two action potentials per minute for each oxytocin neuron.

Bursting activity occurs in oxytocin neurons only during parturition and lactation. Other stimuli that excite oxytocin neurons simply increase the continuous firing rate of the neurons, which rarely exceeds about 15 action potentials per second [27, 28]. Additionally, during lactation, oxytocin neurons will only fire in bursts in response to suckling and continue to respond to other stimuli in a similar way to virgin rats [37]; indeed stimuli that increase the background firing rate can actually inhibit burst firing [38]. Therefore, the emergence of bursting behaviour at the end of pregnancy does not result simply from changes in the structure or intrinsic properties of oxytocin neurons that fundamentally alter the way in which they respond to excitatory inputs. Rather, it is the nature of the stimulus that determines whether bursts will be triggered, and our recent work suggests the possibility that the emergence of a central kisspeptin projection to oxytocin neurons over the course of pregnancy might fulfil this role. Uniquely, milk-ejection bursts, and bursts during parturition, are synchronised throughout all the magnocellular oxytocin neurons, with neurons in both the SON and PVN firing at the same time [39, 40].

Central Effects of Oxytocin

In addition to release into the circulation, oxytocin is also released into the brain from centrally projecting neurons, as well as from the dendrites of magnocellular oxytocin neurons [41]. While centrally projecting oxytocin neurons have important functions, the oxytocin release into the brain from magnocellular neuron dendrites appears most important for regulating peripheral secretion, particularly secretion underpinned by bursts of action potentials [42]; blocking the effects of dendritically released oxytocin by administration of an OTR antagonist SON delays birth [14]. Therefore, oxytocin release from dendrites might contribute to pulsatile oxytocin secretion needed for parturition and lactation by facilitating the occurrence and synchronisation of action potential bursts in oxytocin neurons [43].

Afferent Inputs to Oxytocin Neurons

Like all other neurons, oxytocin neurons receive afferent inputs from many different peripheral and central sources. The relay of sensory information from the uterus and nipples is, of course, particularly important in allowing for the Ferguson reflex (during parturition) and milk-ejection reflex (during lactation). The vagus nerve provides a major route of sensory input from the periphery to the brainstem and in particular the nucleus of the solitary tract (NTS) [44]. In turn, the NTS and the ventrolateral medulla (VLM) within the medulla oblongata of the brainstem provide afferent input to the SON and PVN [45]. Therefore, vagal afferent fibres provide a pathway by which factors in the periphery can relay information to the SON to modulate the secretion of oxytocin. Electrical stimulation of vagal afferents increases the firing rate of some magnocellular neurons [46] and disrupting the vagal pathway reduces oxytocin secretion in response to peripheral modulators [47]. Brainstem projections are mainly noradrenergic from the A1 group of the VLM and the A2 group of noradrenergic neurons in the NTS to the SON [48], and excitation of A2 noradrenergic neurons excites oxytocin neurons in the SON [49]. Over half the projections from the brainstem neurons to the SON are activated during parturition [50], and many of these are noradrenergic. During parturition, the excited A2 neurons release noradrenaline in the SON [51], contributing to the excitation of oxytocin neurons. This is unlikely to be the only link in the Ferguson reflex afferent pathway, but to date the A2 neurons are the only identified population with a demonstrated role in directly exciting oxytocin neurons during parturition.

Peptides secreted in the periphery also excite oxytocin neurons indirectly, via vagal afferents to the NTS and subsequent excitatory noradrenergic input to the SON. Cholecystokinin (CCK) is a peptide hormone that is released from the stomach following food intake and, in addition to inhibiting feeding [47], CCK increases the firing rate of oxytocin neurons [52], resulting in the secretion of oxytocin into the circulation [47]. The effects of CCK on food intake and oxytocin secretion are

mediated though CCKA receptors located on peripheral vagal endings [53], which signal through the NTS in the brainstem to the oxytocin neurons in the hypothalamus [28]. Recently, another gastric peptide hormone, secretin, has also been shown to increase the activity and secretion of oxytocin neurons. This effect appears to also be mediated by the noradrenergic pathway, because intracerebroventricular (icv) injection of the $\alpha 1$ adrenergic antagonist, benoxathian, blocked the increase in firing rate of oxytocin (and vasopressin) neurons [54]. Hence, visceral inputs to oxytocin neurons (including those active in parturition and lactation) converge on the noradrenergic inputs via the vagus nerve.

The SON and PVN also receive prominent inputs from several forebrain areas, including areas in the anteroventral region of the third ventricle (AV3V): the subfornical organ (SFO), the organum vasculosum of the lamina terminalis (OVLT) and the median preoptic nucleus (MnPO) [45]. The SFO and OVLT are located outside the blood brain barrier and so peripheral stimuli can stimulate oxytocin secretion by acting on AV3V inputs. These projections from the AV3V are best characterised as mediating osmoregulation, and lesions of this region reduces oxytocin secretion and consequently impairs sodium excretion, but does not affect the milk-ejection reflex or parturition [55]. In addition to the above afferent inputs that have been extensively studied, the SON and PVN also receive numerous afferent inputs that have been less well characterised and whose physiological function is not well-established. These inputs include (but are not limited to): arcuate nucleus, bed nucleus of the stria terminalis, diagonal band of Broca, raphe nuclei, tuberomammillary nucleus and supra-chiasmatic nucleus.

