The Role of Neurokinin B Signalling in Reproductive Neuroendocrinology

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Abstract

The KNDy neuropeptides, kisspeptin, neurokinin B (NKB) and dynorphin A (Dyn), have been implicated in regulating puls Absatilte ract luteinising hormone (LH) secretion. Studies of the interactions between KNDy signalling systems, however, arecurrently few. Although the stimulatory effect of kisspeptin and the inhibitory effect of Dyn on the gonadotropin-releasing hormone pulse generator are widely accepted, the effects of NKB in rodents are variable and sometimes controversial. Literature describing increased LH secretion in response to NKB receptor agonism predominates and is in line with human physiology, as well as the pathophysiology of pubertal failure associated with disruption of NKB signalling. However, the robust suppression of the LH pulse, induced by the same treatment under hypoestrogenic conditions, may hold clues as to the mechanisms of reproductive inhibition under pathological conditions. This review discusses the recent evidence for this paradox and outlines a revised working model incorporating the mechanisms by which KNDy neuropeptides modulate the reproductive axis.

Key Words
Neurokinin B · Kisspeptin · Dynorphin A · Gonadotropin-releasing hormone · Luteinising hormone

Introduction

Episodic release of gonadotropin-releasing hormone (GnRH) into the hypophyseal portal circulation coordi-nates the pituitary secretion of gonadotropins (luteinising hormone, LH, and follicle-stimulating hormone, FSH), which stimulate the gonads to produce gametes and secrete sex steroids (oestrogens, progesterone and testosterone) and, thereby, drive the reproductive system [1] . Although GnRH neurones have been shown to possess inherent rhythmicity and an ability to synchronise firing [2–5] , bursts of electrical activity of GnRH neurones in vitro/ex vivo rarely occur at frequencies concomitant with LH pulses in the living animal [6] . To describe the pulsatile release of GnRH/LH, the term GnRH pulse generator was coined [7] . Despite over 40 years of ensuing research this elusive entity is still considered a necessary concept to explain the discrepancy in the frequencies of isolated GnRH neurone firing and pulses of LH in the systemic circulation. It is well recognised that peptidergic signalling mechanisms modulate the GnRH pulse generator [8] and are thus important regulatory components of the hypothalamic-pituitary-gonadal (HPG) axis. In particular, the neuropeptides neurokinin B (NKB) [9] and kisspeptin [10, 11] have been shown to be indispensable for physiological development and function of the human reproductive system. Their colocalisation with an opioid peptide, dynorphin A (Dyn) within a subset of neurones in the hypothalamic arcuate nucleus (ARC) [12] , which project to the medial preoptic area (mPOA) [13] and median eminence (ME) [14] where contacts with GnRH neurones have been documented in several species [14–20] (see [21] for a recent review), raises great interest in this kisspeptin-NKB-Dyn neuronal popula-tion (referred to as KNDy neurones) as the possible substrate of the GnRH pulse generator

. Mechanisms of KNDy Signalling

The mechanisms whereby kisspeptin influences the HPG axis seem relatively straightforward: GnRH neurones express kisspeptin receptors (GPR54) [17, 22], are exquisitely sensitive to kisspeptin originating from neurones of the ARC [23] and the anteroventral periventricular nucleus [17, 24, 25], as well as exogenous kisspeptin [26–30], and are consistently driven to secrete GnRH in either a phasic or continuous fashion apparently dependent on the frequency of kisspeptinergic stimulation [31, 32]. However, the kisspeptin-dependent nature of the HPG axis is challenged by a report that mice lacking kisspeptin neurones exhibited normal reproductive development, whereas kisspeptin neurone ablation in adulthood rendered mice infertile, suggesting the development of compensatory mechanisms [33].

