Neural signals that regulate GnRH neurones directly during the oestrous cycle

Matthew J. Smith¹ and Lothar Jennes^{2*}

Departments of ¹Physiology and ²Anatomy and Neurobiology, University of Kentucky School of Medicine, Lexington, KY 40536-0298, USA

GnRH, produced by a loose network of neurones in the basal forebrain, is the primary brain signal responsible for the release of LH and FSH from the anterior pituitary gland. The ovarian steroid hormone oestradiol feeds back at both the central nervous system and the anterior pituitary to regulate the patterns of release of GnRH and the gonadotrophins. Although recent evidence indicates that oestradiol may act directly on some GnRH neurones through classical genomic mechanisms, data from published studies have demonstrated that neurotransmission of afferent neuronal systems that are receptive to oestradiol is necessary to drive reproductive cyclicity. Many classical neurotransmitters and neuropeptides alter GnRH neuronal activity, through direct and sometimes indirect actions. This review focuses on the neurotransmitters that regulate GnRH neurones by binding to and activating specific membrane receptors that are expressed in GnRH neurones. These include the catecholamines, γ -aminobutyric acid, glutamate, neuropeptide Y, neurotensin, β -endorphin and vasoactive intestinal polypeptide. On the basis of recent molecular and neuroanatomical evidence, it is proposed that oestradiol influences the activity of these neurotransmitter and neuropeptide systems within the GnRH network to drive reproductive cyclicity.

The oestrous/menstrual cycle results from an intricate orchestration of neurochemical and endocrine events acting at the central nervous system, anterior pituitary and ovary (Freeman, 1994; Hotchkiss and Knobil, 1994). GnRH neurones represent the final output pathway of the neural network that integrates a multitude of internal and environmental cues to regulate the secretion of LH and FSH from the anterior pituitary gland. Different patterns of pulsatile secretion of GnRH into the hypophyseal portal system, along with changes in the responsiveness of the pituitary gonadotrophs to GnRH, cause the changes in LH and FSH secretion observed over the oestrous cycle. The most marked of these changes is the massive outpouring of LH at mid-cycle, a critical event responsible for initiating ovulation.

The ovary secretes oestradiol and progesterone, which feedback to the central nervous system and anterior pituitary to regulate the synthesis and patterns of release of GnRH and the gonadotrophins, as well as the responsiveness of the gonadotrophs to GnRH (Freeman, 1994; Hotchkiss and Knobil, 1994). During most periods of the oestrous cycle, oestradiol and progesterone restrain GnRH-mediated LH secretion through negative feedback. However, during pro-oestrus, these two steroid hormones, coupled with circadian

input, exert positive feedback on GnRH neurones and the pituitary gonadotrophs to generate the preovulatory LH surge. Although recent data indicate that oestradiol may act directly on certain GnRH neurones through specific nuclear receptors, evidence in published studies has demonstrated that neurotransmission of afferent neuronal systems that are sensitive to oestradiol and progesterone is necessary to stimulate GnRH neurones to induce the mid-cycle LH surge (Kalra, 1993; Kordon *et al.*, 1994).

The purpose of this review is to detail the recent progress made toward deciphering the neurochemical signals involved in the control of cyclic GnRH secretion in the oestrous cycle of rats. Specifically, the current understanding of the functional significance of substances that communicate directly with GnRH neurones will be examined. Emphasis is placed on detailing the means by which oestradiol, neurotransmitters and neuropeptides interact and then communicate directly with GnRH neurones to drive reproductive cyclicity. This review focuses upon the female rat, and does not address the role of progesterone since its influence is entirely dependent upon pre-exposure to oestradiol.

Anatomy of the GnRH neuronal system

Before describing the neurochemical signals that are important in direct regulation of GnRH secretion, it is

*Correspondence
Email: ljenn0@pop.uky.edu

necessary to summarize the anatomical organization of the GnRH neuronal system in the adult rat brain (Jennes and Conn, 1994; Silverman *et al.*, 1994). GnRH cell bodies are not found in compact groups or clusters, but are arranged in loose networks (Fig. 1). In rodents, GnRH cell bodies form a loose continuum from the medial septum and diagonal band of Broca to the ventral anterior hypothalamus. GnRH neurones send axonal projections to many sites within the brain; however, the major projection relevant to the control of anterior pituitary function extends to the external layer of the median eminence through periventricular and subventricular pathways. Here, GnRH is released into the fenestrated capillaries and is carried via the hypophyseal portal system to the anterior pituitary to regulate gonadotrophin secretion. Retrograde transport studies have demonstrated that, in rats, approximately 50–75% of all GnRH neurones project axons or axon collaterals to the median eminence. This percentage is likely to be an underestimate, since a single injection of retrograde tracer is probably unable to fill the entire GnRH terminal field in the median eminence.