Up to this point, we have provided a brief background of the oxytocin system and its importance in pregnancy, parturition and lactation. There is remarkable plasticity in the activity of oxytocin neurons that emerges at the end of pregnancy to facilitate delivery of the offspring and delivery of milk to the offspring. This emergent behaviour requires afferent input for its expression, and so any afferent input that exhibits plasticity over the course of pregnancy is a likely candidate to be involved in the processes that underpin this change in behaviour at a cellular level. Our recent results suggest that kisspeptin might be an important newly discovered player in the regulation of oxytocin neurons in pregnancy and lactation. The remainder of this chapter focuses on how kisspeptin affects the oxytocin neurons and highlights new data that indicates that central kisspeptin regulation of oxytocin neurons emerges towards the end of pregnancy.

Circulating Kisspeptin Concentrations in Pregnancy and Lactation

Investigations of kisspeptin regulation of fertility have focussed on central interactions with GnRH neurons [56]. However, our initial interest was in the effects of circulating kisspeptin on oxytocin neurons because IV kisspeptin administration increases plasma oxytocin levels in non-pregnant female rats [1], and the plasma

concentration of kisspeptin in woman has been reported to increase hugely over the course of pregnancy, with a 900-fold rise in the first trimester that further increases to over 7,000-fold in the third trimester. While the circulating kisspeptin that is found at low concentrations in non-pregnant women (and in men) is likely to be from either vascular endothelial cells [57] or adipose tissue [58], the main source of circulating kisspeptin during pregnancy is likely to be the placenta, because *KISS1* mRNA and kisspeptin protein are detectable in syncytiotrophoblasts in the placental wall [59]. The physiological function of elevated kisspeptin during human pregnancy is unknown; while it is possible that kisspeptin regulates trophoblast invasion, a process that is important for embryonic development [60], we hypothesised that such elevations in circulating kisspeptin might signal the oxytocin neurons to prepare for birth and lactation.

Activation of Oxytocin Neurons by Peripheral Kisspeptin in Non-pregnant Rats

To determine whether circulating kisspeptin might signal the oxytocin neurons to prepare for birth and lactation, we used an *in vivo* electrophysiology preparation to record spontaneous activity from the neurons in the SON of anaesthetised rats. This preparation causes the least disruption to whole animal physiology, allowing us to record near-normal neuron activity that can be altered by their intact peripheral and central inputs. Our work using this preparation was the first to show that neurons in the SON increase their firing rate following IV administration of kisspeptin-10 [2].

First, extracellular single-unit recordings of action potential firing rate were made from oxytocin neurons in non-pregnant rats across all stages of the estrous cycle. Intravenous administration of 25 μg kisspeptin-10 (which should achieve similar circulating concentrations of kisspeptin reported at the end of human pregnancy [57]) increases plasma oxytocin levels in the blood of virgin female rats [1], and so we administered 25 μg IV kisspeptin-10 to investigate whether changes in oxytocin neuron activity underpin the increased plasma hormone concentration; we found that this IV dose of kisspeptin-10 caused a robust, short-lived (~5 min) increase in the firing rate of every single oxytocin neuron tested (Fig. 10.2). The increase in firing rate following the peripheral kisspeptin injection was rapid, with a clear increase in firing rate within 30 s of administration and the peak rate within 60 s. The majority of the neurons showed a return to basal levels between 5 and 10 min following the injection. Hence, we were able to show that oxytocin neurons are able to rapidly respond to fluctuations in circulating kisspeptin, at least in non-pregnant rats. Importantly, repeated IV injections of kisspeptin-10 every few minutes caused a similar increase in firing rate, showing that the oxytocin neuron response does not desensitise over the time course expected for milk-ejection bursts. Given the short duration of excitation, and the lack of down-regulation of the response, it is likely that breakdown of kisspeptin (rather than a long deactivation or