Dyn has long been known to decrease GnRH pulse frequency [34], and its primary role as such has been postulated to be in mediating the negative feedback effects of progesterone (P_4) on the neuroendocrine hypothalamus [35]. The κ-opioid receptor (KOR), through which Dyn preferentially signals [36], is not abundantly expressed in the vicinity of GnRH neurones [37, 38]. It was, therefore, hypothesised that Dyn neurones affect GnRH secretion via a yet undiscovered intermediary signalling system. Currently accepted models propose that kisspeptin acts as a trigger for GnRH release, while Dyn terminates each GnRH pulse [39], although conclusive evidence for the latter notion is currently lacking. A plausible hypothesis is that Dyn, released from ARC KNDy neurones, acts in an autocrine or paracrine modality to inhibit the activity of kisspeptin neurones. Indeed, KOR is expressed by mouse KNDy neurones [35], and application of KOR agonists to ex vivo murine hypothalamic slices strongly suppresses the firing rate of GFP-labelled ARC kisspeptin neurones [40, 41]. However, the stimulatory effects of the KOR antagonist norbinaltorphimine on pulsatile LH secretion in the rat seem to be estradiol (E_2)-dependent [42], suggesting that a Dyn-ergic tone endogenous to the ARC may only participate in GnRH pulse regulation in the presence of physiological levels of gonadal steroids. This observation conflicts with the notion that Dyn might terminate GnRH pulses, since pulsatile LH secretion is preserved in postmenopausal women [43] and ovariectomised (OVX) rodents [44, 45] alike.

In contrast to kisspeptin/GPR54 signalling, the mechanisms by which NKB signalling influences HPG axis function appear more complex. NKB preferentially binds and activates the neurokin 3 receptor (NK3R), although promiscuous binding to other neurokinin receptor subtypes has been implicated in the modulatory actions of NKB signalling in rodents [40, 46]. The initial report of suppressed LH secretion following selective activation of NK3R in OVX + E_2 rats [47] prompted similar experiments in other species. Subsequent reports of stimulation of LH secretion in response to administration of the selective NK3R agonist, senktide, in the mouse [35], rat [48], goat [49], sheep [50, 51] and monkey [52, 53], together with the discovery that inactivating mutations in human genes encoding NKB (*TAC3*) and NK3R (*TACR3*) result in hypogonadotropic hypogonadism [9, 54–56], questioned the previous report of HPG axis inhibition by central administration of the agonist [47]. A mechanism for NKB stimulation of LH secretion was then proposed, when it was shown that the increase in LH secretion associated with NK3R agonism is dependent upon kisspeptin/GPR54 signalling in the prepubertal male monkey [53]. This was supported by the observation that kisspeptin infusion restored LH pulsatility in humans with inactivating mutations in *TAC3* or *TACR3* [54]. In the light of apparent species differences, it was required to determine whether a similar kisspeptinergic mechanism underlies the NKB-induced stimulation of LH secretion in rodents. The LH-secreting action of senktide was indeed absent in GPR54 knockout mice [57]. It was subsequently confirmed that the GnRH pulse generator in ovary-intact prepubertal female rats responds to intracerebroventricular administration of senktide by eliciting single LH pulses in a kisspeptin/GPR54-dependent fashion [58]. The physiological significance of this peptidergic mechanism is, however, questioned by the report that mice lacking functional NK3R are capable of pubertal development and fertility [59], which contrasts with observations in humans [9, 54–56]. Either way, evidence for both stimulatory and inhibitory effects of NKB on the rat reproductive axis [44, 45, 47, 48] highlights the importance of elucidating the underlying mechanisms in different physiological states and in a range of species.

It was demonstrated that, at least in the female, gonadal status determines the response of the HPG axis to treatment with agonists of NK3R. In the OVX rat, intracerebroventricular administration of senktide significantly reduced LH levels [48]. Conversely, senktide caused an increase in LH secretion in OVX rats chronically replaced with proestrus levels of E_2 [48]. Moreover, in follicular-phase ewes, intracerebroventricular injection of senktide was able to increase LH secretion, but it had no effect in luteal-phase ewes [50]. This prompted our studies on the roles of gonadal steroids in modulating the effects of intracerebroventricular administration of senktide in OVX rats. These studies provided definitive evidence that the presence of neither low levels of E_2 (35.8 ± 1.2 pg/ml) nor low levels of P_4 (16.7 ± 1.5 ng/ml) [60] alters the dynamics of senktide-induced suppression of the GnRH pulse generator [44]. Ovary-intact diestrous [44, 48] and prepubertal rats [58] showed a consistent increase in LH levels following intracerebroventricular administration of senktide. Curiously, senktide arrested pulsatile secretion of LH in OVX rats replaced with E_2 levels comparable to diestrous rats (83.3 ± 14.2 pg/ml) [45]. Notably, however, more subtle changes in circulating E_2 levels, such as those occurring through the oestrous cycle, failed to reverse the stimulatory effects of senktide administration on LH secretion [48]. Taken together, these findings suggest that over a broader spectrum of E_2 concentrations the GnRH pulse generator responds differently to central NK3R agonism. The high degree of oestrogen receptor-α colocalisation with NKB within ARC neurones [12, 61] provides the mechanistic machinery for these biphasic effects. There is perhaps a physiological threshold of circulating E_2 levels, which sees reversal of the NKB-induced stimulation of LH secretion. This threshold is likely to be species- and even strain-specific, since the polymorphisms that distinguish the structures of oestrogen receptor-α expressed by different rat strains [62] are potentially capable of giving rise to differences in sensitivity to E_2 [63–67]. However, the mechanism whereby NKB can both stimulate and suppress LH secretion remains cryptic.

