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# NEURAL CIRCUITS INVOLVED IN THE CONTROL OF LHRH SECRETION: A MODEL FOR ESTROUS CYCLE REGULATION

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**Summary**—These studies have identified a core of hypothalamic neuronal systems in the regulation of episodic basal and preovulatory LH release during the estrous cycle. Steroid concentrating neurons under the direction of ovarian steroid milieu promote LHRH accumulation and either independently or in association with the opioid peptide neurons modulate the episodic LHRH discharge. The sequence of neural events that occur preceding the preovulatory LH surge on the afternoon of proestrus are described. Evidence is presented to support the hypothesis that a transient curtailment in the inhibitory influence of hypothalamic opioid peptides in the morning of proestrus, sets in motion a chain of temporally-related events starting with augmentation of adrenergic tone, leading to accumulation of neuropeptide Y and LHRH in the median eminence and culminating in episodic LHRH and LH hypersecretion in the afternoon of proestrus.

## INTRODUCTION

The maintenance of regular estrous cycles of 4–5 day duration in adult female rats depends on two modes of gonadotropin secretion from the anterior pituitary. A basal mode of luteinizing hormone (LH) secretion is evident during each day of the estrous cycle and is characterized by low amplitude pulses discharged once every hour. This basal mode of LH secretion is interrupted on the afternoon of proestrus by fast, high amplitude pulses constituting the preovulatory LH surge lasting for 4–6 h [1–3]. There is compelling evidence to show that periodic hypothalamic LH releasing hormone (LHRH) signal is essential for sustenance of these two modalities of gonadotropin secretion during the estrous cycle [1, 4]. There may be unequal LHRH secretion during the periods of basal and cyclical LH secretion. The amount of LHRH secreted during diestrus II was reported to be considerably smaller than that detected in the afternoon of proestrus [5]. Similar increments in LHRH secretion were evident in association with the LH surge induced by progesterone (P) in estrogen-primed ovariectomized rats [6]. Also, in accord with the notion that LHRH hypersecretion may be responsible for induction of the LH surge, we found that hypothalamic fragments, obtained from estrogen-primed ovariectomized rats, either just prior to or in the midst of P-induced LH discharge, secreted LHRH at high rates *in vitro*. Similar increased LHRH output *in vitro* occurred from the hypothalami of rats undergoing the LH surge induced by estrogen alone [7]. Further, delivery of small amounts of LHRH as 2 min unvarying pulses every 20 min restored the LH surge in pentobarbital-blocked proestrous rats [8]. While these *in vivo* and *in vitro* evidence clearly show that varying patterns of hypothalamic signals may maintain regular ovulatory cycles, the precise modality of LHRH discharge that sustains basal LH secretion on each day of the estrous cycle and abruptly evokes cyclical LH secretion has not been precisely defined.

## CONTROL OF LHRH SECRETION

A survey of the voluminous studies of the past 15 years shows that our knowledge of how a multitude of neural and hormonal factors integrate the varying LHRH secretion patterns during the estrous cycle is scanty [1, 4]. LHRH-producing neurons in the rat brain are sparsely distributed [9, 10]. Those LHRH-producing perikarya which may participate in the control of LH secretion are concentrated anteriorly in the diagonal band of Broca and septal regions, the preoptic and anterior hypothalamic areas and caudally some are scattered in the arcuate nucleus (ARC) and in other regions of the medial basal hypothalamus (MBH) [1]. The LHRH perikarya in the diagonal band of Broca, septal regions and preoptic area innervate regions within and outside the hypothalamus [9, 10]. There are two LHRH nerve terminal beds within the preoptic–tuberal pathway [11, 12] in the organum vasculosum of lamina terminalis (OVLT) and in the median eminence (ME). Evidence obtained from the lesion studies suggested that LHRH nerve terminals in the OVLT may participate in the cyclic LH discharge [13, 14], whereas the ME LHRH innervations may control the two modalities of LH secretion [1, 15]. Also, a few studies have attempted to identify the LHRH neurons which participate in each of the two modalities of LH secretion [1]. The output of the MBH LHRH neurons, which constitute 10–20% of the LHRH cell

population in the rat brain, can maintain indefinitely the basal mode of LH secretion. However, the contribution of an additional population of LHRH neurons, located medially in the preoptic–suprachiasmatic regions, is apparently essential for producing the normal quota of preovulatory LH surge [1].

Thus, one can envisage the complexity of neural control of LHRH secretion when this diffuse distribution pattern of LHRH neurons is considered along with the possibility that a host of diverse neuronal systems in the vicinity may have functional links with them. Systematic attempts have been made in recent years to identify the putative networks [1, 4] involved in regulation of the two modalities of LH secretion [1, 4]. The ones studied in some detail are the following: (1) The steroid concentrating neurons (SCN); (2) the classical adrenergic systems of epinephrine (E) and norepinephrine (NE) producing neurons, (3) the endogenous opioid peptide (EOP) producing neurons and (4) Neuropeptide Y (NPY) producing neurons. Our studies to date suggest that these four neural systems may form a core neuronal circuit for the control of intermittent LHRH secretion [1, 4]. The functional significance of each of these four systems is briefly summarized in this communication. In addition, a model is presented in an attempt to integrate the temporal sequence of interactions among these systems in elicitation of the preovulatory LHRH surge.

