AN INVESTIGATION INTO THE ROLE OF THE ANTERIOR MEDIAL PREOPTIC NUCLEUS AND SUPRACHIASMATIC NUCLEUS IN THE CONTROL OF THE OVULATORY CYCLE: EFFECTS OF LESIONS ON LH, PROLACTIN, PROGnRH AND GnRH.

by

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ABSTRACT

Experimental evidence has demonstrated that lesions of the anterior medial preoptic nucleus (AMPO) or the suprachiasmatic nucleus (SCN) interrupt the reproductive cycles in the female rat, produce a persistent vaginal estrus (PE), and block the preovulatory surges of luteinizing hormone (LH) and prolactin (PRL). Experiments were designed to further investigate the effects of bilateral electrolytic lesions of the AMPO or the SCN on plasma levels of LH and PRL, and on hypothalamic levels of proGnRH and GnRH in the female rat. Approximately 4 weeks after lesion placement, blood samples were withdrawn from catheterized freely moving animals and plasma LH and PRL were determined by radioimmunoassay (RIAs). Two months later, the animals were sacrificed between 0830 and 0930 h. The brains were sectioned, and microdissections were made from the preoptic area (POA) and the basal hypothalamus (BH). The microdissected tissues were extracted, purified and GnRH and proGnRH levels were determined by RIAs.

The preovulatory surges of LH and PRL were eliminated in AMPO- or SCN-lesioned rats. Moreover, the LH surge was eliminated and the PRL surge significantly attenuated following estrogen and progesterone injections. Lesions in the dorsal part of the POA induced persistent diestrus (PD). In these animals, estradiol benzoate (EB) consistently induced LH and PRL surges. Plasma progesterone levels were significantly lower in the PE animals ($P<0.01$), whereas plasma estradiol levels were similar to that of sham lesioned proestrous controls. In contrast, PD animals had high levels of progesterone and low levels of estradiol.

The proGnRH levels in the POA were significantly reduced in AMPO-lesioned (0.95 ± 0.11 fmoles/mg tissue) and in SCN-lesioned (0.87 ± 0.09 fmoles/mg tissue) rats, as compared to sham-lesioned proestrous controls (7.99 ± 1.11 fmoles/mg tissue). In contrast, GnRH levels in the POA or the BH were not significantly different in AMPO-lesioned (15.04 ± 2.94 and 90.05 ± 10.32 fmoles/mg tissue, POA and BH respectively) or in SCN-lesioned (16.23 ± 1.33 and 97.83 ± 10.96 fmoles/ mg tissue, POA and BH respectively) animals, as compared to sham-lesioned proestrous controls (15.03 ± 0.59 and 96.25 ± 8.69 fmoles/mg tissue, POA and BH respectively). Therefore, lesion of the AMPO or the SCN caused persistent estrus without a reduction in GnRH levels, while proGnRH levels are significantly reduced.

These data support the hypothesis that the AMPO and the SCN participate in the control of the estrous cycle and are necessary for the preovulatory surges of PRL and LH to occur. Moreover, the AMPO and/or the SCN participate in the control of the LH surge by regulating GnRH synthesis and release.

INTRODUCTION

Experimental evidence has demonstrated that the anterior medial preoptic nucleus (AMPO)[197,198,256] and suprachiasmatic nucleus (SCN)[27,187,197,198,258] are essential neural components for the normal control of phasic (preovulatory) luteinizing hormone (LH) and prolactin (PRL) secretion. Destruction of the AMPO or SCN block spontaneous ovulation, induce persistent vaginal estrus (PE), and abolish the estrogen- and progesterone-induced surges of LH in female rats[86,119,131,184,204,212,257].

It has been well documented that gonadotropin-releasing hormone (GnRH), also called LHRH, is a primary neural signal in control of pituitary LH secretion[108]. These neurons are primarily located in the preoptic area (POA) and send fiber projections to the median eminence (ME) region where the majority of the GnRH fibers terminate[223]. Numerous hypothalamic deafferentation and SCN-POA lesions have shown that these procedures reduce the GnRH content in the ME[28,93,96,99,131,184,216,254]. Thus, it is postulated that postlesion anovulatory persistent estrus is due to significant reduction of GnRH in the ME[204,212]. However, immunohistochemical studies have shown that immunostaining of GnRH neurons rostral, lateral and caudal to the lesions is increased, especially, the ME exhibited intense fiber stain after lesions of the SCN, AMPO or both[197,242,259]. Therefore, it has been suggested that persistent estrus can be produced without a reduction of hypothalamic GnRH levels by destruction of neurons in the SCN or AMPO, which directly or indirectly control the synthesis, release or both in GnRH cells.

Because, immunohistochemistry is not a quantitative approach to detect the changes of GnRH levels, we designed this study to quantitate GnRH content in the hypothalamus in AMPO- or SCN-lesioned persistent estrous rats. We further designed this study to measure the precursor of GnRH (proGnRH) to evaluate how the lesions of the AMPO or SCN might affect GnRH synthesis. In addition, we also measured plasma hormone levels of LH and PRL to evaluate the lesions and lesion placement.