Mechanisms of NKB Suppression of the GnRH Pulse Generator

Because both stimulatory and inhibitory effects of central senktide administration are observed in the female rat, NKB was hypothesised to have dual and opposite roles in the control of the GnRH pulse generator. The blockade of senktide-induced LH pulses in prepubertal rats by pretreatment with a GPR54 antagonist [58], as well as an absence of senktide-induced stimulation of LH secretion by GPR54 knockout in male mice [57], suggests that GnRH neurones are stimulated by kisspeptin released as a result of NK3R activation. However, the mechanism by which senktide is able to suppress LH pulses under hypoestrogenic conditions (OVX +/– low E_2/P_4) is unclear. Senktide administered intracerebroventricularly can reach and activate NK3R expressed by a wide range of neuronal populations [68, 69]. Therefore, to determine whether the ARC population of KNDy neurones is involved in mediating the observed suppression of pulsatile LH secretion in response to intracerebroventricular administration of senktide, we targeted injections of the NK3R agonist to exclusively reach the ARC. In OVX rats, intra-ARC administration of senktide achieved a similar dose-dependent suppression of LH secretion to that observed with intracerebroventricular administration, albeit at a fraction of the dose [45]. Senktide administered through misplaced cannulae had no effect on LH secretion, thus confirming that ARC NKB neurones are indeed responsible for the observed suppression of the GnRH pulse generator [45]. NK3R is abundantly expressed by NKB/Dyn neurones in the rat ARC [12], as well as in ovine KNDy neurones [70], thus intra-ARC administration of senktide pharmacologically mimics the local effects of NKB-ergic volume transmission events that are suggested to underlie the suppression of the GnRH pulse generator. The notion of NKB-ergic autoregulation in the ARC is supported by the evidence for numerous bilateral interconnections between ARC NKB neurones [13, 14].

The feasibility of direct effects of KNDy peptides on the HPG axis can be assessed by means of a detailed neuroanatomical analysis of the connections between KNDy and GnRH neurones. NKB neurones of the ARC have been shown to innervate both the perikarya and axon termini of GnRH neurones. However, the latter express far more abundant NK3R [14], which strongly suggests that ARC NKB neurones projecting to the ME might suppress neurosecretion of GnRH from axon terminals. Because the extracellular milieu of the ME is separated from that of the ARC by the blood-brain barrier [71, 72], the only possible sources of NKB reaching GnRH axon terminals are the projections of ARC NKB neurones. Systemic administration of senktide should reach all NK3Rs outside the blood-brain barrier, including those expressed at terminals of GnRH axons in the ME. With this assumption, three doses of senktide (0.65–65 nmol/kg) were administered intravenously to conscious, freely moving OVX +

