Minireview: Kisspeptin/Neurokinin B/Dynorphin (KNDy) Cells of the Arcuate Nucleus: A Central Node in the Control of Gonadotropin-Releasing Hormone Secretion

Michael N. Lehman, Lique M. Coolen, and Robert L. Goodman

Department of Anatomy and Cell Biology (M.N.L., L.M.C.), The University of Western Ontario, London, Ontario, Canada N6A 5C1; and Department of Physiology and Pharmacology (R.L.G.), West Virginia University, Morgantown, West Virginia 26506

Recently, a subset of neurons was identified in the arcuate nucleus of the hypothalamus that colocalize three neuropeptides, kisspeptin, neurokinin B, and dynorphin, each of which has been shown to play a critical role in the central control of reproduction. Growing evidence suggests that these neurons, abbreviated as the KNDy subpopulation, are strongly conserved across a range of species from rodents to humans and play a key role in the physiological regulation of GnRH neurons. KNDy cells are a major target for steroid hormones, form a reciprocally interconnected network, and have direct projections to GnRH cell bodies and terminals, features that position them well to convey steroid feedback control to GnRH neurons and potentially serve as a component of the GnRH pulse generator. In addition, recent work suggests that alterations in KNDy cell peptides may underlie neuroendocrine defects seen in clinical reproductive disorders such as polycystic ovarian syndrome. Taken together, this evidence suggests a key role for the KNDy subpopulation as a focal point in the control of reproductive function in health and disease. (***Endocrinology*** **151: 3479–3489, 2010)**

Although it has been nearly 40 yr since the discovery of GnRH and its central role in reproduction (1), identification of the afferent neuronal groups and pathways through which internal hormonal signals (*e.g.* gonadal steroids, stress hormones, and nutrient signals) and external cues in the environment (*e.g.* social cues and day length) regulate GnRH release is still a major unresolved issue in the field of neuroendocrinology. Much of the recent focus on afferent control of GnRH secretion has been upon kisspeptin neurons located in the preoptic area (POA) and hypothalamus, based on the ability of mutations of the kisspeptin receptor to cause hypogonadotropic hypogonadism in humans and animal models (2, 3). Compelling evidence now suggests that kisspeptin plays a key role in conveying the feedback effects of gonadal steroid hormones on GnRH neurosecretory activity during puberty (4, 5), the estrous cycle (6), and seasonal reproductive transitions (7). In the past year, another neuropeptide, neurokinin B (NKB), has come under the same intense spotlight. Although NKB had been studied for its role in steroid feedback control of GnRH release since the 1990s (8–10), its recent celebrity comes from the discovery that human mutations in the gene encoding this peptide (called TAC3), or its receptor (TACR3), like that of the kisspeptin system, leads to a defect in the control of GnRH release and subsequent hypogonadism (11, 12). In 2007 (13), a key observation was made when both NKB and kisspeptin, along with a third peptide, dynorphin (DYN), were shown to be colocalized in a single subpopulation in the hypothalamic arcuate nucleus (ARC) of the sheep (Fig. 1). DYN is an endogenous opioid peptide (EOP) that appears to mediate the inhibitory feedback control of progesterone

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Abbreviations: ARC, Arcuate nucleus; DYN, dynorphin; E_2, estradiol; EOP, endogenous opioid peptide; ERα, α-isoform of the estrogen receptor; KOR, κ-EOP receptor; MBH, mediobasal hypothalamus; MUA, multi-unit electrical activity; NKB, neurokinin B; NK3R, high-affinity receptor for NKB; OVX, ovariectomy; PCOS, polycystic ovarian syndrome; POA, preoptic area; PR, progesterone receptor; T, testosterone; vGLUT-2, vesicular glutamate transporter-2.

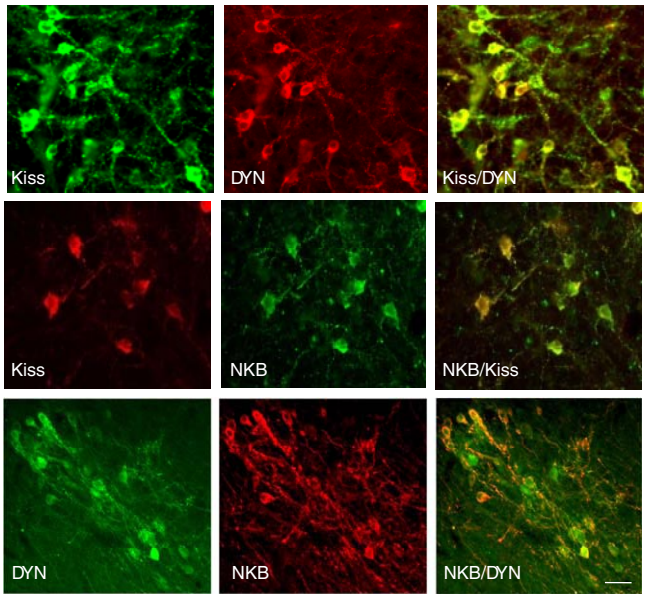

FIG. 1. Fluorescent images showing immunocytochemical colocalization of kisspeptin (Kiss) and DYN, kisspeptin and NKB, and DYN and NKB in KNDy cells of the ovine ARC. *Bar*, 20 μm. (Modified from Refs. 13 and 43.)