## STEROID CONCENTRATING NEURONS

The pioneering studies of Hohlweg [16] in the early thirties suggested that gonadal steroids may act centrally to regulate pituitary gonadotropin secretion. This feedback concept received further strong support from several sources. Lisk [17] and Davidson *et al.*[18] showed that intracranial implantation of small amounts of estradiol benzoate (EB) or testosterone (T) in the MBH produced atrophy of gonads and accessory glands in the rat. Using a similar intracranial implantation approach, it was later affirmed that estradiol 17-$\beta$ ($E_2$) or T implants in the MBH inhibited LH release, while $E_2$ or P implants more rostrally in the medial preoptic area (MPOA) acutely stimulated LH release [19, 20]. The demonstration of $E_2$ and androgen concentrating neurons in the preoptic–tuberal pathway raised the possibility that SCN may mediate the central feedback effects of gonadal steroids [1, 4, 21]. In fact, it is generally believed that activation of the intracellular events in these neurons [22] under the influence of circulating steroids milieu assists in regulation of gonadotropin secretion [1, 4]. However, several important questions crucial in understanding the mode of central feedback action of gonadal steroids have remained unanswered. Do SCN regulate LHRH secretion? While $E_2$, T, or P in synergism with $E_2$, suppress LH release in gonadectomized rats, it is unknown whether there is an accompanying decrease in LHRH secretion. Contradictory effects of estrogens on LHRH secretion were reported when assessed either in the hypophysial portal blood of anesthetized or unanesthetized rats *in vivo* [23, 24], from the ME–ARC by push–pull cannula preparation [6] or by *in vitro* LHRH output studies [7].

On the other hand, considerable evidence shows that the synergistic action of $E_2$ and P may augment LHRH accumulation in the ME–ARC [1, 4]. LHRH concentrations in the ME increase abruptly prior to the preovulatory LH release on proestrus [25, 26]. Similar antecedent LHRH accumulation exclusively in the ME–ARC can be evoked by P treatment of estrogen-primed ovariectomized rats [27–29]. Interestingly, however, this antecedent neurosecretory event is conspicuously absent when the LH surge is induced by estrogen alone in ovariectomized rats [1, 4, 27]. On the basis of these findings it was postulated that circulating concentrations of $E_2$ and P, in the interval between diestrus II and prior to the critical period on proestrus, may evoke antecedent LHRH accumulation in the ME [1, 4]. Presumably, the synergistic action of $E_2$ and P either activated *de novo* synthesis or this milieu may have augmented processing of the immunoreactive and biologically active LHRH from precursor proteins in anticipation of the impending preovulatory LH surge. In general, gonadal steroids appear to be capable of promoting accumulation of ME–LHRH independently of their effects on LHRH output. All three gonadal steroids —$E_2$, T and dihydrotestosterone—uniformly stimulate accumulation of ME–LHRH in male rats (see paper by P. S. Kalra).

Another interesting outcome of our studies was that although LHRH-producing neurons are scattered throughout the diencephalon and about 80% of these cells are located in the diagonal band of Broca, septal and preoptic regions, this remarkable steroid-induced LHRH accumulation occurred far caudally and exclusively in LHRH nerve terminals in the ME [29]. These observations together with the reported congruency of LHRH cell bodies and SCN in the diencephalon suggested two possible ways whereby steroids could promote ME–LHRH accumulation. It is possible that LHRH neurons concentrate steroids wherein the neurosecretory events initiated by $E_2 + P$ lead to LHRH accumulation in nerve terminals. It is also plausible that SCN are a discrete population of neurons which either directly or indirectly activate LHRH accumulation in the ME. A regional specificity of steroid action was seen with intracranial steroid implantation techniques [30]; steroid implants only in the MBH stimulated LHRH levels locally. Since the MBH has only a few sparsely distributed LHRH-containing perikarya in marked contrast to the SCN neurons, which not only outnumber the LHRH neurons but are discretely limited to the ARC and VMH, these results suggested that the MBH SCN may either directly by synaptic contacts with LHRH neurons or indirectly via other

neuronal system(s) promote LHRH accumulation in the ME [4, 19]. Recent studies of Shivers *et al.*[31] who failed to observe $E_2$ accumulation by LHRH neurons in the rat brain are in line with this hypothesis.