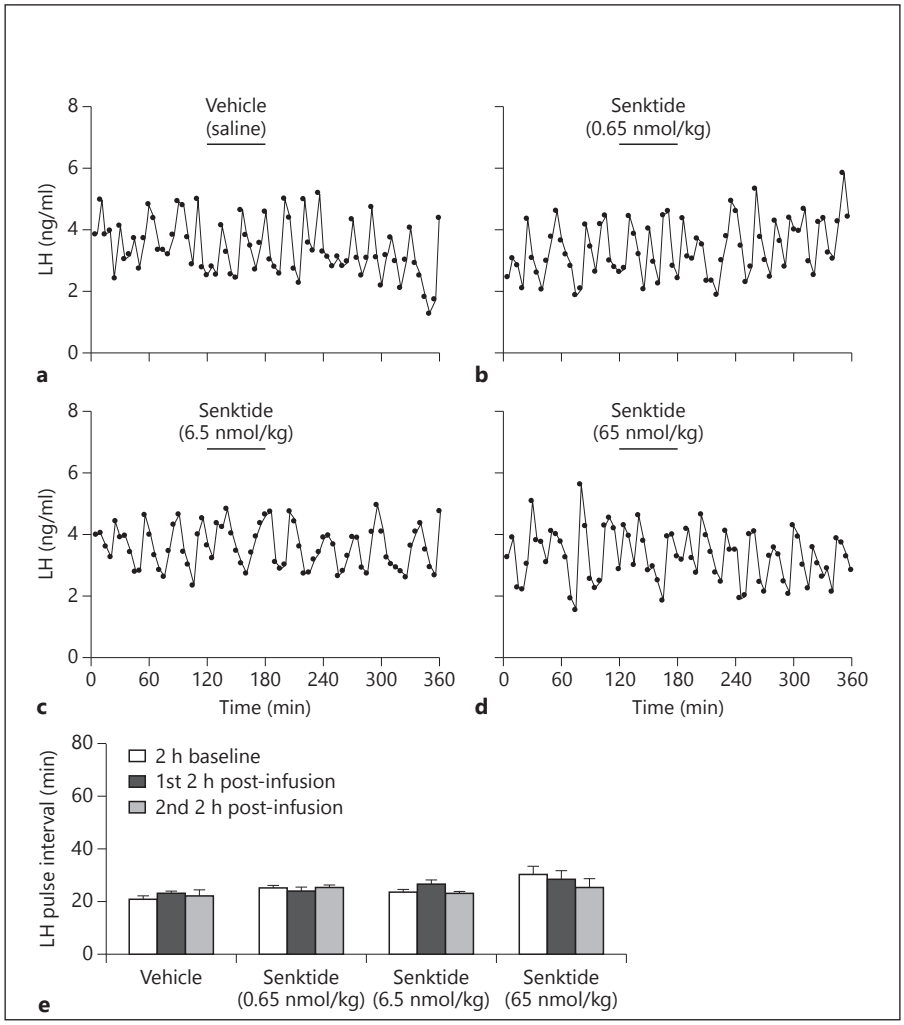

Fig. 1. Effect of peripheral administration of an NK3R agonist on pulsatile LH secre-tion. Representative LH profiles demonstrating the effect of intravenous adminis-tration of a selective NK3R agonist, senk-tide (**b–d**), or vehicle (**a**), on pulsatile LH secretion in OVX + E_2 rats. Peripheral ad-ministration of senktide had no effect on pulsatile LH secretion irrespective of the dose, as summarised in **e** (n = 4–7 per group).

E_2 rats in order to simulate efflux of NKB from projections of ARC NKB neurones into the ME. Surprisingly, none of the three doses of senktide administered this way had a detectable effect on pulsatile LH secretion within 4 h of administration (fig. 1), although comparable doses potently stimulated LH secretion in male monkeys [52]. These data are in agreement with the only previous study investigating the effect of peripheral (intraperitoneal) administration of NKB on LH secretion in the rodent [73], which also suggests a lack of an acute effect of NK3R activation outside the blood-brain barrier, including those expressed at the surface membranes of GnRH axon terminals in the ME. Therefore, the rodent blood-brain barrier is unlikely to be permeable to senktide, and there remains no functional evidence to suggest that ARC neurones secrete NKB into the ME in order to alter the pulsatile pattern of GnRH neurosecretion. Thus, the role of NK3R expressed at GnRH terminals in the ME [14] is a conundrum, particularly since it has recently been shown that GnRH terminals within male mouse brain slices secrete GnRH in response to bath application of senktide [74], but the electrical activity of GnRH neurones within such slices is unaffected by senktide [75]. Furthermore, unlike in the rat, ovine GnRH neurones appear to be altogether devoid of NK3R [70]. It is also important to recognise that the identification of NK3R in GnRH terminals in the ME is based on immunohistochemistry [14], and functional binding has not been demonstrated.

A possibility is that the effects of NKB secreted within the ARC are mediated through NK3R expressed along projection structures of GnRH neurones that pass through the ARC en route to the ME. In the 'artificial follicular-phase' (OVX + E_2 + P_4) ewes, some 17% of ARC KNDy

neurones receive GnRH inputs [51] and, although their origin has not been explored, such structures have been shown to simultaneously display synaptic and conductive properties typically associated with dendrites and axons, respectively [76]. In fact, it was recently reported that as many as half of female mouse ARC kisspeptin neurones are in direct contact with GnRH axon varicosities [77]. Notably, in light of these novel findings, one needs to question the robustness of previous efforts to identify NK3R/GnRH coexpression. Future studies will undoubtedly aim to localise functional NK3R expression to specific regions along GnRH processes, including the ARC.