on GnRH secretion (14, 15). Thus, a single subpopulation of neurons in the ARC contains three distinct neuropeptides, each of which has been strongly implicated in the feedback regulation of GnRH neurons; for ease of reference [and because of its paronomastic relationship to Hershey's kiss, the origin of kisspeptin (16)], we abbreviated the name of this cell group as the KNDy (coexpressing kisspeptin, NKB, and DYN) subpopulation.

Although each of these three peptides is found in separate sets of neurons in other brain regions, recent evidence suggests that the colocalization of KNDy peptides seen in the ARC is unique among brain regions and is conserved across multiple mammalian species that include the rat (17), mouse (18), sheep (13), and goat (19) (for complete list of references, see Supplemental Table 1 published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). Complementary studies (albeit not yet multiple-label studies) suggest that kisspeptin, NKB, and DYN are also colocalized in a single subpopulation in the human infundibular (ARC) nucleus (20). Functional evidence has accumulated in parallel with these anatomical observations and strongly suggest that KNDy cells constitute a conserved, central node in the control of GnRH secretion, playing a key role in normal physiological control of reproduction as well as in abnormalities leading to reproductive endocrine disorders. In this minireview, we will provide an overview of this research, focusing on the anatomical features of KNDy cells and evidence for their roles in steroid feedback control of GnRH neurons in the generation of episodic GnRH secretion and in reproductive health and disease. It should be noted that this focus, and space constraints, preclude a detailed consideration of kisspeptin signaling; interested readers are directed to an excellent recent review on this subject (16).

Anatomical Features of KNDy Cells

A key neuroanatomical observation leading to the identification of the KNDy subpopulation was that each of the component neuropeptides (kisspeptin, NKB, and DYN) when examined in dual-label studies showed a very high degree of colocalization with gonadal hormone steroid receptors, specifically the α-isoform of the estrogen receptor (ERα) (17, 21–23), the progesterone receptors (PR) (24), and the androgen receptor (25, 26). The high degree of colocalization of KNDy cells with each of these receptors (*e.g.* in sheep, >95% for ERα and PR and >85% for androgen receptor) is particularly noteworthy when compared with other neuropeptide cell types in the ARC, such as β-endorphin (20% of which colocalize ERα) (27), neuropeptide Y (15% of which colocalize ERα) (28), and A12 dopaminergic cells (5–15% of which colocalize ERα) (29).

The question of whether KNDy cells directly contact GnRH neurons is key to understanding their functional role but is difficult to examine using standard tract-tracing techniques due to the scattered distribution of the GnRH population. Because colocalization of the three KNDy peptides in the ARC appears to be unique among brain areas examined to date, colocalization of multiple KNDy peptides in the same axon terminal can be used to determine the efferent targets of this subpopulation. Using this approach, we have demonstrated in the sheep that KNDy cells provide direct inputs to GnRH neurons in the POA as well as in the mediobasal hypothalamus (MBH) at the level of their cell bodies and dendrites (Fig. 2, A and B). This is consistent with data in the rat (30, 31), mouse (32), rhesus monkey (33), and human (34) showing that kisspeptin, NKB, or DYN fibers directly contact GnRH cell bodies, although it is not known from these studies whether the inputs derive specifically from KNDy cells or from single-labeled NKB or kisspeptin cells located elsewhere. This evidence that KNDy neurons project to GnRH cell bodies in the POA is consistent with work in ewes using retrograde tract tracing that demonstrated ERα-containing neurons in the ARC projecting to the POA (35). Although one study concluded that ARC neurons do not project directly to POA GnRH neurons (36), the anterograde tracer in these two ewes appears to have been injected into the rostral ARC, where there are few KNDy cell bodies (13).