General Background

I. Regulation of LH secretion

LH is one of the principal glycoprotein hormones and is produced in the basophil cells of anterior pituitary. It is known that LH plays an important role in causing the rupture of the mature ovarian follicle (ovulation). It is well documented that GnRH, is the primary neural signal to activate both LH and follicle stimulating hormone (FSH) secretion from the pituitary gonadotropes[108]. Furthermore, gonadal steroids also act on the pituitary to regulate the responsiveness to GnRH which in turn affect LH release[100].

The patterns of LH secretion

Essentially, two patterns of LH secretion have been identified[76], one is characterized by low levels of pulsatile LH secretion during diestrus and estrus, the other is high frequency and amplitude of LH release during the afternoon of proestrus. Moreover, gonadal steroids play an important role in regulation of LH secretion during the estrous cycle (see below).

Hypothalamic control

<1> Role of GnRH in the regulation of LH release

GnRH is a primary neural signal to activate the secretion of LH and FSH. The first demonstration of an extractable neurohormone (GnRH) from the hypothalamus, which had luteotropin-releasing activity, was done by McCann et al. in 1960[152]. GnRH subsequently was isolated, purified and its structure was identified as (pyro)Glu-His-Tryp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 determined independently by Schally et al.[9,148] and Guillemin et al.[31]. GnRH-producing neurons are located mainly in septal, preoptic, and hypothalamic regions. They project into the ME, and release GnRH into the portal capillaries, which transport and deliver GnRH to the pituitary[96,197,223].

It has been reported that the pituitary stalk plasma immunoreactive GnRH concentrations increase approximately 5 fold during proestrus and decrease to diestrous levels during estrus, when GnRH concentration in pituitary stalk plasma rises, LH concentration in systemic plasma also rises[42]. This suggests that GnRH secretion pattern is associated with the two patterns of LH secretion in intact rats. Wise et al.[263] have demonstrated that during proestrus, GnRH content in POA is increased 4 h prior to the increase in LH secretion. During diestrus, however, no significant changes in GnRH concentrations are found at different time points. Further, Levine and Ramirez[140] also have demonstrated that during proestrus, GnRH secretion rate is enhanced 3 h prior to LH secretion, and when GnRH secretion rate is maximal, LH secretion is also maximal. During diestrus day 1 and day 2, GnRH secretion is low, and rises slightly in the late afternoon. These data suggest that LH secretion is controlled by GnRH.

<2> Role of the AMPO and SCN in the control of LH release

Deafferentation studies have shown that bilateral transection in the coronal plane, caudal to the SCN separating the preoptic region from the basal hypothalamus (BH), blocks the preovulatory surge of LH, prevents ovulation and leads to the development of the PE syndrome[21,131,172]. Further, lesion studies have shown that lesions of the SCN area in the rat result in the PE syndrome[6,239], and these lesions block spontaneous and progesterone-induced ovulation[27] and the progesterone-induced LH surges[16,86,204]. Therefore, it is apparent that the SCN controls phasic (preovulatory) LH secretion. Wiegand et al.[256-258] and others[86,184] have demonstrated that the AMPO is also involved in controlling the estrous cycle and the preovulatory release of LH. More recent findings reported by Ronnekleiv et al. [197,198] not only corroborate these studies, but also indicate the SCN and AMPO act as a functional unit to control the surge of LH and PRL. However, both anatomical and physiological relationships between the SCN and the AMPO are not certain yet.

The AMPO are two small clusters of periventricular cells that lie just caudal to the organum vasculosum of the lamina terminalis (OVLT) and dorsal to the rostral end of the optic chiasm[177]. Simerly et al.[225-227] have demonstrated that, immunoreactive vasopressin, substance P, cholecystokinin (CCK) and somatostatin fibers are densely visualized within the AMPO, while many fewer fibers are present in the zone lateral or rostral to the nucleus. In contrast, stained dense plexuses of leucine-enkephalin, vasoactive intestinal polypeptide (VIP), neurotensin, serotonin, neuropeptide Y and dopamine ß-hydroxylase (norepinephrine) fibers are found in the zone lateral to the AMPO[225-227]. The most common cell type found within the AMPO is leucine-enkephalin-immunoreactive neurons, and a few VIP, CCK, tyrosine hydroxylase (dopamine), dopamine ß-hydroxylase cell bodies are also found in the AMPO[225-227]. There are a few GnRH fibers, but no immunoreactive GnRH cells are found in the AMPO. GnRH neurons are dorsal and lateral to the AMPO[225-227].