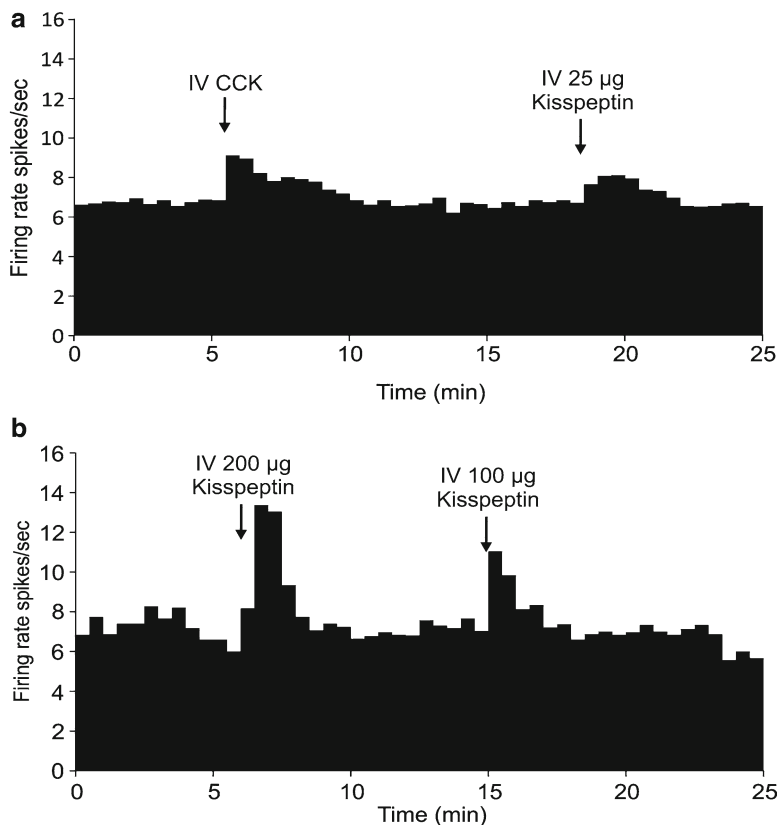


Fig. 10.2 (a) Representative ratemeter record (in 30 s bins) showing an increase in firing rate of an oxytocin neuron (identified by a transient excitation following IV CCK) in response to 25 µg IV kisspeptin-10 in a urethane-anaesthetised virgin female rat. (b) Representative ratemeter record (in 30 s bins) showing a dose-dependent increase in firing rate of an oxytocin neuron (identified by a transient excitation following IV CCK, not shown) in response to IV kisspeptin-10 in a urethane-anaesthetised virgin female rat

down-regulation of *Kiss1r* is the limiting factor in the duration of the oxytocin neuron response to a bolus IV injection of kisspeptin-10.

To further characterise the response of oxytocin neurons to IV kisspeptin, we completed dose-ranging studies that showed a clear dose-dependent increase in oxytocin neuron firing rate to IV kisspeptin. Even at the lowest dose that we tested (5 µg), IV kisspeptin elicited a small but consistent increase in firing rate of oxytocin neurons.

Kisspeptin-10 is known to have comparable, if not greater, biological potency to that of the full length peptide [61, 62], but to eliminate the possibility that our observed responses were specific to kisspeptin-10, we also completed a small number of experiments where we used kisspeptin-54 (the full length peptide). As expected, IV kisspeptin-54 caused a similar increase in oxytocin neuron firing rate to that seen with kisspeptin-10.

As explained earlier, the basal firing rates of individual oxytocin neurons are highly variable, but the responses of oxytocin neurons to IV kisspeptin were completely independent of basal firing rate. This observation, combined with the consistent increase in firing rate evident in every oxytocin neuron challenged with IV kisspeptin-10, makes it probable that, when circulating kisspeptin rises, every single oxytocin neuron increases its firing rate. Hence, circulating kisspeptin is a potential mechanism that could co-ordinate activity across the population of oxytocin neurons; such co-ordinated oxytocin neuron activity is a pre-requisite for successful milk ejection during lactation.

Vagal Mediation of Oxytocin Neuron Activation by Peripheral Kisspeptin

As described earlier, information is relayed to the oxytocin neurons via various afferent input pathways, including noradrenergic pathways from the brainstem that relay vagal signals. This pathway is implicated in the co-ordination of neuroendocrine changes occurring at birth and is well-established as completing the positive feedback loop from the uterus to the oxytocin system in birth and lactation. Hence, we hypothesised that circulating kisspeptin-10 might also converge on this pathway to act on the SON, because Kiss1r are expressed in peripheral tissues, including the stomach and small intestine [1], that are known to be innervated by the vagus nerve.

To test this hypothesis, we repeated our experiments after desensitisation of vagal sensory fibres using the sensory neurotoxin, capsaicin (8-methyl-*N*-vanillyl-6-nonenamide). Capsaicin is a compound found in capsicums and hot chilli peppers that creates a perception of burning in mammals via activation of the transient receptor potential channel-vanilloid receptor subtype 1 (TRPV-1; capsaicin receptor). While capsaicin initially excites thin primary afferent c-fibres expressing TRPV-1, when it is administered in large and/or repeated doses it results in the desensitisation and defunctionalisation of the neurons [63]. TRPV-1 is expressed on gastric primary vagal afferent fibres [64], and capsaicin can act on these fibres to effectively block the vagal pathway into the brain with the advantage that efferent vagal pathways are left intact.

We found that pre-treatment with intraperitoneal (IP) capsaicin completely eliminated the increase in firing rate of oxytocin neurons induced by IV kisspeptin-10, as well as that induced by IV CCK (which is known to be mediated by vagal inputs to the NTS [47]). Thus, it appears that in non-pregnant rats, IV kisspeptin-10 does not directly excite oxytocin neurons, but acts as a hormone on peripheral targets with projections to the SON relayed by vagal afferent fibres. While we do not yet have direct evidence to support the involvement of the NTS, or of noradrenergic neurons, it is likely that NTS relays the vagal input to the SON through noradrenergic input. Of course, further work is still required to fully establish the pathway from the vagus to the SON, which will involve retrograde-labelling from the SON

combined with immunohistochemistry for neuronal activation in the retrogradely labelled neurons after IV administration of kisspeptin.

While we have clearly established that peripheral administration of exogenous kisspeptin-10 consistently increases the firing rate of oxytocin neurons in non-pregnant rats [2], the physiological significance of this excitation remains to be established and this has been a focus of our more recent unpublished work.