Subsequent to LHRH increments in the ME, an increase in LHRH secretion is observed invariably on proestrus and after P treatment of EB-primed ovariectomized rats [5, 6]. The onset and time course of LHRH secretion detected by *in vivo* and *in vitro* techniques is somewhat conflicting. LHRH hypersecretion was detected from the hypothalamus *in vivo* at 4–6 h [6] and *in vitro* at 2 h after P exposure *in vivo* [7]. Thereafter, the hypothalami obtained from these P-treated rats displaying LH surge continued to hypersecrete LHRH *in vitro* [7]. Similar prolonged hypersecretion of LHRH could not be evoked by continuous P exposure *in vitro* of hypothalami from EB-primed rats [7]. On the other hand, brief pulses of P *in vitro* readily elicited LHRH secretion from MBH–POA and ME in a reliable fashion [32]. Although the acute stimulation of LHRH release from the ME *in vitro* by P delivered continuously [7] or in pulses [32] and by $E_2$ [33] clearly suggested that steroids are capable of evoking LHRH release, the physiological significance of these findings is not readily apparent. $E_2$ and P circulate not only at much higher concentrations than used in the *in vitro* studies, but they are present for prolonged periods during the estrous cycle and their ratio varies in accordance with the stage of the cycle [34]. Recent studies show that these changing patterns of $E_2$ and P levels assist in maintaining basal episodic LH secretion on each phase of the estrous cycle. The elegant studies of Gallo *et al.*[35, 36] show that by simulating the physiological concentrations of $E_2$ and P in acutely ovariectomized rats, it is possible to restrain LH secretion to basal levels as observed during each phase of the estrous cycle. Presumably, the braking influence on the frequency of LHRH discharge is imposed predominantly by $E_2$ during diestrus and proestrus [35, 36]. Thus, the stimulatory effects of $E_2$ and P *in vitro* on LHRH release and what is normally seen during the estrous cycle as a result of their inhibitory feedback effects on LH release are obviously contradictory and remain to be resolved.

## ADRENERGIC SYSTEMS

A wealth of information is available on the effects of classical neurotransmitters on LH release in the rat [1, 37]. Since NE and E are excitatory in gonad-intact and steroid-primed rats, the general consensus is that these catecholamines may play a role in the control of the two modalities of LH secretion [1]. The early pharmacologic evidence suggested that NE may participate in the LH surge on proestrus and that induced by ovarian steroids in estrogen-primed ovariectomized rats [38, 39]. Also, it was shown that a functional link between the LHRH neurons and the adrenergic system may occur in the MPOA [40]. The observations of increased NE turnover at crucial sites in the preoptic–tuberal pathway in association with the LH surge occurring either spontaneously or that induced by ovarian steroids [41, 42] supported these conclusions [38, 39]. However, Crowley *et al.*[43] showed that E also may play an important role in the steroid-induced LH surge. Our recent pharmacological experiments have also implicated E neurons in the preovulatory LH discharge on proestrus [44]. Additionally, the fact that of all the three catecholamines tested, only E evoked LH release in proestrous rats [44] clearly underscores the importance of E in triggering the preovulatory LH surge.

Attempts have been made to define the precise role of the two adrenergic neurotransmitters in control of LH secretion. It seems that NE and E may modulate different aspects of LHRH neurosecretion and that these disparate effects may even occur at different loci in the hypothalamus [1, 4]. A careful survey of the literature showed that increments in NE turnover occurred prior to onset of the LH surge and that they coincided with accumulation of LHRH in the ME [25–29, 38, 39]. Interestingly, when NE neurotransmission was decreased pharmacologically, the antecedent ME–LHRH increments failed to occur [28, 29]. On the other hand, prior suppression specifically of E turnover in the MBH failed to disrupt the antecedent ME LHRH increments, but the subsequent LH discharge was blocked [45]. Seemingly, the function of increased NE activity in the preoptic–tuberal pathway may be to provide a microenvironment which facilitates the neuroendocrine circuitry involved in accumulation of LHRH in the ME. On the other hand, the role of E systems in the MBH may be limited to initiation and maintenance of LHRH hypersecretion throughout the LH surge period [44–46]. In line with this view is the observation that E, and not NE, evoked LH release when administered intraventricularly just prior to the preovulatory LH release on proestrus [44].