Another possible mechanism by which ARC NKB neurones might suppress the GnRH pulse generator is through inhibition of kisspeptinergic stimulation of GnRH neurosecretion, perhaps involving Dyn/KOR signalling. Several lines of evidence serve to substantiate this hypothesis: (1) the suppression of the LH pulse by central administration of senktide is dependent on Dyn/KOR signalling [44, 45], (2) the anatomy of KNDy neurones permits direct negative autoregulation by Dyn [12, 35, 39], (3) Dyn or its synthetic analogue, U50488, have been shown to reduce the rate of spontaneous firing of murine KNDy neurones [40, 41] and (4) central administration of senktide or U50488 attenuates kisspeptin-induced LH secretion in male rats [78]. On the other hand, studies addressing this hypothesis have predominantly been conducted in animal models in which the effect of senktide on the HPG axis is stimulatory. It is hardly surprising, therefore, that electrophysiological data, such as that describing NKB- or senktide-induced KNDy neurone firing in male mice [40, 75], do not fit this hypothetical mechanism. Indeed, if similar experiments were to be performed in OVX rats, for instance, it is expected that NKB or senktide would cause local secretion of Dyn, concomitant with a period during which KNDy neurones are silent and the associated lack of kisspeptin secretion disallows episodic neurosecretion of GnRH. These predictions, although speculative, are backed by observations that LH pulses [44, 45] and ARC multiunit electrical activity volleys [44] are abrogated in OVX rats injected centrally with senktide, and are intended to stimulate further studies to interrogate the posed hypothesis.

Intriguingly, intra-ARC administration of senktide also caused a reduction in *Gnrh1* and *Kiss1r* mRNA expression in the mPOA [45]. Thus, increased NKB/NK3R signalling in the ARC can also have long-term repercussions on GnRH neurones. Because these effects were observed several hours after the recovery of the basal LH pulse from a period of senktide-induced suppression, they are likely the result of reduced GnRH neurone activity as opposed to a mechanism by which pulsatile LH secretion is diminished following intra-ARC senktide administration. Following a period of prolonged lack of activation GnRH neurones might downregulate *Gnrh1* mRNA expression in order to attenuate peptide synthesis in the presence of accumulated peptide, although further studies are required to determine whether this is the case. Furthermore, the senktide-induced reduction in mPOA *Gnrh1* and *Kiss1r* mRNA expression is reminiscent of corticotrophin-releasing factor (CRF)-mediated mechanisms that diminish GnRH pulse generator frequency and sensitivity to kisspeptinergic stimulation following acute exposure to stress [79]. For this reason, we investigated the possible involvement of CRF and related stress-associated neuropeptide, arginine vasopressin (AVP), in mediating the suppressive effects of central administration of senktide on the HPG axis. Despite evidence that central administration of NK3R agonists induces expression of c-Fos (marker of neuronal activation) in CRF and AVP neurones [80, 81] and increases adrenocorticotrophic hormone and corticosterone release [82], neither CRF nor AVP receptor antagonists prevented the senktide-induced suppression of the GnRH pulse generator [83]. ARC NKB neurones are therefore unlikely to recruit these elements of the HPA axis to suppress the GnRH pulse generator. Nevertheless, NKB/NK3R signalling does appear to be involved in the stress-induced suppression of the LH pulse, since pretreatment with an NK3R antagonist significantly reduced the extent to which acute inflammation (intravenous endotoxin challenge) suppressed pulsatile LH secretion and stimulated corticosterone release in OVX rats [83]. Future experiments should determine which neuronal populations secrete and respond to NKB as part of a mechanism of GnRH pulse generator suppression by immunological stress.

Dyn and NKB: Partners in Crime?