Activation of Oxytocin Neurons by Peripheral Kisspeptin in Pregnancy and Lactation

We have begun to repeat our experiments by administering IV kisspeptin while recording oxytocin neuron firing rate in rats anaesthetised at various times over pregnancy and lactation. While still preliminary, our results to date show that the excitation of oxytocin neurons by IV kisspeptin is evident throughout pregnancy and into lactation. Superficially, these observations might seem straightforward, but they are difficult to reconcile with the published observations of markedly increased kisspeptin levels over the course of pregnancy, particularly in the third trimester [59]. In the face of hugely increased endogenous kisspeptin levels, one might expect occlusion, or desensitisation to the effects of exogenous kisspeptin. Furthermore, one would expect the endogenous kisspeptin to drive a steady increase in firing rate of oxytocin neurons over the course of pregnancy. We have seen no diminution in the effectiveness of exogenous kisspeptin in pregnant rats, but the progressive rise in circulating oxytocin concentrations over the course of human pregnancy [6, 7] is very much more modest than the increase reported for kisspeptin in humans [59].

However, the published data on circulating kisspeptin levels in pregnancy and lactation are from humans and our experimental model is rats. It appears likely that humans (and possibly higher primates) are the only species in which plasma kisspeptin levels might increase during pregnancy, because kisspeptin does not appear to increase during pregnancy across many non-primate species, including rodents, sheep and horses (Alain Caraty, personal communication). Thus, if the published data on humans genuinely reflect a species difference rather than a lack of specificity of kisspeptin antibodies used in the early studies, the rat might not be the model of choice for kisspeptin regulation of human pregnancy. Notwithstanding any species differences, it appears that kisspeptin probably excites oxytocin neurons in pregnant and lactating rats, and if the placental secretion of kisspeptin is pulsatile at parturition, this might add another level of control for the co-ordination of oxytocin neuron bursts during parturition. Of course, this idea is highly speculative and requires further investigation using peripheral administration of kisspeptin receptor antagonists to determine whether these can disrupt delivery of the offspring (and/or delivery of milk to the new-born).

Activation of Oxytocin Neurons by Central Kisspeptin in Pregnancy and Lactation

Several electrophysiology studies have shown that kisspeptin directly excites various neuronal populations: GnRH neurons, arcuate nucleus neurons, pro-opiomelanocortin neurons and hippocampal neurons are all excited by direct application of kisspeptin to brain slices [65–68]. So, we administered icv kisspeptin-10 in the expectation that we would localise the site of action of IV kisspeptin-10 to the brain. However, to our surprise, we did not find any effect of icv kisspeptin on the firing rate of oxytocin neurons in non-pregnant rats. In fact, in marked contrast to the robust and repeatable excitation following IV kisspeptin, there was no change in firing rate following icv injection at 2 µg or at 40 µg (both in a 2 µL volume) in any oxytocin neuron tested (Fig. 10.3), even in those neurons that were excited by IV kisspeptin-10. The higher dose of icv kisspeptin-10 that we used (40 µg) has been published as inducing a very robust response in the GnRH system to markedly increase circulating luteinising hormone levels [69], so it is very unlikely that the failure of icv kisspeptin-10 to excite oxytocin neurons in non-pregnant rats was due to a failure to deliver sufficient kisspeptin. The unexpected lack of response of oxytocin neurons to icv kisspeptin-10, combined with a robust response to IV kisspeptin, suggests that kisspeptin-10 might not cross the blood brain barrier to act directly on oxytocin neurons, or on Kiss1r that might be expressed by any central inputs to the SON. Consistent with our observed lack of effect of icv kisspeptin-10 on oxytocin neurons in non-pregnant rats, a recent mapping study has shown the presence of only a few kisspeptin fibres in the locality of the SON and it is not known whether they terminate in or form synapses in the area [70]. Additionally, there is no clear indication as to whether the Kiss1r is expressed in the SON [71].

Notwithstanding the lack of effect of icv kisspeptin on oxytocin neurons in non-pregnant rats, we continued to administer icv kisspeptin during our more recent studies into the effects of IV kisspeptin in pregnancy and lactation. The preliminary data that we have generated make the lack of effect of icv kisspeptin on oxytocin neurons in non-pregnant animals even more intriguing because our unpublished observations suggest that a central kisspeptin excitation of oxytocin neurons might emerge over the course of pregnancy in rats and that this is sustained during lactation.

In our latest experiments, we made *in vivo* extracellular single-unit recordings from oxytocin neurons in rats on different days of pregnancy (day 15–21). As described above, the response of oxytocin neurons to peripheral kisspeptin-10 was maintained throughout pregnancy, with IV injections of kisspeptin-10 continuing to cause a short (~5 min) increase in the firing rate of oxytocin neurons recorded from pregnant rats. This excitation was indistinguishable from the response seen in non-pregnant rats (Fig. 10.2), with the increase in firing rate following the peripheral injection occurring within 30 s and returning to basal levels 5–10 min later.