There is also evidence that KNDy cells project to the median eminence in rodents, monkeys, and sheep and es-

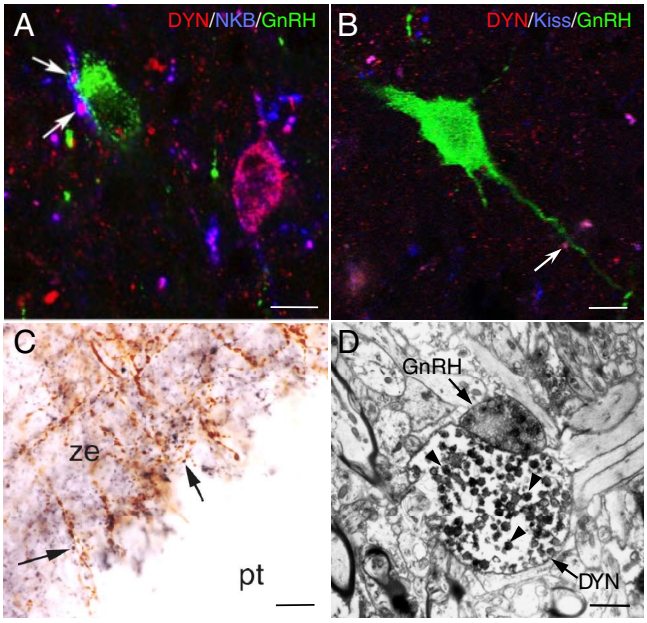

FIG. 2. Evidence that KNDy cells directly contact GnRH cell bodies (A and B) and terminals in the median eminence (C and D) in the sheep. A and B, Confocal images (1-μm optical sections) of triple-labeled sections through the ovine MBH showing DYN/NKB-positive (A) and DYN/Kiss-positive (B) axon terminals in close contact with a GnRH soma (A) and dendrite (B), respectively. Note, in A, the presence of a DYN/NKB-positive KNDy cell body nearby. *Bars* in A and B, 10 μm. C and D, Light microscopic (C) and electron microscopic (D) evidence of direct contacts between DYN and GnRH-positive terminals in the median eminence of a luteal-phase ewe. The section shown in C was processed for dual-label immunoperoxidase detection of DYN (*blue-black*) and GnRH (*brown*) and shows close associations (*arrows*) between DYN and GnRH fibers. D is an electron micrograph of dual-labeled DYN/GnRH section through the median eminence, generated using a preembedding dual-immunoperoxidase technique (110). A terminal containing DYN-positive dense-core vesicles (*arrowheads*) is seen in direct contact to a GnRH terminal that contains large neurosecretory granules. pt, Pars tuberalis; ze, zona externa of the median eminence. *Bars* in C and D, 10 μm (C) and 2 μm (D).

tablish close contacts with GnRH terminals in the external zone (30, 33, 37), although whether these terminals contain the appropriate receptors remains to be determined. In the sheep, fibers containing KNDy peptides form contacts onto GnRH terminals at both the light microscopic and electron microscopic level (Fig. 2, C and D), and preliminary results from retrograde tracing studies have confirmed that these inputs arise from KNDy cells of the ARC (38). Similarly, in the rat and monkey, there are close contacts between terminals containing kisspeptin or NKB and GnRH terminals in the median eminence at both the light microscopic (33, 37) and electron microscopic (30) level, and anterograde tracer injections into the rat ARC label KNDy peptide-containing axons projecting to the median eminence (39).

Thus, a body of evidence (see Supplemental Table 2) strongly suggests that KNDy cells, and individual KNDy peptides, can influence the activity of GnRH cells by acting directly at the level of their cell bodies and/or their neurosecretory terminals in the median eminence. Recent studies on the stimulatory effects of kisspeptin are consistent with actions at both the median eminence (40) and GnRH cell bodies in the POA (41, 42). However, evidence that some KNDy projections directly contact GnRH cells and terminals does not preclude the possibility that projections of KNDy cells to interneurons, such as single-labeled DYN or kisspeptin cells in the POA (13, 43), play a major role in the control of GnRH release.

One of the most interesting features of KNDy cells is that most appear to receive input from other KNDy cells and thus form a reciprocally interconnected network within the ARC (17, 24, 39). KNDy-KNDy cell connections have been detected in the sheep and rat ARC (17, 24), where axon terminals labeled for one or more KNDy peptides are in direct contact with KNDy-positive cell bodies. In the sheep, these contacts are seen in more than 90% of all KNDy neurons and have been confirmed at the electron microscopic level as representing *bona fide* synapses (24). Reciprocal contacts are absent among single-labeled DYN cells (24) and appear much less frequently among single-labeled kisspeptin cells (Lehman, M. N., unpublished observations) located in the POA in sheep; hence, this feature appears to be another distinguishing characteristic of the KNDy subpopulation. Based on this, it has been hypothesized that the KNDy network may play a role in the generation of episodic GnRH (see below) (18, 44) as well as in the coordination/amplification of responses to internal and external signals that KNDy cells are attuned to (*e.g.* gonadal steroids). In either case, one would predict that appropriate postsynaptic receptors to KNDy peptides should also be expressed within KNDy cells. At present, this appears to be true for at least two of the three KNDy peptide receptors: the high-affinity receptor for NKB (NK3R) is colocalized in KNDy cells in rats (37), mice (18), and sheep (45), and the κ-EOP receptor (KOR), the opioid receptor subtype with highest specificity for DYN, is expressed within KNDy neurons in the mouse (18). Although the kisspeptin receptor (*Kiss1r*) is expressed predominantly in GnRH cells, it is also found in other hypothalamic regions but, at least in mice, is not expressed in the ARC nucleus, suggesting a lack of colocalization within KNDy cells (46).