The SCN are two small cell clusters dorsal to the optic chiasm and lateral to the third ventricle[177]. Immunohistochemical studies reported by van den Pol et al.[246,247], Card and Moore[38] have demonstrated that the cell bodies immunoreactive to neurophysin, vasopressin, gastrin-releasing peptide, VIP and somatostatin are located in specific areas within the SCN: <1> Neurophysin and vasopressin are found in greatest densities in dorsomedial part of the SCN; <2> VIP and gastrin-releasing peptide immunoreactive neurons are found predominantly in the ventral part of the SCN and often embedded in the optic chiasm ventral to the

SCN; <3> Somatostatin immunoreactive cell bodies are primarily in the dorsomedial part of the SCN. Immunoreactive GnRH perikarya are not seen within, but lateral to the SCN and dorsal to the supraoptic nucleus[197]. Immunoreactive serotonin and VIP fibers are seen throughout the ventral medial part of the SCN[38,246,247] and serotonin fibers innervate VIP cells in the SCN[24,92]. A few numbers of dopamine immunoreactive fibers are found throughout the SCN, and neurophysin and vasopressin fibers are both seen in dorsomedial part of the SCN[38,247]. GnRH immunoreactive fibers are found either rostral or lateral to SCN or coursing down the midline between the left and right SCN[246,247]. Neurons within the SCN containing neurophysin and vasopressin or somatostatin are found to project to the medial paraventricular nucleus[246,247]. SCN VIP neurons are found to project into the periventricular nucleus and anterior hypothalamic areas[246,247]. Furthermore, VIP fiber-connections between two SCN are also observed[246,247]. The serotoninergic fibers within the SCN originate in the raphe nucleus, and NPY-containing fibers within the SCN originate in the lateral geniculate nucleus[246,247].

A direct neural pathway from the retina to the SCN[59] suggests that light input to SCN may be an important factor in the control of the reproductive cycle in some species, such as rat and golden hamster. It is well documented that the light:dark (LD) cycle determines the time of the preovulatory surge of LH and ovulation in the female rat[5]. For instance, during LD 14:10 or 12:12, LH is released into the peripheral circulation 2-4 h after the midpoint of the light period, or 2-4 h prior to turning the lights off. More importantly, when the 14-h or 12-h light period is advanced 3 to 4 h, LH release is also advanced 3 to 4 h. The 2-4 h duration, after the midpoint of the light period, were defined as "critical period" of LH surge on proestrus by Everett and Sawyer[64,65]. It is only during this critical period that barbiturates can completely block the proestrous LH surge. Wise et al.[263] have found that, in the preoptico-suprachiasmatic-tuberoinfundibular system, GnRH content is increased approximately 3 h prior to LH surge, and the GnRH content are decreased (due to increase release) during the occurrence of LH surge. Therefore, the timing of GnRH/LH release is controlled by the LD cycle. It has also been reported that constant bright light leads to persistent estrus and the failure of ovulation [36]. These phenomena further support the view that the SCN may act as a central pacemaker (biological clock)[234].

Role of gonadal steroids

It is known that gonadal steroids control gonadotropin (LH and FSH) secretion by feedback actions on the hypothalamo-pituitary axis[83,150,154,185]. It is generally believed that basal pulsatile LH secretion is controlled by the negative feedback actions of estradiol and progesterone[81,117], while the preovulatory surge of LH is mainly regulated by the positive feedback action of estradiol[214]. In contrast, progesterone shows a biphasic effect on the surge of LH, either negative[81] or positive action[45]. It is important to know that progesterone negative feedback action is necessary for preventing the surge of LH at other estrous cycle stages[81], whereas the positive action seems only to modulate the magnitude of the surge[181]. Although these steroids play an important role(s) in regulating LH release, it is still uncertain where the sites of feedback actions of the steroids are, and the mechanism of action in regulating the release of LH.

<1> Negative feedback actions of the steroids

Numerous studies have shown that the negative feedback actions of estradiol occur within the hypothalamo-hypophysial axis[19,154,195]. Ovariectomy increases[19] and estradiol decreases[52,249] the levels of LH released in response to exogenous GnRH. Kalra et al.[107] and Libertun et al.[142] demonstrated that a single injection of estrogen, initially decreased the pituitary responsiveness to GnRH, followed by an enhancement for a prolonged period at the pituitary. These findings suggest that estradiol can act at the pituitary. On the other hand, the negative feedback actions of progesterone may occur in the medial basal hypothalamus (MBH) because progesterone implants in the MBH suppress LH without decreasing the pituitary response to GnRH[230]. However, progesterone implants in the POA block estradiol-induced LH release[155] and decrease GnRH output in response to stimulation of the POA[37]. The difference may be due to the diffusion of implants, either from MBH to POA or from POA to MBH. Another explanation is that progesterone may act both at the POA and the MBH.

<2> Positive feedback actions of the steroids

It is evident in the rat that the positive feedback action of estradiol occur both at the pituitary and in the brain. Estrogens can enhance the response of the pituitary to exogenous GnRH about 24 to 72 h after injection (sc) of estradiol benzoate and 5 to 7 h after infusion (iv) of estradiol[142,249]. Estrogens also can promote the priming effect of GnRH to increase the response of the pituitary to subsequent GnRH administration[3]. In intact rats, the elevation of portal blood GnRH levels on the afternoon of proestrus[206] might be due to the positive feedback action of estradiol in the brain[207]. Furthermore, it has been demonstrated that increasing estradiol concentrations in the pituitary to levels greater than those in the pituitary of proestrous rat does not elicit LH release[82]. This would suggest that the effect of estradiol at the pituitary is not sufficient alone to induce the surge of LH. Implant studies further demonstrated that estradiol implanted in the POA elicited a discharge of LH, although the pituitary estradiol concentrations were low[82]. Therefore, estradiol can act at the POA to induce LH release. It is believed that gonadal steroids may affect both GnRH synthesis, storage and/or release[108]. Hypothalamic GnRH secretion from the ME was reduced after ovariectomy[140] and GnRH secretion <u>in</u> <u>vivo</u> can be enhanced from MBH at the time of the LH surge in ovariectomized rats by replacing estradiol[208]. Thus, these findings further confirm that the POA may be one of the areas for the positive feedback action of estradiol to induce the surge of LH.