By contrast to the consistent responses to IV kisspeptin, there appears to be a marked change in the response of oxytocin neurons to icv kisspeptin-10 that arises over the course of pregnancy. In all oxytocin neurons recorded from animals on day

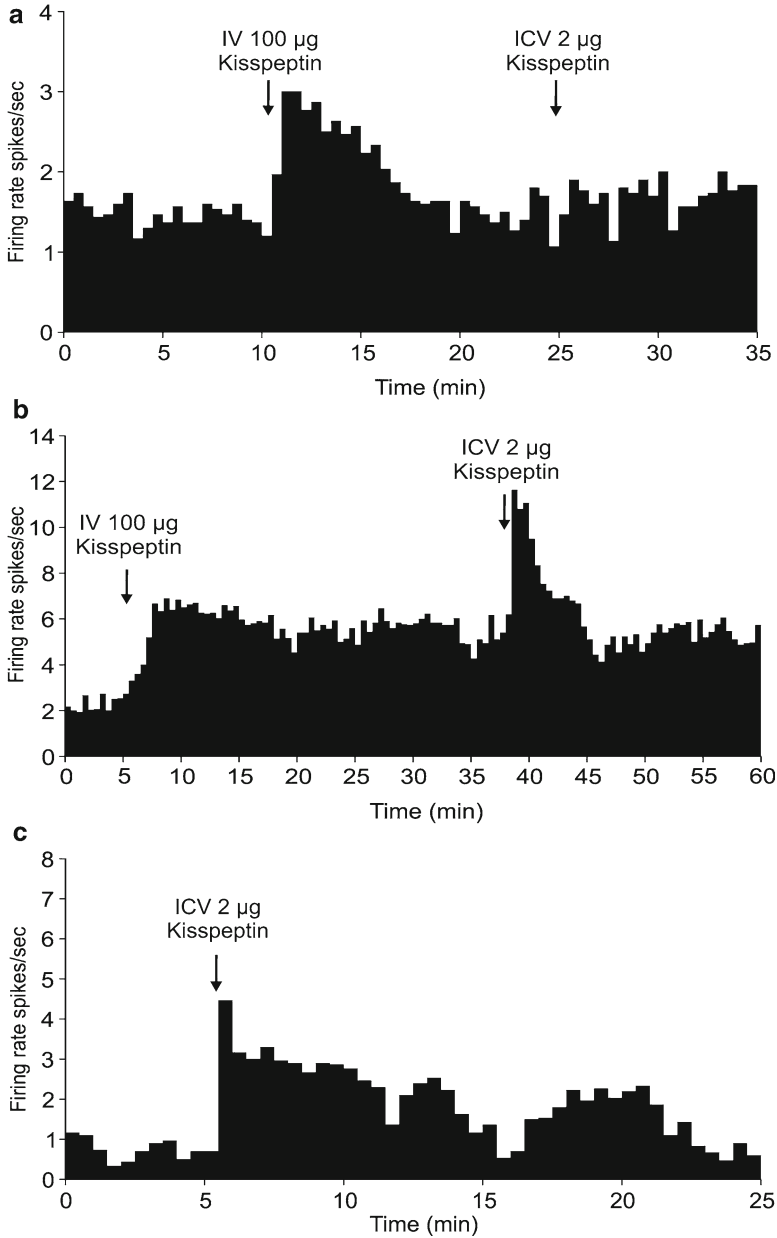


Fig. 10.3 (a) Representative ratemeter record (in 30 s bins) of an oxytocin neuron, showing an increase in firing rate after IV kisspeptin-10 but no response to icv kisspeptin-10, in a urethane-anaesthetised virgin female rat. (b) Representative ratemeter record (in 30 s bins) of an oxytocin neuron, showing a prolonged increase in firing rate after IV kisspeptin-10 and a rapid ~5 min excitation after icv kisspeptin-10, in a urethane-anaesthetised day 20 pregnant rat. (c) Representative ratemeter record (in 30 s bins) of an oxytocin neuron, showing an increase in firing rate after icv kisspeptin-10 in a urethane-anaesthetised day 7 lactating rat

18–21 of pregnancy, icv kisspeptin-10 caused an immediate, robust increase in oxytocin neuron firing rate following the injection. Unlike the response to peripheral kisspeptin-10, the increase in firing rate seen with central administration appeared substantially different between neurons, with some neurons showing a return to basal firing within 10 min, while others showed a sustained shift to a higher firing rate that lasted tens of minutes, somewhat reminiscent of the initial observations of kisspeptin excitation of GnRH neurons *in vitro* [65]. The most remarkable aspect of this response to icv kisspeptin-10 is that it only becomes apparent during pregnancy.

We have no knowledge of the functional consequences of the emergence of this sensitivity of oxytocin neurons to central kisspeptin during pregnancy, but it is tempting to speculate that it might be involved in driving, or facilitating, bursting behaviour of oxytocin neurons during parturition. Consistent with this speculation, the excitation of oxytocin neurons seen in response to icv kisspeptin-10 in late-pregnant rats was also evident in the one oxytocin neuron that we have recorded from a lactating rat, the only other time in a mammal's life that oxytocin neurons are known to exhibit high-frequency co-ordinated bursts of action potentials to release a bolus of oxytocin into the bloodstream.