Although we have defined KNDy cells on the basis of the presence of three peptides, this subpopulation may well contain other neurotransmitters as well as other receptors. Evidence in rats (30) and sheep (47) indicates that KNDy cells and their terminals colocalize the vesicular glutamate transporter-2 (vGLUT-2), suggesting that they are glutamatergic as well as peptidergic in phenotype. A majority of vGLUT-2-positive cells in the ARC of the sheep, like KNDy cells, are colocalized with ERα (48), and

given the role of glutamate in conveying the feedback influence of estradiol (E_2) during the preovulatory GnRH surge (49), it is tempting to speculate that coordinated release of kisspeptin and glutamate provide dual stimulatory signals to activate either the KNDy subpopulation and/or GnRH neurons during the follicular phase of the estrous cycle. Although there is evidence that *N*-methyl-D-aspartic acid receptors are expressed in GnRH cell bodies (50, 51) as well as in GnRH terminals at the level of the median eminence (52), it is not known whether KNDy cells express these or other glutamate receptor subtypes.

Role in Steroid Feedback

In females, two modes of GnRH/LH secretion occur at different times during the ovarian cycle (53). Tonic GnRH/LH is secreted in an episodic pattern throughout most of the cycle and is controlled by the negative feedback actions of ovarian steroids, with E_2 inhibiting pulse amplitude and progesterone inhibiting pulse frequency (54). The preovulatory GnRH/LH surge occurs at the end of the follicular phase and is induced by the positive feedback actions of high E_2 concentrations from the preovulatory follicle(s). There is growing evidence implicating KNDy neurons as mediators for ovarian steroid feedback, but different peptide components within these neurons appear to mediate different types of steroid feedback.

Recent work has provided strong support that kisspeptin in ARC neurons mediates the negative feedback actions of E_2 (16). In mice (16, 23, 55), sheep (56), and primates (57), *in situ* hybridization techniques have demonstrated that ovariectomy (OVX) increases and E_2 decreases the number of *Kiss1*-expressing neurons in the ARC but not in more rostral areas (16, 23, 55, 56), and a similar increase is observed in postmenopausal women (57). In mice, this effect of E_2 is mediated by ERα (23), the receptor implicated in the negative feedback actions of E_2 (58). Moreover, in this species, E_2 inhibition of both tonic LH secretion and *Kiss1* mRNA in the ARC occur via a nonclassical mechanism that does not involve estrogen response element signaling (59). Quantitative RT-PCR has also demonstrated an increase in *Kiss1* mRNA in the ARC after OVX in primates (60) and possibly rats (RNA was extracted from tissue containing both the POA and ARC) (61). Using a similar approach, OVX increased and E_2 treatment inhibited ARC *Kiss1* mRNA in sheep (62). Moreover, a strong positive correlation between ARC *Kiss1* mRNA and LH pulse amplitude was observed (62), suggesting that E_2 inhibits GnRH/LH pulse amplitude by suppressing kisspeptin release from KNDy neurons during the breeding season.

Kisspeptin in KNDy neurons has also been implicated in the photoperiod-controlled changes in E_2 negative feedback responsible for seasonal breeding in the ewe (53). Kisspeptin expression in the ARC, but not the POA, is suppressed under inhibitory photoperiod (63), and exogenous kisspeptin induces ovulation in anestrous ewes (64), suggesting that the low levels of this neuropeptide is limiting fertility at this time of year. Kisspeptin appears to play a similar role in seasonal breeding in male Syrian hamsters (65) but not in male Siberian hamsters (66). In contrast to its important role in E_2 negative feedback, it seems unlikely that kisspeptin plays a role in the negative feedback actions of progesterone. Progesterone had no effect on *Kiss1* mRNA in ewes (56, 62), and there was no correlation between *Kiss1* mRNA and LH pulse frequency (62).