Despite the fact that available evidence, as outlined in the preceding section uniformly supports adrenergic participation in the preovulatory LH surge, there are some instances which assign a minor role, if any, to NE in regulation of LH secretion. As alluded to earlier, increased NE turnover in several crucial regions in the preoptic–tuberal pathway was not found to be temporally associated with the increased LH secretion [25–29, 38, 39]. In fact, increased rate of LH release was seen quite sometime after the increase in hypothalamic NE tone. Clearly then, these findings argue for a role of NE neurons other than in triggering LHRH release. Other studies have suggested that the role of NE system may be "modulatory" rather than mandatory in control of basal and preovulatory LH discharge. Nicholson *et al.*[47] and Clifton and Sawyer [48] noted that a permanent decrease of 80–85% of NE levels in the

hypothalamus failed to disrupt gonadotropin secretion and occurrence of ovulatory cycles. It is quite possible that denervation-induced receptor hypersensitivity, interplay of the compensatory mechanisms within the hypothalamus, along with a possible neural reinnervation by the NE neurons may allow resumption of gonadotropin secretion and ovulatory cycles. It is also quite likely that other hypothalamic neuronal systems, whose potential in LH control has not yet been recognized, and which are not disrupted by these surgical and pharmacological insults, come to play a key role in sustaining normal reproductive function. The newly described neuropeptide Y-containing neurons [49] may be a strong candidate to fill the gaps revealed by these experimental manipulations (see Section on NPY).

## ENDOGENOUS OPIOID PEPTIDES

For a long time considerable indirect evidence existed to suggest that opiates may adversely affect reproductive function in man [50]. The discovery of naturally occurring morphine-like neuropeptides in the rat brain especially in regions innervated by LHRH neurons, has provided the impetus to closely examine their functional significance in reproduction. There are three major subclasses of known EOP, the enkephalins, endorphins and dynorphins. Each of these EOP-containing neurons innervate the preoptic–tuberal pathway to a varying degree [50]. Like morphine, EOP generally inhibit gonadotropin secretion, although the effects of enkephalins have not been consistent [1, 50]. $\beta$-Endorphin ($\beta$E) was found to be the most effective inhibitor of LH release in ovariectomized rats [51]. Continuous intraventricular infusion of $\beta$E rapidly decreased LH release as a result of instantaneous abolition of episodic LH discharge (Fig. 1). More recently, we have shown that intraventricular $\beta$E suppressed preovulatory LH release and ovulation [52]. Dynorphin suppressed LH secretion but the extent of inhibition was less than that seen after $\beta$E. On the other hand, the enkephalins produced disparate effects on LH release. While methionine–enkephalin was ineffective, leucine–enkephalin stimulated LH release [51]. The suppressive effects of the EOP on LH release are