Additional sites in which GnRH axons terminate next to fenestrated capillaries include the organum vasculosum of the lamina terminalis (OVLT) and the subfornical organ. However, since only a venous connection has been found between these regions and the anterior pituitary, the role and targets of GnRH released from these sites are not known. However, owing to the rapid degradation of the decapeptide in the blood ($t_{1/2} = 2$ min), the high dilution factor in the blood and a binding affinity for the GnRH receptor of about 5 nmol l^{-1} , these targets can be expected to be nearby. Less extensive GnRH pathways extend into the medial amygdala through the stria terminalis and the ventral amygdalofugal pathway as well as the periaqueductal central gray through periventricular radiations and the medial habenula-fasciculus retroflexus-interpenduncular nucleus pathway. It should be noted that studies using electron microscopy have shown that GnRH is contained in presynaptic terminals in many of these regions, indicating a role as neuromodulator or neurotransmitter in the above terminal fields.

Ultrastructural analyses of GnRH neurones have revealed several morphological features that may have important functional implications. For example, the synaptic input to GnRH neurones is very limited compared with other neurones in the preoptic area (Jennes and Conn, 1994; Silverman *et al.*, 1994). It has been estimated that about 0.4% of the surface area GnRH perikaryon is occupied by presynaptic specialization compared with 6.6% of neighbouring cells. This percentage corresponds to approximately five to seven synapses per GnRH perikaryon. Thus, the neurotransmitters and neuropeptides that communicate directly with GnRH neurones through synapses probably exert quite a powerful effect on GnRH neuronal activity. An alternative explanation is that direct presynaptic inputs to GnRH nerve terminals at the level of the median eminence play a more crucial role in determining the

Fig. 1. Tracings of GnRH immunoreactive perikarya and neurites in coronal sections of the rat brain at the medial septum-diagonal band of Broca (a) and the organum vasculosum of the lamina terminalis (OVLT) (b).

activity of the GnRH neurones. However, evidence is limited in this regard and additional research is necessary.

GnRH neurones are enveloped by thin glial sheaths and it can be envisioned that such sheaths could prevent axon terminals from forming temporary synaptic complexes with the plasma membrane of the GnRH neurones. Such a scenario is reminiscent of the neurohypophyseal system in which pituicytes can separate temporarily the oxytocin- and vasopressin-containing nerve terminals from the site of release into the perivascular space (Hatton *et al.*, 1984). At present it is not clear whether, in rats, dynamic changes occur in the degree of glial ensheathment of GnRH neurones under various endocrine conditions, as has been described for monkeys (Romero *et al.*, 1994). However, ovariectomy induces morphological changes in the physical contact between GnRH axon terminals and the basal lamina of fenestrated capillaries in the median eminence (King and Letourneau, 1994).

The role of oestradiol in the induction of the preovulatory LH surge and subsequent ovulation is well recognized (Herbison, 1998). However, owing to the paucity of information on the temporal and spatial nature of its interaction in the central nervous system, the exact nature of the influence of oestradiol on GnRH neurones remains undetermined. For the past two decades, it was thought that the actions of oestradiol on GnRH neurones were indirect and that oestradiol altered GnRH neuronal activity by activating or inhibiting afferent neurones. This view was based on the inability of immunocytochemical studies to detect oestrogen receptor protein consistently in GnRH neurones of a number of species, as well as on the lack of accumulation of radioactive oestrogen in nuclei of GnRH neurones *in vivo*. Recent evidence based on improvements in the sensitivity of the methods indicates that some GnRH

Table 1. Neuronal cell populations implicated in communicating directly with GnRH neurones

Neurochemical signal	Receptors present in GnRH neurones	Likely site of origin
Catecholamines (noradrenaline, adrenaline)	α_{1B} (80%); α_{2A}	BS
5-Hydroxytryptamine (5HT)	–	Raphe nuclei
Glutamate	KA ₂ (50%); NMDAR ₁ (?)	POA
γ -Aminobutyric acid (GABA)	GABA _A (75%); GABA _B	POA
Neuropeptide Y	NPY Y-1	BS and ARC
Neurotensin	NT1	POA
Opioid peptides (β -endorphin)	–	ARC
Vasoactive intestinal polypeptide (VIP)	VIP ₂ (40%)	SCN

ARC: arcuate nucleus; BS: brainstem; POA: preoptic area; SCN: suprachiasmatic nucleus; –: indicates insufficient evidence at present; ?: indicates discrepancies in published studies with regard to the percentage. See text for references.

neurones may contain oestrogen receptors (Skynner *et al.*, 1999; Hrabovsky *et al.*, 2000). Large discrepancies are apparent among these studies and, therefore, there is no concrete evidence for the direct regulation of GnRH neurones by oestradiol. However, it is clear that afferent input to GnRH neurones is required for the maintenance of cyclic GnRH secretion, since treatments with specific antagonists to several key neurotransmitters and neuropeptides abolish the preovulatory LH surge (Kalra, 1993; Kordon *et al.*, 1994).