Although the kisspeptin produced by KNDy neurons does not appear to mediate progesterone negative feedback, there is considerable evidence that DYN from KNDy cells plays this role. It is now generally accepted (53, 54, 67) that the inhibition of GnRH/LH pulse frequency by progesterone is mediated by an inhibitory EOP, and recent work in the ewe has implicated DYN as this EOP. Data supporting this hypothesis include 1) virtually all KNDy neurons contain PR (24); 2) local administration of an antagonist to the DYN receptor (KOR) to the MBH increased LH pulse frequency in luteal phase ewes, whereas antagonists to other EOP receptors did not (14); 3) OVX decreased prepro-DYN mRNA in the ARC (15); and 4) local administration of the PR antagonist RU486 to the ARC disrupted the negative feedback actions of progesterone (Goodman, R. L., unpublished data). This hypothesis is also consistent with work in humans that EOP mediate progesterone negative feedback (67) and that expression of prepro-DYN mRNA in ARC neurons increased in postmenopausal women (68). However, the data in rodents are contradictory. Pharmacological evidence supports an inhibitory role for DYN (18, 69), but prepro-DYN mRNA in the ARC is inhibited by ovarian steroids in mice (18), and knockout of the KOR decreased LH secretion in OVX mice (18). It is also unlikely that ARC DYN plays a role in E_2 negative feedback in mice because the effects of this steroid on DYN expression occur via the classical estrogen response element (59), which is not required for inhibition of LH secretion by E_2 in this species. Thus, although DYN appears to mediate progesterone negative feedback in ewes and primates, its role in rodents is less clear, possibly because of the abbreviated luteal phase in cycling rats and mice.

Although there may be minor species differences in neural mediators of the negative feedback actions of ovarian steroids, there are major differences between rodents and

larger mammals in the neural systems mediating the positive feedback actions of E_2. In rodents, E_2 acts in the anteroventral periventricular nucleus (AVPV) and adjacent areas (70) to induce the preovulatory GnRH/LH surge, and there is strong evidence that AVPV kisspeptin neurons play a critical role in this action of E_2 (16). In contrast, in sheep (71) and primates (72, 73), the positive feedback action of E_2 occurs in the MBH, probably in either the ventromedial nucleus or the ARC. Moreover, recent work using the early immediate gene product Fos as an index of neural activation has implicated KNDy neurons in sheep in the induction of the preovulatory surge. The expression of Fos in KNDy neurons is dramatically increased both 1 h after injection of a surge-inducing dose of E_2 (74) and during the preovulatory LH surge in ovary-intact ewes (47). Interestingly, Fos was also elevated in kisspeptin neurons in the POA in the latter, but not the former, ewes. This observation, together with the recent report that *Kiss1* mRNA is increased in these neurons late in the follicular phase (74), suggests that this POA kisspeptin subpopulation may also contribute to the stimulation of GnRH secretion during the surge. Although these data implicate KNDy neurons in both the initial and final phases of E_2 positive feedback, they do not indicate which neurotransmitter is involved. One obvious possibility is kisspeptin; exogenous kisspeptin induced an LH surge in follicular phase ewes (64), and *Kiss1* mRNA levels increased in KNDy neurons late in the follicular phase (74). Glutamate is another possibility (53), and vGLUT-2 is colocalized in kisspeptin terminals that contact POA and MBH GnRH neurons (47). NKB is the third possibility because we have observed that activation of NK3R with an agonist (senktide) consistently stimulates LH secretion in the ewe (75). Even a brief treatment with senktide during the follicular phase produced a prolonged increase in LH concentrations to levels close to those seen during the preovulatory LH surge. The recent report that mutations in the gene for NK3R are associated with infertility in humans (11) is also consistent with a key stimulatory role for NKB. In contrast to sheep and humans, NKB appears to be predominantly inhibitory in rodents because senktide suppressed LH secretion in both rats (76) and mice (18).

Based on these data, the current working hypothesis for the role of KNDy neurons in the preovulatory LH surge in ewes is that E_2 acts on these neurons to trigger a sequence of events that leads to release of NKB and kisspeptin 12–18 h later that then drives GnRH secretion during the surge. As noted above, the stimulatory as well as inhibitory effects of steroids mediated by KNDy neurons may be conveyed directly to GnRH neurons via KNDy efferents (Figs. 2 and 3) or indirectly via projections to other neurons, which then in turn project to GnRH cells or terminals. The data implicating KNDy neurons in both positive and negative feedback actions of E_2 in ewes raise intriguing questions as to how the same set of neurons can have both inhibitory and stimulatory effects on GnRH secretion. The two most likely explanations are that subsets of KNDY neurons mediate different actions of E_2 or that the same set of neurons respond differently to low and high concentrations of this steroid, perhaps through different intracellular signaling mechanisms (77). It is also important to note that other neural systems, possibly including noradrenergic neurons in the brainstem and somatostatin-containing neurons in the ventromedial nucleus, have also been implicated in the positive feedback actions of E_2 in sheep (53); thus, it is likely E_2 that acts via multiple pathways to induce the preovulatory LH surge in the ewe, as it does in the rat.