Both in vivo[68] and in vitro[135] studies have demonstrated that progesterone can increase the pituitary response to GnRH. The pituitary responsiveness to GnRH is enhanced, just prior to and following the preovulatory LH surge, when progesterone secretion rises[101], or shortly after progesterone injection to estrogen-primed ovariectomized rats[73]. However, other studies have found that progesterone is also able to induce GnRH secretion from the hypothalami of estrogen-primed rats[125,136]. Administration of progesterone to MBH-POA fragments prepared from intact rats results in increased GnRH release[136], and in vivo it can promote GnRH release in estrogen-primed ovariectomized rats[139]. It is known that progesterone secretion rises prior to and during the preovulatory LH surge[101]; thus, it is likely that in combination with the neural trigger from the brain, it may facilitate GnRH hypersecretion during the afternoon of proestrus. Another interesting aspect of progesterone is that the latency (3 to 4 h) of the stimulatory effect on GnRH accumulation is much shorter than estradiol (3 to 4 days)[2].

<3> Possible mechanism of gonadal steroid on GnRH

Numerous studies have indicated that gonadal steroids may modulate some intraneuronal events related to neurohormone production and storage in the GnRH neurons[102,106,108,122]. Gonadectomy invariably causes reduction in the content of GnRH in the MBH, which is restored by administration of estradiol[102]. Interestingly, during long periods of uninterrupted low basal LH secretion in gonadectomized rats treated with estradiol, GnRH accumulates in the ME terminals[103]. Thus, one interpretation is that the steroid-induced accumulation of GnRH may be mainly due to the inhibition of release by steroids acting on the cell membrane as demonstrated by Kelly et al.[123]. Another possibility is that the steroids may induce the production of new GnRH which is transported to the ME. Whether the accumulation of GnRH is caused by the production of new precursor GnRH protein or by acceleration of processing the precursor into bioassayable or radioimmunoreactive GnRH, is still not clear. Recent results have shown that GnRH and proGnRH levels are significantly reduced in 2-week ovariectomized rats as compared to intact proestrus rats; however, no significant difference was found in GnRH mRNA levels[122]. This would indicate that gonadal steroid(s) may act on post-transcriptional processing of GnRH. However, **in situ** hybridization findings have shown that estrogen treatment to ovariectomized rats can either increase[180] or decrease[266] GnRH mRNA levels. Further studies are needed to reconcile these different results.

Role of monoamines

It has been known that the monoamines may be involved in controlling LH secretion[71,111,252]. Neuronal projections containing different monoamines are found in the diencephalon[71,143,220], and each of these monoaminergic systems innervate areas in association with GnRH neurons in the septal-preoptic-tuberal pathway[97]. It has been proposed that the nerve terminals of monoaminergic neurons may be the final connection to GnRH neurons that transmit messages in response to hormonal and environmental changes[111].

It is generally believed that dopamine (DA) may be either facilitatory or inhibitory to LH secretion[188]. It was reported that DA exhibited an inhibitory effect on LH release in ovariectomized rats, since activation of DA receptors with apomorphine and ET-495, inhibited LH release[56,74]. Pimozide, a DA receptor blocker, reversed the effects of apomorphine[77], and HA-966, a DA release inhibitor, enhanced the amplitude of LH release in ovariectomized rats[80]. Furthermore, the DA turnover decreased prior to the preovulatory LH surge[75]. These studies indicate that increases in DA transmission may suppress frequency and/or amplitude of LH release. On the other hand, excitatory effects of DA on LH release were also reported. Thus DA can stimulate GnRH release <u>in vitro</u> from ME fragments of steroid-primed ovariectomized rats[160]. Other studies have demonstrated that intraventricular injection of DA enhanced the release of LH from steroid-primed ovariectomized rats[213,250]. Moreover, the DA receptor blockers haloperidol and chlorpromazine suppressed LH release in ovariectomized rhesus macaques[15] when these drugs were administered at dosages that produced sedation. Therefore, these studies suggest that DA transmission may also facilitate LH release. The conflicting results still can not be explained, but the data suggest that DA is involved in the regulation of LH release.