Because of the substantial amount of physiological and pharmacological evidence in support of multiple controls of GnRH and LH release by neurotransmitters and neuropeptides, the nature of synaptic inputs to GnRH neurones has received a great deal of attention. A number of studies using dual-label immunohistochemistry and *in situ* hybridization histochemistry has shown that several neurotransmitter and neuropeptide receptor mRNAs and proteins are expressed in GnRH neurones, including receptors for catecholamines, γ -aminobutyric acid (GABA), glutamate, neuropeptide Y, neurotensin and vasoactive intestinal polypeptide (VIP) (Table 1).

Direct regulation of oestrogen-dependent GnRH neural activity by brainstem catecholamines and indolamines

Extensive evidence indicates that noradrenaline, adrenaline and 5-hydroxytryptamine, which originate from neurones in the brainstem, play a stimulatory role in the regulation of GnRH neuronal functioning.

Noradrenaline and adrenaline

Destruction of noradrenaline neurones in the brainstem or local inhibition of α -adrenergic receptors decreases pulsatile tonic secretion of LH as well as cyclic LH release during the LH surge. However, the precise mechanisms by which the noradrenergic neurones drive GnRH secretion are not completely understood. Noradrenaline appears to allow or promote interactions between other neurones that, in turn, alter GnRH neuronal functioning (Herbison,

1997a). Support for this hypothesis arises from the observation that lesion of the noradrenaline neuronal system abolishes the LH surge for several weeks before cyclicity is reinstated. These experiments also reveal the principle that there are compensatory mechanisms or redundancies in the brain that can take over the neuroendocrine control of the reproductive cycle.

Noradrenaline may also play a role in mediating part of the stimulatory effects of oestradiol on GnRH secretion during the preovulatory period (Herbison, 1997a). Serum oestradiol concentrations correlate with noradrenaline turnover or release in regions of the hypothalamus that regulate GnRH synthesis or release at the time of the preovulatory LH surge (Mohankumar *et al.*, 1994). Noradrenergic neurones are direct targets for oestradiol and since the percentage of noradrenaline neurones in the A2 cell group expressing Fos (an indicator of neuronal activity) increases in pro-oestrus before the onset of the preovulatory LH surge, it is suggested that oestradiol directly activates transcription or transmitter release in these neurones.

Most of the studies on the role of noradrenaline in the control of GnRH secretion have used α -adrenergic receptor antagonists as well as inhibitors of dopamine- β -hydroxylase activity and it is thus possible that the observed effects on GnRH release were actually caused by adrenaline. This view is supported by the finding that both noradrenaline and adrenaline are contained in axons that are juxtaposed to GnRH neurones in the septum-diagonal band. Although certain aspects of the role of adrenaline remain controversial, most studies have demonstrated that adrenaline also stimulates both pulsatile and preovulatory release of GnRH. Adrenaline is important in regulating LH pulse frequency, whereas noradrenaline stimulated both the amplitude and frequency of LH pulses (Gallo *et al.*, 1989). Furthermore, inhibition of adrenaline synthesis, in the presence of adequate noradrenaline concentrations, prevents the pro-oestrous LH surge.

Recent studies have begun to decipher the exact catecholaminergic pathways through which oestradiol affects GnRH neuronal activity. Retrograde tracing techniques revealed that noradrenergic neurones projecting to the immediate vicinity of the GnRH cell bodies in the rostral

Fig. 2. GnRH neurones are apposed by noradrenergic and adrenergic axons. Immunohistochemical triple labelling showing a GnRH neurone (a), dopamine- β -hydroxylase immunoreactive axons (b) and phenylethanolamine-*N*-methyl transferase-containing axons (c) in the medial septum of the female rat. The overlay of the three images (d) shows sites of potential noradrenergic input to the GnRH neurone (arrow) and adrenergic input (arrowheads).

preoptic area appear to be located exclusively in the ventrolateral medulla (A1 cell group) and the nucleus tractus solitarii (A2 cell group) (Wright and Jennes, 1993a). Only the A2 noradrenergic neurones that project to the preoptic area express oestrogen receptor protein, whereas the A1 neuronal afferents do not (Simonian *et al.*, 1999). With respect to adrenaline, most hypothalamic nuclei receive adrenergic afferents from all three cell groups (that is, C1, C2 and C3) in the medulla, although their relative contributions vary. Lee *et al.* (2000) demonstrated that many adrenergic neurones in all cell groups express oestrogen receptors and that a significant percentage of adrenergic neurones in the brainstem express Fos during the initiation of the steroid-induced LH surge. Additional work is necessary to determine whether the adrenergic neurones

that project to the vicinity of GnRH cell bodies are the neurones that also contain oestrogen receptor α protein.