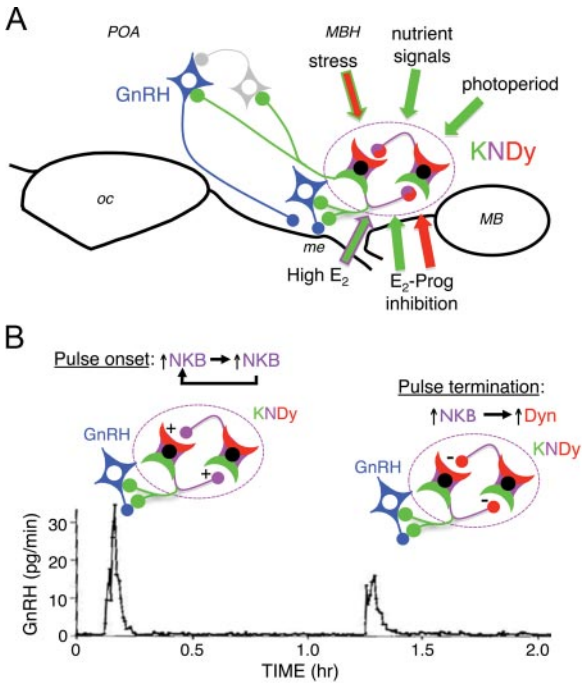

FIG. 3. A, Schematic diagram showing potential regulatory signals conveyed by KNDy cells to GnRH neurons and the KNDy peptides (kisspeptin, *green*; NKB, *magenta*; DYN, *red*) currently implicated in each action. Note that in some cases (*e.g.* positive feedback actions of E_2 and stress), two colors indicate that more than one KNDy peptide may be involved. In addition to direct contacts onto GnRH neurons in the POA and MBH, the potential for indirect influence via interneurons (gray cell) is also shown. MB, Mammillary bodies; me, median eminence; oc, optic chiasm; Prog, progesterone. B. Hypothetical model by which synchronous activity among KNDy cells may regulate GnRH pulse frequency (see text for details). GnRH pulse data was redrawn from Moenter *et al.* (85). Prog, Progesterone.

In summary, KNDy neurons appear to play a key role in steroid negative feedback in a number of species, but their role in E_2 positive feedback is more species specific. There is now strong and growing evidence that KNDy neurons mediate the positive feedback actions of E_2 in

ewes, although other neural systems are also involved. In contrast, it is equally clear that KNDy neurons are not part of the mechanisms responsible for the LH surge in rodents. Data in primates showing that E_2 acts in the MBH and that kisspeptin and NKB are necessary for normal fertility raise the possibility that KNDy neurons participate in the positive feedback actions of E_2, but there is no direct evidence for such a role in primates at this time.

Role in Generation of Episodic GnRH Secretion

Although the importance of the episodic pattern of GnRH secretion has been recognized for several decades (50), the mechanisms responsible for this pattern *in vivo* remain unclear. There is strong *in vitro* evidence that GnRH neurons are capable of episodic secretion without external input (78, 79), but the ability of genetic disruptions of kisspeptin signaling to produce infertility argues that episodic secretion of GnRH *in vivo* is dependent on some input. The observation that KNDy neurons form an interconnected network presumably capable of producing a synchronous burst of firing has led to speculation that they may represent an important component of the hypothalamic pulse generator that drives episodic secretion of GnRH (18, 19). The evolutionary conservation of KNDy neurons, and the data that at least two of their neuropeptides (kisspeptin and NKB) are essential for normal GnRH secretion in humans (2, 3, 11, 12), point to a key role for this neural population in reproductive neuroendocrinology. Lesion data (54), and recordings of multi-unit electrical activity (MUA) coincident with LH pulses (80), point to the MBH as the site of the hypothalamic pulse generator in a number of species. Although speculative, the following observations allow the development of a testable working hypothesis for how KNDy neurons could help synchronize the activity of the GnRH neurons responsible for episodic secretion in sheep: 1) KNDy neurons contain NK3R in sheep (45) and mice (18); 2) NK3R agonists increase LH secretion in ewes (75) and MUA in goats (19); 3) ovine GnRH neurons contain *Kiss1r* (69) but not NK3R (45); 4) kisspeptin is required for episodic LH secretion (44, 81) and kisspeptin pulses correlate with those of GnRH in the primate median eminence (82), but kisspeptin does not affect MUA in goats (83); 5) a nonspecific EOP receptor antagonist increases the amplitude and duration, but does not affect the rising phase, of each GnRH pulse in ewes and increases GnRH secretion between pulses (84); and 6) a KOR antagonist increased the frequency of MUA in goats (19).