Sawyer first observed that norepinephrine (NE) stimulates ovulation in the rabbits[210]. Since then numerous studies indicated that NE-producing neurons in the brainstem[174], which project to the hypothalamus, may participate either in the excitatory action[105], or in the inhibitory action of LH release[78,137], in which the excitatory effects is mediated by α_1-adrenergic receptors, and the inhibitory effect is mediated by both α_2- and ß-adrenergic receptors. Intraventricular injection of NE induced LH release by activation of α_1-adrenergic receptors in hypothalamus and MPOA of gonadectomized or steroid-treated gonadectomized rats[78]. It was found that in ovariectomized rats in which NE depletion was produced by FLA-63 or reserpine, LH release was suppressed and LH release could be restored by administration of the α-receptor stimulator clonidine[62,128]. Furthermore, both _in vitro_ and _in vivo_ studies demonstrated that the stimulatory effect of NE on LH release may be due to the effect of NE on GnRH secretion into the hypophyseal portal system[132,160]. Decreased NE turnover in the preoptic-tuberal pathway was found to suppress GnRH levels in ovariectomized rats[112], and disrupted the progesterone-induced accumulation of GnRH in estrogen-primed ovariectomized rats[228]. Anatomical evidence further confirm the effect of NE on GnRH release, that the entire diagonal band of Broca-preoptic-tuberal pathway may be a site of interaction between NE and GnRH neurons[138]. It has been found that NE nerves terminate in close proximity of dendrites, axons, and perikarya of GnRH neurons in hypothalamus[97]. On the other hand, some studies showed inhibitory effects of NE on LH release in intact and in ovariectomized rats[78,137], thus intraventricular injection of NE or administration of adrenergic agonists suppressed the amplitude and frequency of LH release. Also, it was found that NE may inhibit the preovulatory and ovarian steroid-induced LH release which may be mediated by ß-adrenergic receptors[33]. Therefore, it can be concluded that NE may participate in regulation of LH secretion. However, the precise physiological mechanisms of NE on LH release is still not clear.

Unlike NE, epinephrine (E) may only participate in the control of preovulatory LH secretion[46,49] and ovarian steroid-induced LH surges[49]. Intraventricular injection of E on proestrus and in steroid-primed ovariectomized rats induced LH release[201,250].

5-hydroxytryptamine (5-HT) containing fibers, which are derived from serotonin-producing cell bodies in dorsal and median midbrain raphe nuclei, are present in various regions of the preoptic-tuberal pathway[8]. As previous described, 5-HT containing fibers are distributed throughout the SCN, especially in the ventral area where they form dense networks and synapse on VIP neurons[38,92,246,247]. Thus, the effect of 5-HT on LH release, is likely through the SCN. Intraventricular or systemic administration of 5-HT suppresses LH release in ovariectomized rats[113]. Furthermore, an increase of 5-HT in the vicinity of GnRH neurons in the preoptic tuberal pathway by means of blocking reuptake with Fluxetine (chemical name) or by electrical stimulation of midbrain raphe nucleus suppresses basal (episodic) LH release[20,189]. Thus, these findings indicate that there may be an inhibitory effect of 5-HT on basal LH secretion in the rats. On the other hand, many studies have shown an excitatory effect of 5-HT on preovulatory LH release[94]. Disruption of central 5-HT releasing rhythmicity by pharmacologic means (with reserpine) block ovulation[252] and the estrogen-induced LH surge in ovariectomized rats[94]. Furthermore, lesions of midbrain raphe nucleus, which reduced hypothalamic 5-HT levels, decrease the estrogen-induced LH surge[94]. In general, increased activity of 5-HT neurons facilitates the preovulatory LH secretion, but it suppresses basal LH release.

Roles of neuropeptides and neuroactive amino acids

Recently, numerous studies have shown that neuropeptides and neuroactive amino acids may be involved in the control of GnRH and LH release[108,111]. Neuropeptides and neuroactive amino acids have been localized in the diencephalon, especially in those regions that are innervated by GnRH neurons[104]. Recently, neuropeptide Y (NPY), VIP and endogenous opioid peptides have been found to be involved in the regulation of LH release [108,237,251].

NPY consists of a 36-amino acid residue peptide and its structure is similar to the pancreatic polypeptide[241]. NPY has been localized in hypothalamus by means of immunohistochemistry. Distributions of NPY-like immunoreactive cells and fibers display considerable overlap with the GnRH network. Mainly, the immunoreactive cell bodies are visualized in ARC, periventricular nucleus, and septum; their fiber terminals are found in the ME, AMPO, SCN, and other sites along the preoptic-tuberal pathway[38,66,247]. NPY either stimulates or inhibits GnRH and LH release depending upon the physiological conditions. Intraventricular injection of NPY results in suppression of GnRH and LH release in ovariectomized rats, however, priming the rats with E_2 and progesterone resulted in an enhancement of GnRH and LH release [109,110]. This indicates that ovarian steroids may modulate the effects of NPY on GnRH and LH release.