In rats, noradrenergic and adrenergic axons are present in large numbers in the rostral preoptic area and the medial septum-diagonal band of Broca region, in which most GnRH neurones are located (Wright and Jennes, 1993a; Fig. 2), and some of the tyrosine hydroxylase immunoreactive terminals form presynaptic terminals on GnRH neurones. That these synapses are functional is indicated by the finding that many GnRH neurones express the α_{1B} -adrenergic receptor subtype at the cell body and to a lesser extent at the nerve terminal in the median eminence (Hosny and Jennes, 1998; Fig. 3). However, since the α_{1B} -adrenergic receptor protein is frequently localized to sites on the GnRH neurones that are not in close apposition to noradrenergic

Fig. 3. GnRH neurones express α_{1B} adrenergic receptor protein and are apposed by dopamine- β -hydroxylase containing axons. Immunohistochemical triple labelling shows that most GnRH neurones (a; green) contain α_{1B} adrenergic receptor protein (b; red) and that many of these receptor patches are next to noradrenergic or adrenergic axons (c; blue). (d) Composite image. Arrows indicate sites at which α_{1B} adrenergic receptor protein is located next to dopamine- β -hydroxylase immunoreactive axons.

or adrenergic axons, asynaptic release of the catecholamines followed by diffusion to the receptors is indicated. In addition, Lee *et al.* (1995) reported the presence of α_{2A} -adrenergic receptors in GnRH neurones. However, it should be noted that, thus far, the stimulatory influence of noradrenaline on GnRH neuronal activity has been attributed only to the α_1 -adrenergic receptors.

5-Hydroxytryptamine

5-Hydroxytryptamine (5HT), synthesized by neurones in the raphe nuclei, plays an important role in the generation of the LH surge (Kordon *et al.*, 1994) acting through the 5HT_{2A} receptor (Fink *et al.*, 1999). Serotonergic activity is regulated by ovarian steroids, since treatment of ovariectomized rats with oestrogen and progesterone significantly increases 5HT concentrations in the preoptic area and hypothalamus. Furthermore, a significant proportion of serotonergic cell bodies in the median raphe nucleus (Leranth *et al.*, 1999), but not the dorsal raphe nucleus (Alves *et al.*, 1998), express oestrogen receptor β immunoreactivity. It is not clear whether the effects of 5HT on GnRH release are caused by direct actions on GnRH neurones or whether other interneurons are involved, since anatomical studies have shown that only about 5% of nerve terminal boutons apposed to GnRH neurones are serotonergic and dual *in situ* hybridization studies have

failed to detect mRNA for 5HT_{1A}, 5HT_{1C} and 5HT₂ receptors in GnRH neurones (Wright and Jennes, 1993b).

Direct regulation of oestrogen-dependent GnRH neural activity by local circuits

Much of the regulation of GnRH neurones appears to be accomplished by local circuits. Anatomical studies have demonstrated that extensive, oestrogen-sensitive afferent projections to the vicinity of GnRH neurones originate from cell bodies residing within the preoptic area and hypothalamus, particularly the arcuate nucleus (Simonian *et al.*, 1999). The major neurotransmitters and neuropeptides in this category include excitatory amino acids, GABA, neurotensin, neuropeptide Y, β -endorphin and VIP.

Excitatory amino acids

Neurotransmission of excitatory amino acids in the brain principally involves glutamate and aspartate. Excitatory amino acids induce a rapid increase in GnRH or LH release and enhance GnRH mRNA and protein content (Brann and Mahesh, 1997), whereas inhibition of the different glutamate receptor subunit families has the converse effect. Increased glutamate and aspartate release in the preoptic region slightly precedes or parallels the LH surge.

Glutamatergic input to the GnRH neuronal system is likely

Fig. 4. The subpopulation of GnRH neurones that expresses Fos during the LH surge contains glutamate receptor subunits kainate-2 and GluR5. Immunohistochemical quadruple staining for GnRH (a), GluR5, 6, 7 (b), kainate-2 (c) and Fos (d). Scale bar represents 10 μ m.

to be mediated by direct synapses at the GnRH cell bodies or dendrites, as has been described in monkeys, or by asynaptic neurotransmission at the median eminence (Kawakami *et al.*, 1998). In further support of a direct action of excitatory amino acids on GnRH neurones, kainic acid 2 (KA₂) receptor mRNA and protein is present in approximately 50% of all GnRH neurones, and the KA₂-receptor protein is preferentially expressed in GnRH neurones that synthesize c-Fos during the LH surge (Eyigor and Jennes, 1996, 2000; Fig. 4). Whereas several investigators have reported that only approximately 5% of all GnRH neurones express NMDAR₁ mRNA (Abbud and Smith, 1995; Eyigor and Jennes, 1996), a recent publication indicates that approximately 80% of all GnRH neurones may express this particular receptor subtype (Ottem and Petersen, 2000). Spergel *et al.* (1999) demonstrated that glutamate evoked currents in GnRH neurones in mice, providing strong evidence that these neurones express functional glutamate receptors.

Glutamate neurones in the hypothalamus are regulated in part by oestrogen. Thus, glutamate content is increased after short-term treatment with oestradiol and the steroid treatment is required for glutamate to exert a stimulatory effect on LH secretion. These effects of oestrogen on glutamate neurones appear to be mediated by binding to, and activation of, nuclear oestrogen receptor α protein that

is expressed in many hypothalamic glutamatergic neurones, especially in the preoptic region (C. T. Moore and L. Jennes, unpublished).