Of the three KNDy neuropeptides, Dyn has been the longest subject of neuroendocrine research [84] and its suppressive effects on LH secretion have been well documented over the past three decades [85]. However, the interaction of this opioid peptide with kisspeptin and NKB signalling systems has not been extensively studied. In the female rat most Dyn-immunoreactive neurones in the ARC express NK3R [12], thus it has been postulated that Dyn/KOR signalling is involved in the senktide-induced suppression of pulsatile LH secretion. Indeed, the

inhibitory effect of intracerebroventricular senktide administration on LH secretion was blocked as effectively by pretreatment with a KOR antagonist as by an NK3R antagonist [44]. Subsequently, the Dyn/KOR-dependent nature of senktide-induced LH pulse suppression was confirmed by targeting KOR antagonist and senktide administration to the ARC bilaterally [45]. These findings suggest that the intimate coexistence of NKB and Dyn within KNDy neurones is what enables ARC NKB/NK3R signalling events to generate inhibitory signals that subsequently suppress pulsatile secretion of GnRH and therefore LH.

Confirmation of the involvement of Dyn/KOR signalling did not provide a comprehensive understanding of the mechanism of senktide-induced suppression of the GnRH pulse generator. Although Dyn/NKB fibres have been detected (using immunocytochemistry) throughout the hypothalamus, including the ME and mPOA [12], in situ hybridisation studies have not detected KOR mRNA in GnRH neurones [37, 38]. Moreover, although Goodman et al. [86] revealed Dyn-ergic synapses between Dyn axon terminals and mPOA GnRH somata using electron microscopy, they were reluctant to comment on the functional importance of this observation without evidence of KOR expression by ovine GnRH neurones, which to date has not materialised. In contrast, publications from the Gallo laboratory provide extensive functional evidence for sensitivity of GnRH neurones to Dyn [87–89]. Indeed, the authors show that perfusion of the mPOA with a selective KOR antagonist increased frequency of LH pulses in pregnant rats [87] and prevented the Dyn-mediated suppression of the LH surge in proestrus rats [88]. Furthermore, they demonstrated that anti-Dyn antibodies infused into the mPOA are capable of advancing the LH surge [89]. Together with the evidence of decreased mPOA expression of Dyn mRNA immediately prior to the LH surge [89], these findings strongly support the notion of dynorphinergic inhibition of GnRH neurone activity in the female rat. However, it is evident that the authors of these reports have been unable to provide adequate morphological evidence of KOR expression within mPOA GnRH neurones, since the only reference to such literature is a review article that mentions KOR expression in the POA without distinguishing between substructures or neuronal populations found therein [90]. Moreover, Zhen and Gallo [87, 91] make reference to previous efforts from their laboratory that showed that disinhibition of GnRH secretion following KOR blockade is a noradrenaline-dependent phenomenon, which contradicts their claims of direct action of Dyn on GnRH neurones. The authors cite a poster abstract as the basis of these experiments, which implies that intra-mPOA administration of Dyn suppresses the local noradrenaline tone [92]. The credibility of claims that GnRH neurones possess inherent (and direct) sensitivity to Dyn, arising solely from the laboratory of Gallo [87–89, 91], is compromised by the observation that μ- and Δ-opioid receptor agonists, but not KOR agonists, were able to suppress the noradrenaline tone within rat mPOA slices [93]. Collectively, these data do not dispel the controversy over the mechanisms of GnRH pulse generator modulation by KNDy neuropeptide systems. Future studies will need to detect KOR expression within GnRH neurones using methods other than in situ hybridisation, such as dual-label immunocytochemistry and labelled ligand binding, to demonstrate more robustly the presence of KOR on GnRH neurones in support of a direct mechanism of Dyn-induced suppression of the GnRH pulse generator. Furthermore, the notion of noradrenergic disinhibition, as a consequence of decreased KNB/Dyn-mediated inhibitory tone, should be scrutinised as the mechanism of increased LH secretion, especially since mPOA noradrenaline signalling appears to play a key role in facilitating the transmission of various afferent signals that converge on GnRH neurones [94].