Not only do we not know the function of central kisspeptin excitation of oxytocin neurons in late pregnancy, we also do not know the mechanisms that underpin the emergence of this excitation. One possibility is an increased accessibility of kisspeptin to Kiss1r that leads to the excitation of oxytocin neurons by icv kisspeptin. In addition, there might be up-regulation of Kiss1r expression in oxytocin neurons (or their afferent inputs), the laying down of a new kisspeptin projection to oxytocin neurons, and/or the up-regulation of kisspeptin expression in an existing projection to oxytocin neurons. These possibilities are a focus of current work in our laboratory.

Conclusion

Kisspeptin and its receptor, Kiss1r (in rodents), have been described as an essential gatekeeper of reproductive function [72]. Our recent work has expanded our knowledge of the critical role that kisspeptin plays in reproductive function via excitation of oxytocin neurons. During pregnancy and lactation, dynamic and transient changes occur within the oxytocin system. The synthesis, dendritic release and peripheral secretion are all modified in order to facilitate and synchronise the function of oxytocin neurons in labour, birth and lactation, as well as in maternal behaviour. We have shown that circulating kisspeptin excites oxytocin neurons throughout life, which might become important when the placenta secretes kisspeptin during pregnancy. Possibly of more importance for successful reproduction, we have also shown that the excitation of oxytocin neurons by central administration of kisspeptin appears to be dramatically changed over the course of pregnancy, emerging only in late pregnancy. The mechanisms behind this change have yet to be established

but may underlie an important new role for kisspeptin in reproduction, the activation and modification of the oxytocin system during pregnancy, parturition and lactation.

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References

1. Kotani M, Detheux M, Vandenberghe A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R, Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann SN, Vassart G, Parmentier M (2001) The metastasis suppressor gene *KiSS-1* encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem* 276:34631–34636
2. Scott V, Brown CH (2011) Kisspeptin activation of supraoptic nucleus neurons in vivo. *Endocrinology* 152:3862–3870
3. Neumann ID (2008) Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. *J Neuroendocrinol* 20:858–865
4. Russell JA, Leng G, Douglas AJ (2003) The magnocellular oxytocin system, the fount of maternity: adaptations in pregnancy. *Front Neuroendocrinol* 24:27–61
5. Mitchell BF, Fang X, Wong S (1998) Oxytocin: a paracrine hormone in the regulation of parturition? *Rev Reprod* 3:113–122
6. Otsuki Y, Yamaji K, Fujita M, Takagi T, Tanizawa O (1983) Serial plasma oxytocin levels during pregnancy and labor. *Acta Obstet Gynecol Scand* 62:15–18
7. de Geest K, Thiery M, Piron-Possuyt G, Vanden Driessche R (1985) Plasma oxytocin in human pregnancy and parturition. *J Perinat Med* 13:3–13
8. Kimura T, Takemura M, Nomura S, Nobunaga T, Kubota Y, Inoue T, Hashimoto K, Kumazawa I, Ito Y, Ohashi K, Koyama M, Azuma C, Kitamura Y, Saji F (1996) Expression of oxytocin receptor in human pregnant myometrium. *Endocrinology* 137:780–785
9. Renthal NE, Chen CC, Williams KC, Gerard RD, Prange-Kiel J, Mendelson CR (2010) miR-200 family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor. *Proc Natl Acad Sci U S A* 107:20828–20833
10. Ferguson JKW (1941) A study of the motility of the intact uterus at term. *Surg Gynecol Obstet* 73:7
11. Nishimori K, Young LJ, Guo Q, Wang Z, Insel TR, Matzuk MM (1996) Oxytocin is required for nursing but is not essential for parturition or reproductive behavior. *Proc Natl Acad Sci U S A* 93:11699–11704
12. Roizen J, Luedke CE, Herzog ED, Muglia LJ (2007) Oxytocin in the circadian timing of birth. *PLoS One* 2:e922
13. Antonijevic IA, Douglas AJ, Dye S, Bicknell RJ, Leng G, Russell JA (1995) Oxytocin antagonists delay the initiation of parturition and prolong its active phase in rats. *J Endocrinol* 145:97–103
14. Neumann I, Douglas AJ, Pittman QJ, Russell JA, Landgraf R (1996) Oxytocin released within the supraoptic nucleus of the rat brain by positive feedback action is involved in parturition-related events. *J Neuroendocrinol* 8:227–233
15. Kendrick KM (2000) Oxytocin, motherhood and bonding. *Exp Physiol* 85 Spec No:111S–124S
16. Lincoln DW, Paisley AC (1982) Neuroendocrine control of milk ejection. *J Reprod Fertil* 65:571–586