γ -Aminobutyric acid (GABA)

GABA, the major inhibitory neurotransmitter of the brain, plays an important role in the regulation of GnRH secretion. Removal of GABAergic tone on the afternoon of pro-oestrus is an important neural signal for the generation of the LH surge (Kimura and Jinnai, 1994). Extracellular GABA concentrations in the medial preoptic area, but not in the medial basal hypothalamus, decrease before and during the LH surge induced by oestradiol in ovariectomized rats (Jarry *et al.*, 1995). The co-localization of oestrogen receptor α in preoptic GABAergic cell bodies as well as the effects of oestradiol on GABA release, re-uptake and GABA_A receptor gene expression indicate that the GABAergic neuronal network in the rostral hypothalamus is extremely sensitive to circulating concentrations of oestradiol (Herbison, 1997b). Thus, oestrogen decreases the hyperpolarizing response of GABAergic neurones to GABA_B receptor agonists for at least 24 h and it reduces glutamic acid decarboxylase mRNA content after 42 h but not after 24 h. These data indicate that oestrogen reduces the autoinhibition of GABAergic neurones

during negative feedback conditions, whereas during positive oestrogen feedback conditions GABAergic activity is reduced (Wagner *et al.*, 2001). GABA probably inhibits GnRH release through a direct action on GnRH neurones since nerve terminals containing glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis, make synaptic contacts with GnRH cell bodies in the rat medial preoptic area, and a significant proportion of GnRH neurones express mRNA for GABA_A (Petersen *et al.*, 1993; Jung *et al.*, 1998) and GABA_B (S. L. Petersen, personal communication) receptors. Recent electrophysiological studies (Spergel *et al.*, 1999; Sim *et al.*, 2000) have shown that all GnRH neurones in mice express functional GABA_A receptors, which underlines the importance of this neurotransmitter in the control of GnRH neurones.

Neuropeptide Y

Neuropeptide Y, a 36 amino acid peptide, facilitates GnRH release and potentiates the responsiveness of gonadotrophs to GnRH (Kalra, 1993; Herbison, 1998). Neuropeptide Y innervation of the hypothalamus and preoptic area originates from two discrete cell populations, one in the arcuate nucleus and the other in the brainstem, in which neuropeptide Y is co-localized with noradrenaline. In rats, approximately 15% of neuropeptide Y neurones in the arcuate nucleus express oestrogen receptor α , whereas the neuropeptide Y neurones in the brainstem do not (Simonian and Herbison, 1997). Neuropeptide Y gene expression appears to be regulated in part by oestrogen since synthesis and release of the peptide are high before the pro-oestrous LH surge (Sahu and Kalra, 1998). Although neuropeptide Y influences pulsatile LH release, most attention has focused on involvement of neuropeptide Y in the generation of the LH surge. Thus, immunoneutralization of neuropeptide Y or inhibition of neuropeptide Y synthesis with antisense oligonucleotides prevents the LH surge in steroid-treated rats. Neuropeptide Y-containing terminals originating in both the arcuate nucleus and brainstem project to the median eminence and to the septum-preoptic region, in which some immunoreactive axons make synaptic contacts with GnRH cell bodies and processes. The functional significance of neuropeptide Y innervation of GnRH neurones is not clear at present, since there is no convincing evidence for the co-localization of neuropeptide Y1 receptors in GnRH perikarya and the receptor is only found in certain GnRH terminals in the median eminence (Li *et al.*, 1999).

Neurotensin

Neurotensin is found in many neurones of the preoptic area and numerous studies indicate that this peptide mediates the stimulatory effects of oestrogen on GnRH secretion (Rostene and Alexander, 1997; Herbison, 1998). Administration of neurotensin amplifies, whereas immunoneutralization reduces, the magnitude of the LH surge. In addition, a large proportion of neurotensin-

Fig. 5. GnRH neurones express vasoactive intestinal polypeptide 2 (VIP₂) receptors and are apposed by VIP-containing axons. Immunohistochemical triple staining shows that about 40% of the GnRH neurones (a) contain VIP₂ receptor protein (b) and are in close apposition to VIP-containing axons (c). (d) Overlay of a–c.

containing neurones in the preoptic area express oestrogen receptors and oestradiol stimulates neurotensin gene expression in this region.

The ability of neurotensin neurones in the preoptic area to stimulate GnRH neuronal activity appears to be mediated, at least in part, by direct communication with GnRH neurones. Neurotensin immunoreactive fibres closely appose GnRH neurones in mice (Hoffman, 1985). Neurotensin exerts its effects through at least three receptors that have been cloned and designated NT1 (high affinity), nt2 and nt3. Only NT1 is thought to mediate the physiological effects of neurotensin (Rostene and Alexander, 1997). Smith and Wise (in press) demonstrated that some GnRH neurones in the OVLT/rPOA express mRNA for the NT1 receptor.

Endogenous opioid peptides

Endogenous opioid peptides constitute an important inhibitory component of the neural circuitry that regulates GnRH secretion, and a significant decrease in the inhibitory opioid tone is critical for the generation of the LH surge (Kalra, 1993). Evidence from published studies supports a role for β -endorphin acting via μ -opioid receptors in transmitting information about the steroidal milieu to GnRH neurones. Cell bodies containing β -endorphin are located exclusively in the periarculate region of the medial basal hypothalamus, and a small percentage of these neurones concentrate oestradiol in their nuclei.