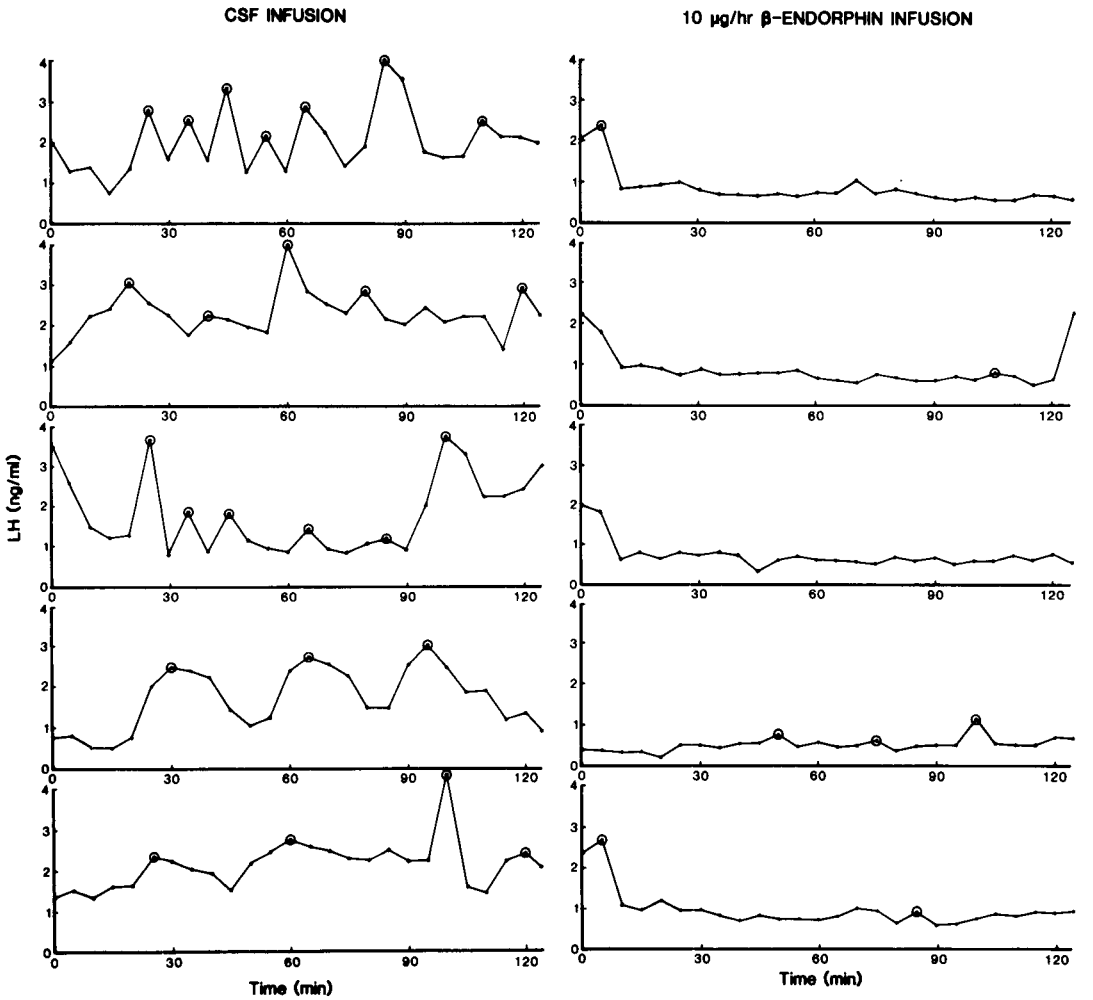


Fig. 1. Effects of intraventricular infusion of $\beta$-endorphin on episodic LH release in ovariectomized rats. Control rats received artificial cerebrospinal fluid (CSF).

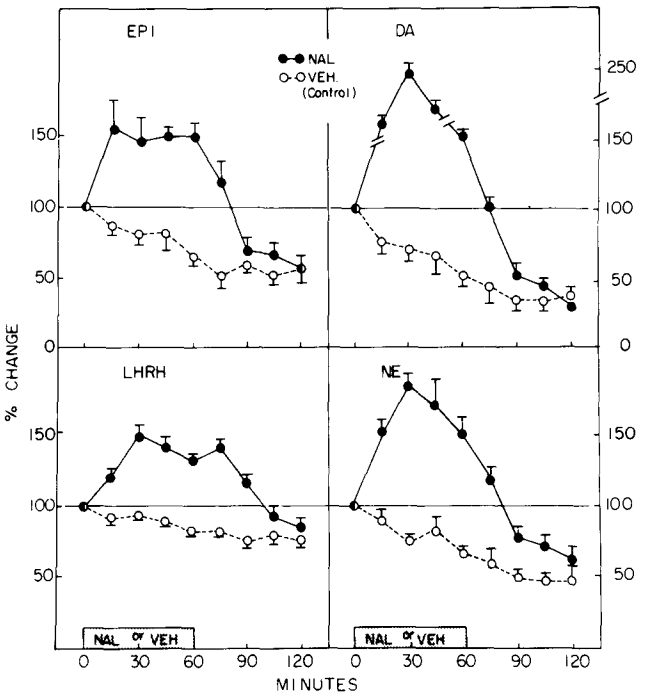


Fig. 3. Stimulation of LHRH, epinephrine (EPI), dopamine (DA) and norepinephrine (NE) release by naloxone (NAL) from the hypothalami *in vitro*. Veh (control).

apparently mediated by specific opiate receptors in the brain as an opiate receptor antagonist, naltrexone, successfully antagonized these effects [51]. Attempts have also been made to identify the subclass of opiate receptors that might mediate the LH suppressive effects of EOP. Our studies indicated that $\beta$E-induced suppression of LH release may be mediated by either $\varepsilon$ receptor type or a combination of $\mu$ and $\delta$ receptor types [51]. Supporting these observations are the findings that the $\mu$ receptor agonist, morphiceptin, and the $\delta$ receptor agonist, delta receptor peptide, inhibited LH release with marked facility [51]. In addition, it is likely that dynorphin-induced decrease in LH release was specifically mediated by $\kappa$ receptors since the specific $\kappa$-receptors agonist FK 33824, also suppressed LH release [51].

The observations of the potent inhibitory effects of $\beta$E and dynorphin posed questions of where and how they act to suppress LH release. Although $\beta$E and dynorphin-containing neurons innervate many hypothalamic and extrahypothalamic areas in the rat brain, it is more likely that they act locally within the preoptic–tuberal pathway [1, 50, 53]. The opiate receptor antagonist naloxone [53] and $\beta$E [54] when administered anywhere in the preoptic–tuberal pathway modified LH release.

Within the preoptic–tuberal pathway, there are several ways EOP may act to suppress LH release. Close proximity of the LHRH and EOP innervations in the preoptic–tuberal pathway suggested direct communication between the two systems. It is possible that subsequent to their discharge from innervations in the vicinity of LHRH neurons, EOP may bind to receptors on LHRH neurons to inhibit neurohormone release. Since opiates and $\beta$E inhibited dopamine-induced LHRH discharge from the ME nerve terminals *in vitro*, it was suggested that EOP may act at the level of LHRH neurons to modulate the excitatory effects of dopamine [55, 56]. Since the role of dopamine in the control of LH release is uncertain [1, 4, 44], it is difficult to assess the physiological significance of these *in vitro* findings. In contrast, a large body of evidence is in accord with the view that EOP may exert their suppressive effects indirectly via those neural systems which also innervate the preoptic–tuberal pathway. We found that pretreatment with either morphine [57] or $\beta$E [52] did not compromise the response of LHRH neurons to adrenergic transmitter actions. Furthermore, the post-adrenergic steps involved in LHRH discharge were also not adversely affected by $\beta$E treatment since prostaglandin-$E_2$ readily overcame the $\beta$E blockade of the preovulatory LH surge and ovulation [52].