17. Brownstein MJ, Russell JT, Gainer H (1980) Synthesis, transport, and release of posterior pituitary hormones. *Science* 207:373–378
18. Hatton GI, Yang QZ, Smithson KG (1988) Synaptic inputs and electrical coupling among magnocellular neuroendocrine cells. *Brain Res Bull* 20:751–755
19. Bourque CW (1990) Intraterminal recordings from the rat neurohypophysis in vitro. *J Physiol* 421:247–262
20. Marrero HG, Lemos JR (2010) Ionic conditions modulate stimulus-induced capacitance changes in isolated neurohypophysial terminals of the rat. *J Physiol* 588:287–300
21. Lemos JR, Wang G (2000) Excitatory versus inhibitory modulation by ATP of neurohypophysial terminal activity in the rat. *Exp Physiol* 85 Spec No:67S–74S
22. Ortiz-Miranda S, Dayanithi G, Custer E, Treistman SN, Lemos JR (2005) Micro-opioid receptor preferentially inhibits oxytocin release from neurohypophysial terminals by blocking R-type Ca²⁺ channels. *J Neuroendocrinol* 17:583–590
23. Brown CH, Bourque CW (2006) Mechanisms of rhythmogenesis: insights from hypothalamic vasopressin neurons. *Trends Neurosci* 29:108–115
24. Bicknell RJ (1988) Optimizing release from peptide hormone secretory nerve terminals. *J Exp Biol* 139:51–65
25. Dutton A, Dyball RE (1979) Phasic firing enhances vasopressin release from the rat neurohypophysis. *J Physiol* 290:433–440
26. Bicknell RJ, Brown D, Chapman C, Hancock PD, Leng G (1984) Reversible fatigue of stimulus-secretion coupling in the rat neurohypophysis. *J Physiol* 348:601–613
27. Leng G, Brown D (1997) The origins and significance of pulsatility in hormone secretion from the pituitary. *J Neuroendocrinol* 9:493–513
28. Leng G, Brown CH, Russell JA (1999) Physiological pathways regulating the activity of magnocellular neurosecretory cells. *Prog Neurobiol* 57:625–655
29. Lincoln DW, Wakerley JB (1975) Neurosecretory activation in the rat: correlation of the suckling stimulus with the pulsatile release of oxytocin. *J Physiol* 245:42P–43P
30. Belin V, Moos F (1986) Paired recordings from supraoptic and paraventricular oxytocin cells in suckled rats: recruitment and synchronization. *J Physiol* 377:369–390
31. Wakerley JB, Lincoln DW (1973) Proceedings: unit activity in the supra-optic nucleus during reflex milk ejection. *J Endocrinol* 59:xlvi–xlvi
32. Lincoln DW, Wakerley JB (1974) Electrophysiological evidence for the activation of supraoptic neurones during the release of oxytocin. *J Physiol* 242:533–554
33. Ingram CD, Wakerley JB (1993) Post-partum increase in oxytocin-induced excitation of neurones in the bed nuclei of the stria terminalis in vitro. *Brain Res* 602:325–330
34. Dyball RE, Leng G (1986) Regulation of the milk ejection reflex in the rat. *J Physiol* 380:239–256
35. Summerlee AJ (1981) Extracellular recordings from oxytocin neurones during the expulsive phase of birth in unanaesthetized rats. *J Physiol* 321:1–9
36. Summerlee AJ, Lincoln DW (1981) Electrophysiological recordings from oxytocinergic neurones during suckling in the unanaesthetized lactating rat. *J Endocrinol* 90:255–265
37. Brown D, Fontanaud P, Moos FC (2000) The variability of basal action potential firing is positively correlated with bursting in hypothalamic oxytocin neurones. *J Neuroendocrinol* 12:506–520
38. Moos F, Poulain DA, Rodriguez F, Guerne Y, Vincent JD, Richard P (1989) Release of oxytocin within the supraoptic nucleus during the milk ejection reflex in rats. *Exp Brain Res* 76:593–602
39. Moos F, Richard P (1988) Characteristics of early- and late-recruited oxytocin bursting cells at the beginning of suckling in rats. *J Physiol* 399:1–12
40. Moos F, Richard P (1989) Paraventricular and supraoptic bursting oxytocin cells in rat are locally regulated by oxytocin and functionally related. *J Physiol* 408:1–18
41. Ludwig M, Leng G (2006) Dendritic peptide release and peptide-dependent behaviours. *Nat Rev Neurosci* 7:126–136
42. Ludwig M, Sabatier N, Bull PM, Landgraf R, Dayanithi G, Leng G (2002) Intracellular calcium stores regulate activity-dependent neuropeptide release from dendrites. *Nature* 418:85–89