Nerve terminals containing β -endorphin synapse on

Fig. 6. Schematic representation of the numerous neuromodulators that could regulate GnRH neuronal activity directly by binding to and activating specific membrane receptors. Projections to GnRH cell bodies arise from local circuits within the hypothalamic network as well as from neurones originating in the brainstem. Neurones with red nuclei express nuclear oestrogen receptors. GABA_{A,B}: γ-aminobutyric acid A and B receptors; KA₂: kainic acid 2 receptor; NT1: neurotensin 1 receptor; VIP₂R: vasoactive intestinal polypeptide 2 receptor.

perikarya and dendrites of GnRH neurones in rats; however, opioid receptor mRNAs (that is μ , δ and κ) have not been found in GnRH neurones (Mitchell *et al.*, 1997; Sannella and Petersen, 1997). However, the receptors are present in many cells within the preoptic area and hypothalamus, which indicates that β -endorphin may influence GnRH neurones through inhibition of interneurons. It should be noted that Eckersell and Micevych (1998) identified μ - and δ -opioid receptor immunoreactivity in GnRH fibres throughout the hypothalamus; however, the GnRH perikarya did not show such immunoreactivity.

Vasoactive intestinal polypeptide (VIP)

Oestradiol drives the hypersecretion of GnRH by coupling a circadian neuronal signal from the suprachiasmatic nucleus (SCN) to the neurotransmitters and neuropeptides that regulate GnRH secretion. The precise mechanisms by which the SCN transmits circadian information to the GnRH neuronal network are not known. However, recent evidence indicates that VIP, synthesized in the ventrolateral aspect of the SCN, is a critical neurochemical messenger that relays time-of-day information from the SCN to GnRH neurones (van der Beek, 1996). Thus, infusion of VIP antisense oligonucleotides

directly into the SCN (Harney *et al.*, 1996) or central administration of antiserum to VIP (van der Beek *et al.*, 1999) delays and attenuates the steroid-induced LH surge. In addition, morphological studies have shown that VIP-containing fibres closely appose GnRH cell bodies and dendrites, and that this input to GnRH neurones originates in the SCN. A substantial proportion of the GnRH neurones that receive VIP input express c-Fos during the afternoon of pro-oestrus, which indicates that these neurones are activated. Approximately 40% of all GnRH neurones express the VIP₂ receptor protein (Smith *et al.*, 2000; Fig. 5), indicating that most of the VIP input to GnRH is direct and probably synaptic. Whether oestradiol has a direct influence on VIP neuronal activity is not known, although oestrogen receptor β is expressed in low amounts in the SCN.

Conclusions

At present, it is clear that oestradiol, coupled with a daily neural signal originating from the SCN, orchestrates the activity of a number of neurotransmitter and neuropeptide systems resulting in a cyclical activation of the GnRH network (Fig. 6). It is apparent that each of these neural components, either acting in series or in parallel with one

another, must be functional for the GnRH/LH surge to occur, since the acute pharmacological manipulation of any one of these components prevents the GnRH/LH surge. This review has focused on those neuromodulators that alter GnRH neuronal activity directly by binding to membrane receptors present on GnRH perikarya; much remains to be learned about other potential participants and the precise mechanisms by which oestradiol communicates with these messenger systems.

This work was supported by NIH grants AG05847 (to M. J. Smith) and MH59890 and AG17164 (to L. Jennes).

References

Key references are indicated by asterisks.