Thus, our numerous investigations favor the notion that adrenergic systems by axo–axonic contacts may mediate the EOP effects of LH release. Further tests strongly supported this hypothesis. Blockade of opiate receptors with naloxone promptly evoked NE and E release from the hypothalamus *in vitro*, a response concurrent with the increments in LHRH secretion (Fig. 2 [58]). Furthermore, blockade of adrenergic receptors with appropriate antagonists or prior suppression of releasable pools of hypothalamic NE and E [59] or specifically of E [60], rendered naloxone ineffective in evoking LH release.

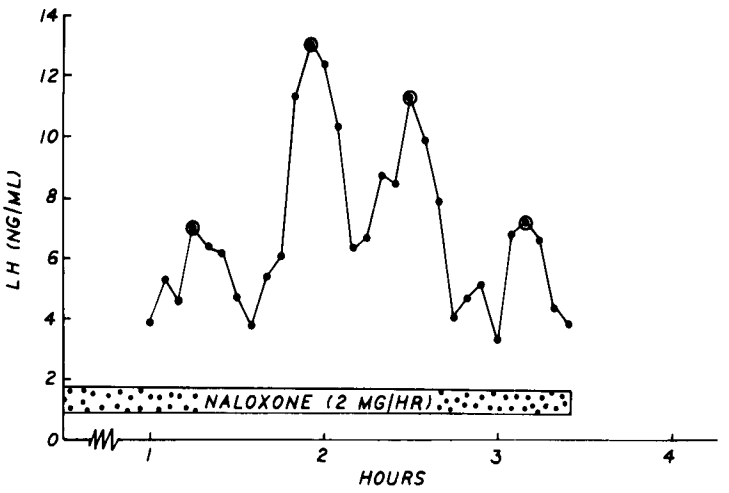


Fig. 3. Stimulation of episodic LH release by naloxone infusion on proestrous morning. Circles identify peak LH levels of an episode.

Consequently, these disclosures led us to suggest that EOP neurons "are part of a complex neuronal network locally modulating the delivery of adrenergic signals in the vicinity of LHRH neurons in the preoptic–tuberal pathway. Conceivably, a transient curtailment in that control on proestrus may enhance adrenergic influx in the preoptic–tuberal pathway and result in triggering of the neurosecretory events in LHRH neurons responsible for the afternoon LH discharge" [57].

The proposal, that a decrease in EOP influence may trigger preovulatory LH surge, was recently tested [8]. We found that continuous intravenous infusion of naloxone on proestrous morning, to achieve a decrease in EOP influence in the preoptic–tuberal pathway, induced hypersecretion of LH. The pattern of naloxone-induced LH hypersecretion resembled the pattern normally seen during the proestrous afternoon. After naloxone infusion, LH levels rose slowly during the first 45–60 min, but thereafter, a marked incremental rise in LH secretion ensued. The rapid rise in LH release was composed of discrete LH episodes, an observation reminiscent of the pattern seen in the afternoon of proestrus (Fig. 3; [3, 12]). It is well known that basal episodic LH secretion on proestrous morning is characterized by hourly LH discharge [2, 3]. We found that the frequency of episodic LH secretion was accelerated to two to three episodes/h after naloxone infusion. That this accelerated LH periodicity was due to increased LHRH discharge was indicated by another line of investigation. We were able to reproduce the pattern of LH surge seen after naloxone infusion with LHRH infusion at a frequency of 20 min in pentobarbital-blocked proestrous rats [8]. Seemingly, sometime prior to the critical period on proestrus, a decrease in the inhibitory opioid signals in the vicinity of LHRH neurons in the preoptic–tuberal pathway, curtails the restraining influence on the excitatory neural circuitry so as to allow rapid episodic LHRH discharge for induction of the LH surge.

## NEUROPEPTIDE Y

The foregoing account provides an insight into possible intimate functional links between EOP and adrenergic systems in triggering the preovulatory discharge of gonadotropin. Are there other neural signals that impinge upon this link to decrease EOP influence? Or, are there excitatory systems that supplement the adrenergic involvement or interpose between the EOP and adrenergic systems?