Based on these observations, we propose that synchronous activity of KNDy neurons is controlled by stimulatory actions of NKB and inhibitory actions of DYN on these neurons and that their output to GnRH neurons is primarily via kisspeptin. The model shown in Fig. 3 predicts that each GnRH pulse is triggered by an initial increase in NKB from a few KNDy neurons, which stimulates further NKB release; the resulting positive feedback loop produces release of kisspeptin onto GnRH neurons and hence the extremely rapid increase in GnRH secretion that occurs at the onset of a pulse (85). NKB stimulation of KNDy neurons is predicted to also stimulate release of DYN, and the inhibitory actions of this EOP on KNDy neurons first begins to hold kisspeptin release in check and, after a few minutes, completely suppresses the activity of KNDy neurons, terminating the GnRH secretory episode and preventing any GnRH secretion between pulses. The action of DYN on KNDy neurons will also suppress DYN release, which eventually allows increased firing and the NKB release that triggers the next GnRH pulse. DYN could also act directly on GnRH neurons, but the model (Fig. 3) proposes an action on KNDy neurons based on data in rodents that KNDy neurons contain KOR (18), whereas GnRH neurons do not (86) (at this time, there are no data on the cellular location of KOR in ewes). Thus, we speculate that kisspeptin in KNDy cells is the output that drives GnRH pulses, that NKB is the trigger that initiates synchronous firing of KNDy neurons and the onset of each pulse, and that DYN is the peptide that shuts off the firing of KNDy neurons and terminates each pulse. It is interesting to note that a similar, albeit somewhat more complicated, model has recently been proposed for the role of KNDy neurons in episodic GnRH secretion in mice (18).

The hypothesis that episodic release of kisspeptin from KNDy neurons is important for pulsatile secretion of GnRH is apparently not consistent with reports that brief exposure of murine GnRH cell bodies to kisspeptin produced a sustained increase in firing rate lasting more than 20 min (41, 42). One simple, albeit speculative, explanation for this apparent paradox is that in mice, kisspeptin actions at the cell body drive the preovulatory GnRH surge, whereas actions at the GnRH terminals (18, 40) are important for episodic GnRH secretion. In ewes, KNDy neurons could act on GnRH terminals in the median eminence, because, as noted above, ovine KNDy neurons project to this area (38). Alternatively, GnRH cell bodies in the MBH receive abundant input from KNDy neurons and are selectively activated (based on Fos expression) when episodic LH secretion is stimulated (87).

Although this model must be rigorously tested, it does provide a simple explanation for the differential roles of kisspeptin and DYN in the negative feedback actions of E_2 and progesterone, respectively, described above. Because

kisspeptin release from KNDy neurons is driving GnRH release during a pulse, E_2 inhibition of kisspeptin expression would be expected to inhibit GnRH pulse amplitude. In contrast, DYN terminates and prevents GnRH secretion between pulses. Thus, a stimulation of DYN release by progesterone might be expected to prolong the interval between pulses and thus reduce GnRH pulse frequency.

Role in Humans

Given the evolutionarily conserved role that KNDy cells appear to serve in the negative feedback control of GnRH secretion, and the observation that mutations in the genes for at least two components of KNDy cell signaling are associated with human infertility (2, 3, 11, 12), it would not be surprising if other clinical neuroendocrine disorders were at least partly due to malfunctions of this cell group. One such disease that may be tied in part to the KNDy subpopulation is polycystic ovarian syndrome (PCOS). PCOS is among the most common of adult reproductive endocrine disorders (88) and is characterized by both reproductive and metabolic deficits (89), the former of which include defects in the ability of gonadal steroid hormones to exert appropriate feedback control on GnRH neurons (90). PCOS is likely a disease of prenatal origin (91), based on epidemiological evidence, suggesting that excess androgens during fetal life can lead to an increased risk of this disorder later in adulthood (92). Animal models of PCOS include the prenatally androgenized ewe (89), monkey (93), rat (94), and mouse (95), and prenatal testosterone (T) treatment of female sheep leads to a constellation of deficits that very closely resemble the symptoms of PCOS in women (89). Among these, again, are pronounced deficits in the gonadal hormone feedback control of GnRH neurons, including the inhibitory influence of progesterone on GnRH pulses and the stimulatory influence of E_2, leading to the generation of the preovulatory GnRH/LH surge (96, 97).