Vasoactive intestinal peptide (VIP) shows a stimulatory effect on LH secretion in ovariectomized rats[251]. Furthermore, VIP has been found to stimulate GnRH release from rat hypothalami _in vitro_[171]. Since VIP neurons in the SCN are densely innervated by serotoninergic fibers and serotonin exerts a direct stimulatory influence on VIP[219], the stimulatory effect of VIP on LH is likely associated with serotonin. However, Alexander et al.[4] have demonstrated that VIP exhibits an inhibitory effect on LH secretion in the ovariectomized rat, probably via an inhibitory effect on GnRH secretion because the inhibitory effect is not due to reduced pituitary responsiveness to GnRH[4]. Recently, Maegawa et al.[146] have demonstrated that synaptic junctions are localized between somatostatin-containing nerve terminals and VIP-containing neurons in the SCN. It is known that somatostatin exerts an inhibitory effect on brain and pituitary cells[61]. Thus, it is possible that VIP neurons are stimulated by serotonin and inhibited by somatostatin.

Experimental evidence indicate that endogenous opioid peptides may be directly or indirectly involved in control of GnRH and LH release[108,111]. Anatomical findings show that the three different opioid peptide neurons are present in the proximity of the GnRH neuronal network in the preoptic-tuberal pathway[124].

Cell bodies containing ß-endorphin are localized in the arcuate and more lateral regions, and send fiber projections to the POA[124]. It was found that the basal episodic [129] and preovulatory[43] LH release were inhibited following intraventricular injections of ß-endorphin. Interestingly, ß-endorphin effects may be modulated by ovarian steroids in control of LH release. Ovarian steroids have been shown to alter ß-endorphin levels. There is a rise in the ME and decrease in the POA during the early phase of preovulatory LH release[10]. The decrease in ß-endorphin in POA parallels the loss of tonic inhibition, and the increase in GnRH synthesis and LH release. In addition, ovariectomy reduces, whereas combined estradiol and progesterone treatment restores, the ß-endorphin secretion rate in hypophyseal portal blood[253]. This suggests that the negative feedback effects of ovarian steroids on GnRH may be indirectly through ß-endorphin inhibition of GnRH. It has been suggested that two types of opiate receptors (delta and Kappa receptor) may be involved in modulating the effects of ß-endorphin on LH release[264].

Dynorphin-immunoreactive cell bodies are localized in the supraoptic nucleus, paraventricular nucleus and limbic nuclei[124]. Intraventricular injections of dynorphin suppressed LH release, but the effect is less pronounced than ß-endorphin[111]. The effects of dynorphin on LH release may be via K-type opioid receptors[264].

Enkephalin-containing cell bodies are widely distributed in the brain and a large population of cells are found within the AMPO[225]. Systemic injection of methionine-enkephalin results in decreased LH release, but intraventricular injection in ovariectomized rats produces little changes in basal LH release [30]. In contrast leucine-enkephalin is found to stimulate LH release[30]. Thus, leucine-enkephalin within the AMPO may be involved in the regulation of LH secretion. In addition, these two enkephalins show preferential affinity for δ-type opioid receptors in the regions of MPOA, AHA and ME[69]. However, the role of enkephalins is still poorly understood.

One can conclude that neuropeptides may participate in regulation of GnRH and LH release. However, the specific inputs of neuropeptides in control of GnRH and LH are still ambiguous.

Summary

In summary of GnRH/LH secretion: <1> GnRH is the primary neurohumoral signal involved in the secretion of LH and FSH. <2> Gonadal steroids act in various ways to modulate GnRH/LH secretion. Generally, gonadal steroids may directly act at hypothalamic level to affect synthesis and storage within the GnRH neurons and in turn influence LH secretion. Gonadal steroids may also interact with other neurons within or outside the hypothalamus to indirectly affect GnRH/LH secretion. Also the steroids may act at pituitary level to modulate the sensitivity to GnRH.

II. Regulations of prolactin (PRL)

Prolactin, a 198-amino-acid peptide hormone, is produced primarily within the anterior pituitary lactotrope cell, and has many physiological functions[166]. Various experimental results have shown that the secretion of PRL is likely regulated by many factors, although the exact role of some of them remains uncertain. Generally, it is believed that secretion of PRL is regulated through the hypothalamic-hypophysiotropic axis[145,151,244]. Other factors, such as steroids, neuropeptides, and neurotransmitters, affect the secretion of PRL directly or indirectly by acting on the hypothalamic-hypophysiotropic axis.

The patterns of PRL secretion during reproductive cycle

In the rat estrous cycle, plasma PRL is characterized by low levels of secretion during diestrus and estrus, and by high levels of secretion during late proestrus (proestrous surge) [162,163,165]. The proestrous surge of PRL is accompanied by similar surges of LH, FSH, and progesterone[231]. It is known that estrogen feedback is an essential component of the preovulatory surge of PRL, as well as LH and FSH as discussed previously. Moreover, PRL secretion shows an unique pattern during pseudopregnancy, pregnancy and lactation: <1> pseudopregnancy: In rat, it is known that artificial stimulation of the uterine cervix at proestrus induces a pseudopregnancy lasting 12 or 14 days, which is characterized by maintenance of progesterone secretion from corpora lutea due to an increase in serum PRL levels[70]. <2> pregnancy: The pattern of PRL secretion during the first 9 days of pregnancy is essentially identical to that of pseudopregnancy[32]. Beyond day 11 of pregnancy, PRL levels in serum remain low until 1 or 2 days before parturition, when a significant increase in PRL levels occurs[209]. <3> lactation: During lactation, PRL secretion is initiated by the suckling stimulus and the secretion shows a rapid discharge within 2 to 5 min following the suckling[90].