The NPY neuronal system in the brain may be an excitatory circuit involved in stimulation of LH release [49]. NPY is a 36 amino acid residue polypeptide recently isolated from the porcine brain [61]. It has been localized in diverse sites in the rat brain by radioimmunoassay [62] and by immunocytochemistry [63]. An examination of the NPY neuronal pathway revealed a close anatomical association with LHRH neurons in the preoptic–tuberal pathway. A group of cells containing NPY in the ARC and their extensive innervations in the MPOA and ME have been observed [63]. A more provocative revelation is the coexistence of NPY and classical adrenergic transmitters in many cells in the brain stem [63]. Among the notable ones are E and NE-containing cells which project into hypothalamic sites.

Prompted by these anatomical findings, we hypothesized that NPY may act like adrenergic transmitters in regulation of LH secretion in the rat. This indeed turned out to be the case when NPY and human pancreatic polypeptide (hPP), a peptide with which it bears close structural homologies, behaved like NE in two experimental designs [49, 64]. Similar to the effects of NE in a steroid-free environment [65], intraventricular injections of NPY or hPP promptly suppressed LH release. In the steroid-primed ovari-

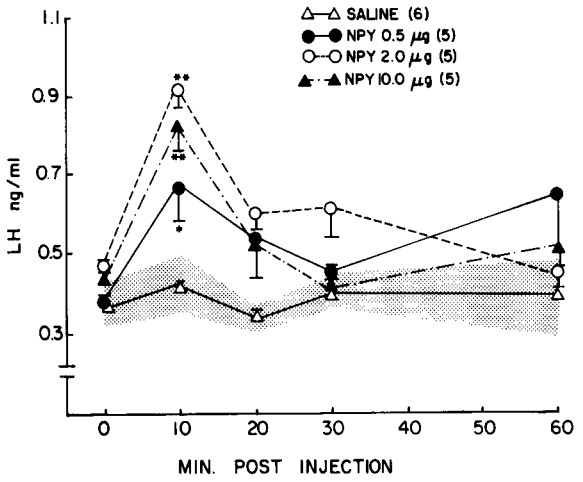


Fig. 4. Stimulation of LH release by intraventricular neuropeptide Y (NPY) in estrogen–progesterone pretreated ovariectomized rats.

ectomized rats, on the other hand, they stimulated LH release in a dose-related fashion (Fig. 4). We have extended these studies to find that NPY stimulated LH release in proestrous females and intact male rats (unpublished). Thus, the uniformly excitatory nature of NPY in intact and steroid-primed ovariectomized rats raised the possibility that the NPY peptidergic system may be an integral component of the excitatory hypothalamic neural circuitry involved in the control of LHRH secretion.

Further evidence has implicated NPY in preovulatory LHRH secretion. It was found that administration of P acutely raised NPY levels in the ME prior to the LH release and as LH levels rose, the ME NPY levels decreased gradually [66]. Since a similar picture of dynamic fluctuations in the ME LHRH concentrations preceding and following the LH surge was also seen, it appeared likely that NPY neurons may play a role in preovulatory gonadotropin discharge. In addition, the observations that P treatment acutely and concurrently raised NPY and LHRH levels in the ME, strongly supported the thesis that gonadal steroids can affect neurosecretion in those peptidergic and aminergic neurons which participate in LH release.

Because of the demonstrated neuronal coexistence of NPY and classical adrenergic neurotransmitters, we have also explored the nature of interaction, if any, between these two excitatory neuronal systems. Preliminary studies showed that NPY and adrenergic systems may act independently, and the adrenergic systems may not mediate the excitatory effects of NPY on LH release [Allen and Kalra, unpublished]. However, these observations do not rule out the possibility of co-action and/or co-operativity between the two systems in regulation of LH secretion.

*Steroid-opioid-neuropeptide Y-adrenergic connection in regulation of LHRH secretion, a Model*

In summary, concerted efforts have unraveled a complex neural circuitry which employs a variety of neurochemical signals within a narrow band in the preoptic–tuberal pathway to direct LHRH secretion. It is likely that LHRH producing neurons discharge their product into the hypophysial portal system at an inherent frequency of 20–30 min to impart a similar rhythmicity in pituitary gonadotropin secretion [67]. Evidently, a similar set of hypothalamic neurochemical signals modulate episodic LHRH signalling in gonadectomized and intact, male and female rats. In addition, peripheral gonadal hormone signals play a crucial role in governing the two modalities of LH secretion in female rats. On the basis of the available evidence, it appears that the gonadal steroid milieu decelerates the frequency of LH discharge from one every 20 min as seen in ovariectomized rats, to one every hour during each day of the estrous cycle. Under the combined action of the neural clock (NC) and the steroid milieu favoring $E_2$ over P, the LH discharge frequency reverts back to one every 20 min during the critical period on proestrus so as to give rise to the preovulatory LH surge. Whether deceleration of episodic LH discharge during each day of the estrous cycle is due to changes in the frequency and amplitude of LHRH signals reaching the pituitary or due to modulation of the pituitary response to incoming LHRH signals has not yet been satisfactorily established. However, convincing evidence exists to support the notion that a shift in the ovarian steroid milieu favoring $E_2$ over P facilitates the occurrence of accelerated episodic LHRH discharge during the critical period on proestrus. In addition, this ovarian steroid milieu also promotes formation and accumulation of a host of neuropeptides in anticipation of LHRH hypersecretion on proestrous afternoon. Our studies show that $E_2 + P$ milieu augments LHRH storage in the ME, possibly by stepping up *de novo* synthesis. Studies show that a similar steroid milieu may modulate EOP levels, especially $\beta$E [68]. The list of peptides regulated by $E_2 + P$ milieu has grown now to include NPY [66], (Fig. 5).