Based on the functional role of the KNDy subpopulation in mediating progesterone negative feedback in sheep (14), we recently tested the hypothesis that alterations in KNDy cell peptides may be associated with the prenatal T model of PCOS in this species (98). Prenatal T treatment between d 30 and 90 of fetal life (the sheep gestation period is 147 d) resulted in long-lasting changes in KNDy cell peptide expression in these animals examined as adults, reducing by half the number of NKB and DYN cells; in contrast, the number of kisspeptin cells remained at levels comparable to that seen in the KNDy subpopulation of normal control females (98). The results suggest that an imbalance between inhibitory (DYN) and stimulatory (kisspeptin) neuropeptides in this subpopulation may be responsible for the deficits in progesterone negative feedback seen in this model and that normalizing the balance of KNDy peptide expression and release in prenatal T sheep may help to ameliorate the neuroendocrine deficit. Kisspeptin antagonists (99) may therefore represent a potential clinical treatment for PCOS and other disorders where pulse frequency is elevated (90, 100). Observations that microinjections of kisspeptin antagonists into the ARC are capable of inhibiting LH pulse frequency (44, 81) are consistent with this possibility as well as the proposed role of KNDy cells as part of the GnRH pulse generator (see above).

Although we have focused here on alterations in KNDy cells that may underlie reproductive disease, there is also compelling evidence that changes in KNDy cells occur as a part of the process of normal aging in association with menopause. Specifically, it has long been known that in the brains of postmenopausal women, there is selective hypertrophy of neurons of the infundibular (arcuate) nucleus of the human hypothalamus (101). Rance and colleagues have shown in single-label studies that the large majority of these hypertrophied neurons each contain *KISS1* (57), NKB (8), and DYN (68) as well as ERα (102) mRNA; thus, they likely represent KNDy cells of the human hypothalamus. In postmenopausal women, there is increased gene expression of NKB (8) and *KISS1* (57) in these cells, along with decreased gene expression of DYN (68), consistent with an alteration in the balance between stimulatory (kisspeptin and NKB) and inhibitory (DYN) KNDy peptides that would lead to the GnRH and LH hypersecretion characteristic of postmenopausal women (20). Because similar changes in KNDy peptide gene expression are seen in young OVX monkeys (20), it may be that these changes in postmenopausal women are a response to the ovarian failure and depletion of ovarian steroids that occurs during menopause. Thus, KNDy cells likely play a key role in normal physiological regulation of steroid negative feedback in humans, just as in experimental animals.

Conclusion and Unresolved Questions

There is now strong evidence that KNDy neurons and their projections play a central role in steroid negative feedback of GnRH secretion in rodents, sheep, and primates and growing evidence that they also participate in the positive feedback actions of E_2 to induce the preovulatory GnRH/LH surge in the ewe (Fig. 3). Although the actions of NKB remain controversial, it is clear that kisspeptin stimulates and DYN inhibits GnRH secretion. This raises the possibility that differential effects of ovarian steroids on these two peptides or their receptors could have a dra-

matic effect on the overall control of GnRH release. This in turn points to the need for studies to determine the effects of steroids on expression of receptors for KNDy peptides in GnRH neurons and whether individual KNDy peptides are sequestered in the same or separate secretory vesicles.

Other possible functions for these neurons remain largely speculative at this time. However, there is evidence that KNDY neurons may mediate the effects of nutritional status and stress on the reproductive neuroendocrine axis. KNDy neurons contain leptin receptors (103) and nutrient restriction inhibits *Kiss1* mRNA expression in rodents (104, 105). Similarly, ovine KNDy neurons contain glucocorticoid receptors (106), and recent work in the rat has implicated ARC kisspeptin neurons in the suppression of GnRH in response to a variety of stressors (107). Thus, KNDy cells may serve as a central node in the control of GnRH secretion, acting as conduits for a variety of intrinsic and extrinsic regulatory signals (Fig. 3A).

Finally, the important role that KNDy neurons appear to play in the control of the GnRH system is interesting in light of the history of reproductive neuroendocrinology. The ARC was the hypothalamic nucleus that Ernst Knobil (108, 109) and others originally identified as the site of the GnRH pulse generator and has long been considered a key locus in steroidal control of GnRH secretion. A central role for KNDy cells in the control of GnRH secretion is therefore a return of the ARC to the neuroendocrine spotlight, albeit at a level of greater cellular detail in both phenotype and circuitry. Understanding how the synthesis and release of individual KNDy peptide is orchestrated within KNDy neurons and their projections, and ultimately translated into control of the reproductive neuroendocrine axis, will likely have key relevance for a wide range of issues affecting reproductive health and disease.

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Address all correspondence and requests for reprints to: Michael N. Lehman, Department of Anatomy and Cell Biology, Medical Sciences Building, University of Western Ontario, London, Ontario, Canada N 6A 5C1. E-mail: michael.lehman@schulich.uwo.ca.

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