- Abbud R and Smith MS** (1995) Do GnRH neurons express the gene for the NMDA receptor? *Brain Research* **690** 117–120
- Alves SE, Weiland NG, Hayashi S and McEwen BS** (1998) Immunocytochemical localization of nuclear estrogen receptors and progesterin receptors within the rat dorsal raphe nucleus *Journal of Comparative Neurology* **391** 322–334
- *Brann DW and Mahesh VB** (1997) Excitatory amino acids: evidence for a role in the control of reproduction and anterior pituitary hormone secretion *Endocrine Reviews* **18** 678–700
- Eckersell CB and Micevych PE** (1998) Colocalization of immunoreactivity for mu- and delta-opioid receptors with cholecystokinin, LHRH and tyrosine hydroxylase in the hypothalamus and limbic system of female rats *Society for Neuroscience* **28** 632.7 (Abstract)
- Eyigor O and Jennes L** (1996) Identification of glutamate receptor subtype mRNAs in gonadotropin-releasing hormone neurons in rat brain *Endocrine* **4** 133–139
- Eyigor O and Jennes L** (2000) Kainate receptor subunit-positive gonadotropin-releasing hormone neurons express c-Fos during the steroid-induced luteinizing hormone surge in the female rat *Endocrinology* **141** 779–786
- Fink G, Dow RC, McQueen JK, Bennie JG and Carroll SM** (1999) Serotonergic 5-HT_{2A} receptors important for the oestradiol-induced surge of luteinizing hormone-releasing hormone in the rat *Journal of Neuroendocrinology* **11** 63–69
- *Freeman ME** (1994) The neuroendocrine control of the ovarian cycle of the rat. In *The Physiology of Reproduction* pp 613–658 Eds E Knobil and JD Neill. Raven Press, Ltd, New York
- Gallo RV, Bona-Gallo A and O'Sullivan D** (1989) Adrenergic and noradrenergic regulation of pulsatile luteinizing hormone release *Journal of Neuroendocrinology* **1** 157–162
- Harney JP, Scarbrough K, Rosewell KL and Wise PM** (1996) *In vivo* antisense antagonism of vasoactive intestinal peptide in the suprachiasmatic nuclei causes aging-like changes in the estradiol-induced luteinizing hormone and prolactin surges *Endocrinology* **137** 3696–3701
- Hatton GI, Perlmutter LS, Salm AK and Tweedle CD** (1984) Dynamic neuronal-glial interactions in hypothalamus and pituitary: implications for control of hormone synthesis and release *Peptides* **5** Supplement 1 121–138
- *Herbison AE** (1997a) Noradrenergic regulation of cyclic GnRH secretion *Reviews of Reproduction* **2** 1–6
- *Herbison AE** (1997b) Estrogen regulation of GABA transmission in rat preoptic area *Brain Research Bulletin* **44** 321–326
- *Herbison AE** (1998) Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons *Endocrine Reviews* **19** 302–330
- Hoffman GE** (1985) Organization of LHRH cells: differential apposition of neurotensin, substance P and catecholamine axons *Peptides* **6** 439–461
- Hosny S and Jennes L** (1998) Identification of α_{1B} adrenergic receptor protein in gonadotropin releasing hormone neurones of the female rat *Journal of Neuroendocrinology* **10** 687–692
- *Hotchkiss J and Knobil E** (1994) The menstrual cycle and its neuroendocrine control. In *The Physiology of Reproduction* pp 711–749 Eds E Knobil and JD Neill. Raven Press, Ltd, New York
- Hrabovszky E, Shughrue PJ, Merchenthaler I, Hajszan T, Carpenter CD, Liposits Z and Petersen SL** (2000) Detection of estrogen receptor-beta messenger ribonucleic acid and ¹²⁵I-estrogen binding sites in luteinizing hormone-releasing hormone neurons of the rat brain *Endocrinology* **141** 3506–3509
- Jarry H, Leonhardt S, Schwarze T and Wuttke W** (1995) Preoptic rather than mediobasal hypothalamic amino acid neurotransmitter release regulates GnRH secretion during the estrogen-induced LH surge in the ovariectomized rat *Neuroendocrinology* **62** 479–486
- *Jennes L and Conn PM** (1994) Gonadotropin-releasing hormone and its receptors in rat brain *Frontiers in Neuroendocrinology* **15** 51–77
- Jung H, Shannon EM, Fritschy JM and Ojeda SR** (1998) Several GABA_A receptor subunits are expressed in LHRH neurons of juvenile female rats *Brain Research* **780** 218–229
- *Kalra SP** (1993) Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge *Endocrine Reviews* **14** 507–537
- Kawakami SI, Hirunagi K, Ichikawa M, Tsukamura H and Maeda KI** (1998) Evidence for terminal regulation of GnRH release by excitatory amino acids in the median eminence in female rats: a dual immunoelectron microscopic study *Endocrinology* **139** 1458–1461
- Kimura F and Jinnai K** (1994) Bicuculline infusions advance the timing of luteinizing hormone surge in proestrous rats: comparisons with naloxone effects *Hormones and Behavior* **28** 424–430
- King JC and Letourneau RJ** (1994) Luteinizing hormone-releasing hormone terminals in the median eminence of rats undergo dramatic changes after gonadectomy, as revealed by electron microscopic image analysis *Endocrinology* **134** 1340–1351
- *Kordon C, Drouva SV, Martinez de la Escalera G, Weiner RI, Knobil E and Neill JD** (1994) Role of classic and peptide neurotransmitters in the neuroendocrine regulation of luteinizing hormone and prolactin. In *The Physiology of Reproduction* pp 1621–1681 Eds E Knobil and JD Neill. Raven Press, Ltd, New York
- Lee A, Talley E, Rosin DL and Lynch KR** (1995) Characterization of α_{2A} -adrenergic receptors in GT1 neurosecretory cells *Neuroendocrinology* **62** 215–225
- Lee E, Moore CT, Hosny S, Centers A and Jennes L** (2000) Expression of estrogen receptor-alpha and c-Fos in adrenergic neurons of the female rat during the steroid-induced LH surge *Brain Research* **875** 56–65
- Leranth C, Shanabrough M and Horvath TL** (1999) Estrogen receptor- α in the raphe serotonergic and supramammillary area calretinin-containing neurons in the female rat *Experimental Brain Research* **128** 417–420
- Li C, Chen P and Smith MS** (1999) Morphological evidence for direct interaction between arcuate nucleus neuropeptide Y (NPY) neurons and gonadotropin-releasing hormone neurons and the possible involvement of NPY Y1 receptors *Endocrinology* **140** 5382–5390
- Mitchell V, Prevot V, Jennes L, Aubert JP, Croix D and Beauvillain JC** (1997) Presence of mu and kappa opioid receptors in galanin but not in GnRH neurons in the female rat *Neuroreport* **8** 3167–3172
- Mohankumar PS, Thyagarajan S and Quadri SK** (1994) Correlations of catecholamine release in the medial preoptic area with proestrous surges of luteinizing hormone and prolactin: effects of aging *Endocrinology* **135** 119–126
- Ottten EN and Petersen SL** (2000) The majority of LHRH neurons in the MEPO/OVLT express NMDAR1 *Society for Neuroscience* **30** 540.4 (Abstract)
- Petersen SL, McCrone S, Adelman JP and Mahan LC** (1993) GABA_A receptor subunit mRNA in cells of the preoptic area: colocalization with LHRH mRNA using dual-label *in situ* hybridization histochemistry *Endocrine* **1** 29–34
- Romero MT, Silverman AJ, Wise PM and Witkin JW** (1994) Ultrastructural changes in gonadotropin-releasing hormone neurons as a function of age and ovariectomy in rats *Neuroscience* **58** 217–225
- Rostene WH and Alexander MJ** (1997) Neurotensin and neuroendocrine regulation *Frontiers in Neuroendocrinology* **18** 115–173
- Sahu A and Kalra SP** (1998) Absence of increased neuropeptide Y neuronal activity before and during the luteinizing hormone (LH) surge may