Hypothalamic control

Experimental evidence supporting the view that the hypothalamus is inhibitory to PRL secretion, is well documented[145]. So far, dopamine is the most important PRL inhibitory factor (PIF)[151]. Dopamine-immunoreactive neurons have been localized in the mediodorsal portion of the arcuate nucleus[182]. Moreover, dopaminergic neurons also have been found in the AMPO[226] and the axons are present in the SCN[247]. These findings suggest that dopamine may be involved in modulation of PRL secretion via the AMPO and /or SCN. Dopamine can act directly on the pituitary to inhibit PRL secretion either *in vitro*[7] or infused directly into a portal vessel *in vivo*[238]. It is found that dopamine is present in high concentrations in the median eminence (ME)[145] and that dopamine receptors are present on anterior pituitary cells[34]. It seems likely that a PIF is at least stored in the ME, since lesions in this region readily cause release of PRL[40]. Numerous studies had shown that dopamine concentrations in the range of 1 to 10 ng/ml were sufficient to inhibit PRL release from pituitaries incubated in vitro. These concentrations are similar to those found in hypophysial stalk plasma[7,11,217]. Moreover, infusion of dopamine antagonists such as haloperidol and sulpiride into a hypophyseal portal system increases PRL levels[17]. In addition, it has reported that dopamine appears to reduce the concentration of PRL mRNA[149,235]. Therefore, it is likely that dopamine is a physiologically significant PIF. One question which needs to be answered is whether a decrease in dopamine secretion accounts for the major surges of PRL secretion at proestrus, during pseudopregnancy, and after suckling. It has been reported[11] that dopamine levels in stalk blood are slightly decreased during proestrus compared to estrus, the converse of the expected rate of PRL secretion. But another report[79] showed that no significant changes in dopamine levels between diestrus and proestrus, were found, despite significant elevations of PRL levels at proestrus. One explanation could be that the rise of estrogen during proestrus, may antagonize the inhibitory effects of dopamine on the pituitary[190].

In addition, it was found that γ-aminobutyric acid (GABA) could be a nondopaminergic PIF[211]. Some studies have demonstrated that drugs such as amino-oxyacetic acid, which inhibits the degradation of GABA, lower plasma PRL[153] and intravenous injection of bicuculline as a blocker of GABA receptors, result in increased plasma PRL[153]. These findings indicate that GABA may also play a role(s) as a PIF. However, the amount of GABA, required to inhibit PRL release *in vitro*[7] is considerably larger as compared to dopamine. Therefore, the physiological significance of GABA is still uncertain.

Recently, Nikolics et al.[169] have demonstrated that the 56-amino acid GnRH-associated peptide (GAP), which is the final 56-amino acid residue of proGnRH, is a potent inhibitor of PRL secretion acting directly on the pituitary. GAP can inhibit the basal secretion of PRL by approximately 50%, which is comparable to inhibition levels reported for dopamine. Thus, GAP might be additional PIF, although *in vivo* evidence does not support this hypothesis[183].

Role of prolactin-releasing factor(PRF)

Evidence for the existence of a PRF was first presented in 1970[167]. The increase in PRL that follows estrogen and suckling might be due to hypothalamic secretion of a PRF rather than a decrease in dopamine secretion. Experimental evidence has continued to support the existence of PRF activity in the hypothalamus[159,245]. There are at least three candidates for PRF which are thyrotropin-releasing hormone (TRF), vasoactive intestinal polypeptide (VIP) and peptide histidine isoleucine (PHI).

TRH has been shown to stimulate PRL release from PRL-secreting cells both *in vitro*[240] and *in vivo*[25]. Also, TRH antiserum administered to the rat attenuates the proestrus PRL surge[130].

Moreover, TRH stimulates prolactin synthesis in cultured cells[51] and the concentration of PRL mRNA as measured by hybridization to prolactin cDNA[63]. Thus, TRH could be a physiological PRF. However, the pattern of PRL secretion does not parallel thyroid-stimulating hormone (TSH) secretion, suggesting the existence of non-TRH PRF[17].

VIP, a 28 amino acid residue peptide originally isolated from porcine duodenum by Said and Mutt in 1970[202], was found to stimulate PRL secretion in the rat, probably via a hypothalamic site of action[118,251]. VIP as a PRL releasing factor has been hypothesized[1,200]. As discussed above, VIP has been localized in neurons in the central nervous system[72]. Specifically, the immunoreactive VIP cell bodies have been found in the basal portion of the SCN[229] and in the cell-sparse zone lateral to the AMPO[175]. Moreover, VIP administration is able to induce PRL release in rats[118], monkeys[67] and humans[47], acting directly at the pituitary level after binding to specific receptors[147]. Thus, these findings indicate that VIP might act, as a PRF, on both hypothalamus and pituitary.