Revising the Model

Dyn/KOR signalling appears to be of less importance under conditions that favour kisspeptin/GPR54-dependent LH pulse generation in response to stimulation of central NK3R. This is the case in ovary-intact prepubertal rats: neither augmentation nor attenuation of Dyn/KOR signalling (by intracerebroventricular administration of KOR agonist or antagonist) had an effect on basal LH secretion [58]. Furthermore, KOR agonism/antagonism did not affect the senktide-induced LH pulses in this animal model [58]. These findings are consistent with previous reports of reduced ability of the non-selective opioid receptor antagonist, naloxone, to increase LH secretion in prepubertal mammals [95–101] and suggest that a reduction in sensitivity to Dyn might disinhibit the HPG axis, facilitating progression into puberty. Likewise, GnRH neurones are apparently able to respond to exogenous kisspeptinergic stimulation during a period of senktide-induced, Dyn/KOR-dependent suppression of pulsatile LH secretion [44]. This suggests that ARC NK3R activation diminishes the endogenous kisspeptin tone under hypoestrogenic conditions and confirms that kis-

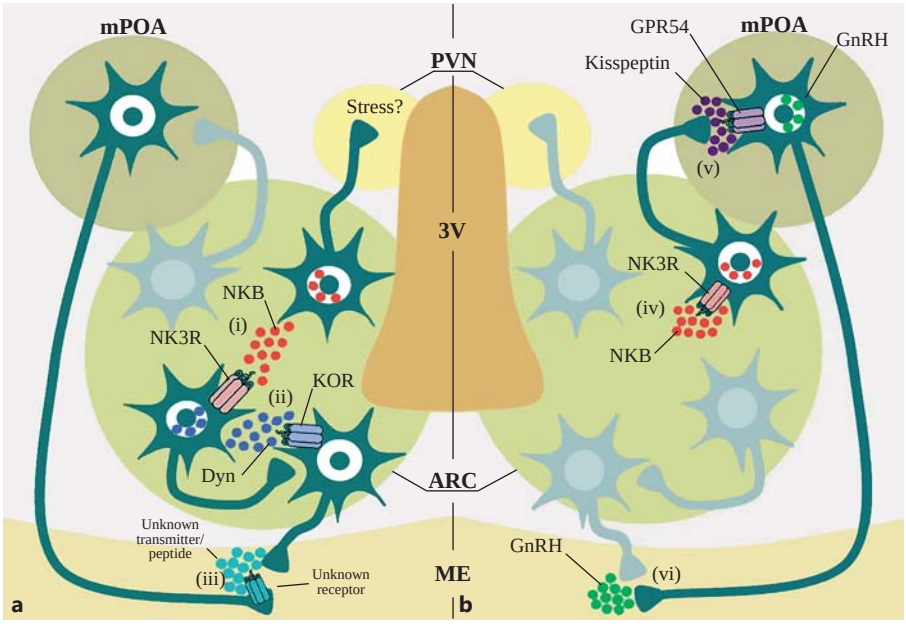

Fig. 2. A model summarising the hierarchical functional relation-ship between the KNDy signalling systems in the regulation of the HPG axis. NKB is the upstream bimodal regulator of GnRH neurosecretion through kisspeptin/GPR54 or Dyn/KOR signalling systems. Under conditions favouring the suppression of the HPG axis (**a**), ARC NK3R activation (i) mobilises Dyn, which ac-tivates KOR expressed by interneurones (ii) that project to termi-nals of GnRH neurones in the ME. This generates an NKB-driven, Dyn/KOR-dependent inhibitory stimulus that suppresses GnRH secretion via an unknown signalling system (iii). Activation of parvocellular neurones in the PVN in response to stress might activate such inhibitory circuitry to suppress the GnRH pulse gen-erator. Conversely, under conditions that preferentially increase GnRH secretion (b) the binding of NKB to NK3R on ARC kiss-peptin neurones (iv) projecting to GnRH perikarya in the mPOA stimulates kisspeptin secretion, which activates GPR54 on GnRH neurones (v), augmenting GnRH synthesis and/or release (vi). KNDy neurones may function as the GnRH pulse generator, me-diating the influence of extrinsic stimuli, which prescribe the con-ditions for the switch between the stimulatory and inhibitory pathways in this system, on the GnRH pulse. PVN = Paraventric-ular nucleus.

speptin is downstream of NKB in the functional hierarchy of KNDy neuropeptide signalling systems. It is apparent that two distinct downstream signalling systems that mediate the opposite effects of central senktide administration on LH secretion are differentially regulated by the gonadal steroid milieu: in a low-E_2 environment (such as that in OVX rats with or without replacement of E_2 to yield circulating levels comparable to those at diestrus) senktide recruits a Dyn/KOR signalling mechanism to suppress the LH pulse [44, 45] (fig. 2a), while activation of NK3R elicits kisspeptin-induced LH pulses in intact prepubertal female rats [58] and increases LH levels in diestrous rats [44] (fig. 2b).