43. Russell JA, Leng G (2000) Veni, vidi, vici: the neurohypophysis in the twentieth century. *Exp Physiol* 85 Spec No:1S–6S
44. Kalia M, Sullivan JM (1982) Brainstem projections of sensory and motor components of the vagus nerve in the rat. *J Comp Neurol* 211:248–265
45. Weiss ML, Hattori GI (1990) Collateral input to the paraventricular and supraoptic nuclei in rat. II. Afferents from the ventral lateral medulla and nucleus tractus solitarius. *Brain Res Bull* 25:561–567
46. Ueta Y, Kannan H, Yamashita H (1991) Gastric afferents to the paraventricular nucleus in the rat. *Exp Brain Res* 84:487–494
47. Verbalis JG, McCann MJ, McHale CM, Stricker EM (1986) Oxytocin secretion in response to cholecystokinin and food: differentiation of nausea from satiety. *Science* 232:1417–1419
48. Day TA, Sibbald JR (1988) Direct catecholaminergic projection from nucleus tractus solitarius to supraoptic nucleus. *Brain Res* 454:387–392
49. Day TA, Sibbald JR (1988) Solitary nucleus excitation of supraoptic vasopressin cells via adrenergic afferents. *Am J Physiol* 254:R711–R716
50. Meddle SL, Leng G, Selvarajah JR, Bicknell RJ, Russell JA (2000) Direct pathways to the supraoptic nucleus from the brainstem and the main olfactory bulb are activated at parturition in the rat. *Neuroscience* 101:1013–1021
51. Herbison AE, Voisin DL, Douglas AJ, Chapman C (1997) Profile of monoamine and excitatory amino acid release in rat supraoptic nucleus over parturition. *Endocrinology* 138:33–40
52. Renaud LP, Tang M, McCann MJ, Stricker EM, Verbalis JG (1987) Cholecystokinin and gastric distension activate oxytocinergic cells in rat hypothalamus. *Am J Physiol* 253:R661–R665
53. Moriarty P, Dimaline R, Thompson DG, Dockray GJ (1997) Characterization of cholecystokinin A and cholecystokinin B receptors expressed by vagal afferent neurons. *Neuroscience* 79:905–913
54. Velmurugan S, Brunton PJ, Leng G, Russell JA (2010) Circulating secretin activates supraoptic nucleus oxytocin and vasopressin neurons via noradrenergic pathways in the rat. *Endocrinology* 151:2681–2688
55. Russell JA, Blackburn RE, Leng G (1988) The role of the AV3V region in the control of magnocellular oxytocin neurons. *Brain Res Bull* 20:803–810
56. Clarkson J, Han SK, Liu X, Lee K, Herbison AE (2010) Neurobiological mechanisms underlying kisspeptin activation of gonadotropin-releasing hormone (GnRH) neurons at puberty. *Mol Cell Endocrinol* 324:45–50
57. Dhillo WS, Murphy KG, Bloom SR (2007) The neuroendocrine physiology of kisspeptin in the human. *Rev Endocr Metab Disord* 8:41–46
58. Brown RE, Imran SA, Ur E, Wilkinson M (2008) KiSS-1 mRNA in adipose tissue is regulated by sex hormones and food intake. *Mol Cell Endocrinol* 281:64–72
59. Horikoshi Y, Matsumoto H, Takatsu Y, Ohtaki T, Kitada C, Usuki S, Fujino M (2003) Dramatic elevation of plasma metastin concentrations in human pregnancy: metastin as a novel placenta-derived hormone in humans. *J Clin Endocrinol Metab* 88:914–919
60. Bilban M, Ghaffari-Tabrizi N, Hintermann E, Bauer S, Molzer S, Zoratti C, Malli R, Sharabi A, Hiden U, Graier W, Knofler M, Andrae F, Wagner O, Quaranta V, Desoye G (2004) Kisspeptin-10, a KiSS-1/metastin-derived decapeptide, is a physiological invasion inhibitor of primary human trophoblasts. *J Cell Sci* 117:1319–1328
61. Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, Calamari A, Szekeres PG, Sarau HM, Chambers JK, Murdock P, Steplewski K, Shabon U, Miller JE, Middleton SE, Darker JG, Larminie CG, Wilson S, Bergsma DJ, Emson P, Faull R, Philpott KL, Harrison DC (2001) AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *J Biol Chem* 276:28969–28975
62. Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M (2001) Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* 411:613–617

63. Holzer P (1991) Capsaicin as a tool for studying sensory neuron functions. *Adv Exp Med Biol* 298:3–16
64. Patterson LM, Zheng H, Ward SM, Berthoud HR (2003) Vanilloid receptor (VR1) expression in vagal afferent neurons innervating the gastrointestinal tract. *Cell Tissue Res* 311:277–287
65. Han SK, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, Clifton DK, Steiner RA, Herbison AE (2005) Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J Neurosci* 25:11349–11356
66. Liu X, Lee K, Herbison AE (2008) Kisspeptin excites gonadotropin-releasing hormone neurons through a phospholipase C/calcium-dependent pathway regulating multiple ion channels. *Endocrinology* 149:4605–4614
67. Fu LY, van den Pol AN (2010) Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism. *J Neurosci* 30:10205–10219
68. Arai AC, Xia YF, Suzuki E, Kessler M, Civelli O, Nothacker HP (2005) Cancer metastasis-suppressing peptide metastatin upregulates excitatory synaptic transmission in hippocampal dentate granule cells. *J Neurophysiol* 94:3648–3652
69. Roa J, Vigo E, Castellano JM, Navarro VM, Fernandez-Fernandez R, Casanueva FF, Dieguez C, Aguilar E, Pinilla L, Tena-Sempere M (2006) Hypothalamic expression of KiSS-1 system and gonadotropin-releasing effects of kisspeptin in different reproductive states of the female rat. *Endocrinology* 147:2864–2878
70. Desroziers E, Mikkelsen J, Simonneaux V, Keller M, Tillet Y, Caraty A, Franceschini I (2010) Mapping of kisspeptin fibres in the brain of the pro-oestrous rat. *J Neuroendocrinol* 22:1101–1112
71. Herbison AE, de Tassigny X, Doran J, Colledge WH (2010) Distribution and postnatal development of Gpr54 gene expression in mouse brain and gonadotropin-releasing hormone neurons. *Endocrinology* 151:312–321
72. Reynolds RM, Logie JJ, Roseweir AK, McKnight AJ, Millar RP (2009) A role for kisspeptins in pregnancy: facts and speculations. *Reproduction* 138:1–7