Information available so far is compatible with our view that under the influence of $P + E_2$ milieu favoring P, EOP may decelerate LHRH discharge frequency. Our findings that continuous intraventricular $\beta$E infusion decelerated episodic LH discharge, while conversely, continuous intravenous naloxone infusion to curtail the inhibitory opioid influence, accelerated the episodic LHRH discharge clearly support our postulate that "a multitude of known and unknown neural events, inhibitory and excitatory, may take place prior to discharge of the preovulatory LH surge. Some of these may occur in strict temporally-related sequence perhaps initiated by the internal clock" [50]. Accordingly, it is likely that the NC, either directly or through another neural circuit, decreases EOP influence prior to the critical period to allow a train of facilitory circuits to operate and eventuate in LHRH hypersecretion (Figs 5 and 6).

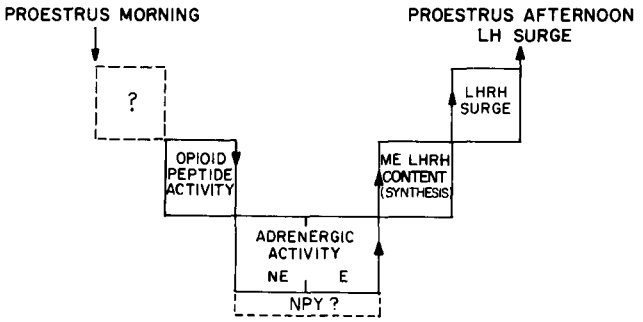


Fig. 5. Cascade of neuroendocrine events preceding the preovulatory LH surge on proestrus.

We have also identified those chain of neural events which may interpose between NC–EOP and LHRH hypersecretion in a strict temporal sequence (Figs 5 and 6). A decrease in the EOP influence apparently stimulates adrenergic activity in the preoptic–tuberal pathway. The NE component of the adrenergic system provides an environment which promotes formation and accumulation of LHRH in the ME. Perhaps a similar neurosecretory response is evoked in the NPY neurons. As the accumulation of these neuropeptides reaches a threshold, increased release of E, and perhaps of NPY, may accelerate and sustain LHRH discharge at 20 min frequency through most of the proestrous afternoon. As a consequence of the cumulative effects of high frequency hypothalamic signals on the hypersensitive pituitary, plasma LH levels climb and reach a plateau within 2 h. These elevated levels are sustained for an additional 60–90 min before the descending phase gradually terminates LH hypersecretion. Depletion of releasable LHRH stores [26–29] and pituitary refractoriness to repeated LHRH stimulus may terminate the LH surge.

Consequently, by varying the delivery of LHRH signals to the pituitary, the brain may control reproductive cycles in the female rat. We have begun to decipher the interactions among the four components of the hypothalamic neural circuitry which may regulate varying, episodic LHRH signalling. A difficult task ahead is to unravel further the underlying intracellular events which assist in this intercommunication.

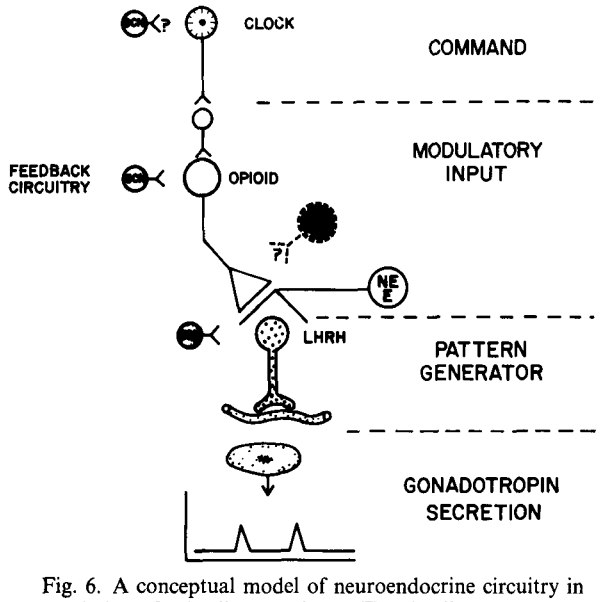


Fig. 6. A conceptual model of neuroendocrine circuitry in regulation of episodic LH release. (For details see Text and References 1, 4 and 50).

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