The E_2 threshold at which there is a reversal of the effects of senktide on LH secretion has yet to be determined. However, such a threshold is apparent from the direct comparison of the response of OVX rats, replaced with low (diestrus) [45] and high (proestrus) [48] levels of E_2, to central senktide administration. In ovary-intact animals experiencing perhaps less extreme and/or comparatively short-term changes in E_2 as part of the oestrous cycle [48] than those conferred by OVX and chronic E_2 replacement, there is no obvious reversal of the effects of central senktide administration on LH secretion. Studies to address this discrepancy, as well as the underlying mechanism, are currently ongoing in our laboratory.

The notion of a stimulatory role of NKB in driving HPG axis function through partnership with kisspeptin concurs with the situation in human physiology and pathophysiology. Levels of NKB message [102] and immunoreactivity [103], as well as *Kiss1* and *Kiss1r* mRNA expression [104, 105], increase through the prepubertal period, peaking at puberty. Moreover, exogenous kisspeptin is able to advance pubertal onset in the rat [106], while administration of a kisspeptin antagonist delays puberty in this animal model [107]. Similarly, hypertrophy of kisspeptin and NKB neurones can be observed in postmenopausal women and OVX rats, concomitant with an

increase in GnRH secretion and LH levels following the decline in E_2 negative feedback [108]. Likewise, a role for NKB in stimulating GnRH neurosecretion via a kisspeptinergic mechanism in experimental animal models is supported by reports of human loss-of-function mutations in *TAC3* and *TACR3*, causing decreased gonadotropin levels, hypogonadism and infertility [9, 55, 56]. Moreover, pulsatile secretion of LH is restored in these patients by administration of kisspeptin-10 [54], suggesting that NKB operates upstream of kisspeptin in an autostimulatory fashion. Inactivating mutations in *KiSS1R* cause a similar hypogonadotropic hypogonadism phenotype in humans [10, 109], and gain-of-function mutations in *KiSS1* and *KiSS1R* result in GnRH-dependent precocious puberty [110, 111]. Taken together, these data highlight the intimate synergistic relationship between the kisspeptin/GPR54 and NKB/NK3R signalling systems in the regulation of reproductive function. However, the physiological relevance of the ability of NKB to suppress the HPG axis through recruitment of Dyn/KOR signalling is less clear.

It is apparent that absent or low E_2 levels convert the stimulatory role of hypothalamic NKB/NK3R signalling in intact female rats to one of inhibition. Under such conditions of absent or weak negative and/or positive feedback effects of gonadal steroids, basal LH levels are high and an LH surge or ovulation are impossible. Incidence of such conditions in women are perhaps restricted to conditions of which hypoestrogenicity is either a feature or a cause (such as amenorrhoea, menopause, ovarian failure and ovariectomy), decline of ovarian function through pregnancy, lactation, hyperprolactinaemia, infection, immune pathology, stress, malnutrition, or a side effect of chronic and/or aggressive therapies. The roles of NKB/NK3R signalling under such conditions are far from clear and it remains to be established whether NKB/NK3R signalling contributes to pathology or is brought about as a protective mechanism. In either case, NK3R would resemble a promising therapeutic target.

Evidence for the importance of hypothalamic NKB signalling in the regulation of reproductive function is mounting, and recent research has documented stimulatory and inhibitory effects in a wide range of species. Although further research would be necessary to reach a consensus on the precise role of NKB signalling in reproductive neuroendocrinology, mainly to reconcile the evident species differences encountered in this field, a vast body of literature suggests that NKB/NK3R signalling contributes significantly to the peptidergic control of pulsatile GnRH neurosecretion. Emerging reports that conflict with the predominating opinion of a stimulatory role for NKB raise important questions as to the pleiotropy of neural networks that involve NKB/NK3R signalling and their potential significance in reproductive regulation under a diversity of physiological and pathophysiological conditions. In order for reliable conclusions regarding the various regulatory roles of NKB/NK3R signalling to be drawn, future research efforts should substantiate existing data from each of the commonly used animal models, as well as clinical cases, ensuring the following: (1) that observations from in vitro experiments are consolidated by data gathered using whole (ideally conscious and behaving) animals, (2) that in vivo approaches encompass a range of physiological conditions with regards to gonadal status, age and environmental factors and (3) that each proposed mechanism of action is accompanied by clear anatomical evidence for each component of a proposed neural signalling pathway in a given species.

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