- underlie the attenuated preovulatory LH surge in middle-aged rats *Endocrinology* **139** 696–702
- Sannella MI and Petersen SL** (1997) Dual label *in situ* hybridization studies provide evidence that luteinizing hormone-releasing hormone neurons do not synthesize messenger ribonucleic acid for mu, kappa, or delta opiate receptors *Endocrinology* **138** 1667–1672
- *Silverman A-J, Livne I and Witkin JW** (1994) The gonadotropin-releasing hormone neuronal systems: immunocytochemistry and *in situ* hybridization. In *The Physiology of Reproduction* pp 1683–1709 Eds E Knobil and JD Neill. Raven Press, Ltd, New York
- Sim JA, Skynner MJ, Pape J-R and Herbison AE** (2000) Late postnatal reorganization of GABA_A receptor signaling in native GnRH neurons *European Journal of Neuroscience* **12** 3497–3504
- Simonian SX and Herbison AE** (1997) Differential expression of estrogen receptor and neuropeptide Y by brainstem A1 and A2 noradrenaline neurons *Neuroscience* **76** 517–529
- Simonian SX, Spratt DP and Herbison AE** (1999) Identification and characterization of estrogen receptor alpha-containing neurons projecting to the vicinity of the gonadotropin-releasing hormone perikarya in the rostral preoptic area of the rat *Journal of Comparative Neurology* **411** 346–358
- Skynner MJ, Sim JA and Herbison AE** (1999) Detection of estrogen receptor alpha and beta messenger ribonucleic acids in adult gonadotropin-releasing hormone neurons *Endocrinology* **140** 5195–5201
- Smith MJ and Wise PM** Neurotensin gene expression increases during proestrus in the rostral medial preoptic nucleus: potential for direct communication with GnRH neurons *Endocrinology* (in press)
- Smith MJ, Jennes L and Wise PM** (2000) Localization of the VIP₂ receptor protein on GnRH neurons in the female rat *Endocrinology* **141** 4317–4320
- Spergel DJ, Kruth U, Hanley DF, Sprengel R and Seeburg PH** (1999) GABA- and glutamate-activated channels in green fluorescent protein-tagged gonadotropin-releasing hormone neurons in transgenic mice *Journal of Neuroscience* **19** 2037–2050
- van der Beek EM** (1996) Circadian control of reproduction in the female rat *Progress in Brain Research* **111** 295–320
- van der Beek EM, Swarts HJ and Wiegant VM** (1999) Central administration of antiserum to vasoactive intestinal peptide delays and reduces luteinizing hormone and prolactin surges in ovariectomized, estrogen-treated rats *Neuroendocrinology* **69** 227–237
- Wagner EJ, Ronnekleiv OK, Bosch MA and Kelly MJ** (2001) Estrogen biphasically modifies hypothalamic GABAergic function concomitantly with negative and positive control of luteinizing hormone release *Journal of Neuroscience* **21** 2085–2093
- Wright DE and Jennes L** (1993a) Origin of noradrenergic projections to GnRH perikarya-containing areas in the medial septum-diagonal band and preoptic area *Brain Research* **621** 272–278
- Wright DE and Jennes L** (1993b) Lack of expression of serotonin receptor subtype-1a, -1c, and -2 mRNAs in gonadotropin-releasing hormone producing neurons of the rat *Neuroscience Letters* **163** 1–4