PHI, a 27 amino acid residue peptide, which is member of the same family of gastrointestinal peptides, has been immunohistochemically localized in the external layer of the ME[95]. In vivo[218] and in vitro[203] studies have shown that PHI stimulates PRL release. These findings suggest that PHI may play a physiologic role in the control of PRL release. However, PHI is less effective than VIP in regulation of PRL release[151].

Role of estrogen

Estrogens have long been known to cause hypertrophy of the pituitary gland and increase prolactin secretion[163]. It has also been demonstrated that ovariectomy is accompanied by a decrease in PRL production, which is reversed by injecting estrogen[144]. It has been shown that estrogen is responsible for the surge of PRL during proestrus, since administration of an antiserum to estradiol at diestrus-2 reversibly blocks the proestrus surges of PRL in 4-day cycling rat[164]. According to Neill[164] the stimulatory effect of estrogen on PRL secretion is not a simple linear relationship, since silastic implants that maintain constant elevated levels of estradiol in serum within the physiologic range result in repeated discharges of prolactin[164] in the form of daily surges rather than in continuous elevation of PRL.

Interestingly, the effects of estrogen on PRL release are comparable to the effects of estrogen on LH release, as described in previous section. This suggests that the proestrous surges of PRL and LH may be controlled via a common clock mechanism. This clock is believed to reside within the SCN because complete lesions of the SCN abolish proestrous prolactin and LH surges[12,58,197,198]. Moreover, Kawakami et al.[121] have demonstrated that lesions of the SCN abolish the estrogen-induced daily surges of PRL and LH in ovariectomized rat. This would indicate that the site of estrogen action to induce surges of PRL and LH may be within the SCN. However, autoradiographic studies have provided evidence that tritiated estradiol is not concentrated by neurons in the SCN[179,236]. This would rule out a direct genomic action of estradiol in the SCN. Recently, Wiegand et al.[255] have demonstrated that tritiated estradiol is highly concentrated in the AMPO, suggesting that estrogen action on PRL and LH surges may occur through the AMPO.

Recent studies using dispersed pituitary cells showed that estrogen regulated the PRL gene transcription, in which the PRL mRNA was rapidly increased following injection of 17ß-estradiol[84]. Thus, estrogen also stimulates PRL production at the level of the pituitary.

Role of neurotransmitter

It has been well documented that activation of central serotoninergic system promotes the release of PRL. Direct stimulation of serotonin receptors by injection of 5-hydrotryptamine (5-HT) into the third ventricle[114] or by administration of the receptor stimulant quipazine[156] results invariably in increases of plasma PRL. A recent study[261] has further demonstrated that 5-HT injection into the medial basal hypothalamus stimulates the secretion of PRL in a dose response pattern, and this effect of 5-HT is blocked by metergoline (serotonin receptor blocker). Anatomical evidence has shown that both the arcuate and ventromedial hypothalamic nuclei and the posterior periarcuate region receive serotonin input[233]. In addition, both the SCN[38] and the AMPO[225] are innervated by serotoninergic fibers. Of particular interest is the finding that serotonin fibers synapse on VIP cells within the SCN[38,246,247]. Therefore, the stimulatory action of 5-HT on PRL secretion could be possibly via the interaction between serotoninergic input and VIP cells in the SCN. However, the mechanism on PRL secretion is not understood yet.

Effects of "stress" on secretion of PRL

It has been known for a long time that various noxious stimuli would induce secretion of PRL[89,161,168]. During the afternoon of proestrus in the rat, the high levels of PRL are decreased by applying stress[161]. In contrast, the low levels of PRL at diestrus are sharply increased after stress[161]. Pregnant rats also show a prompt reduction of serum PRL levels when they are stressed during the diurnal peak of PRL[194]. Thus, the initial level of circulating PRL appears to be important in determining the affect of stress, the PRL secretion being stimulated when the prestress levels are low and depressed when they are high. Grosvenor et al.[89] observed in lactating rats that laparotomy and bleeding under ether anesthesia or stunning by a blow to the head followed by decapitation rapidly increase PRL secretion. Nicoll et al.[168] found that applying a number of stresses, such as severe cold, intense light and heat, restraint and injection of formaldehyde, induced lactation in estrogen-primed rats. The increase in plasma PRL levels in response to various types of stress have been proposed to be modulated by several peptides.

Recent studies have suggested a stimulatory role of VIP and PHI in PRL secretion in rats exposed to ether vapors[98]. Also, histamine has been found to stimulate PRL secretion by a receptor-specific action in response to stress [141,193]. Furthermore, the observation that naloxone inhibits the PRL rise induced by heat exposure[248], ether vapors[55], immobilization[248], swimming[186] and footshock[85] suggest that the endogenous opioid peptides may be involved in the stress-induced PRL release. There are at least three classes of endogenous opioid peptides, i.e. endorphin, dynorphin, and enkephalin, which bind preferentially to particular opiate receptor subtypes (μ/ϵ, K, and δ, respectively) in the hypothalamus[48]. Currently, it is believed that the ß-endorphin-μ/ϵ pathway modulates PRL secretion in rats under basal conditions.[232] and during stress[85,186,248]. Petraglia et al. [178] has further demonstrated that ß-endorphin and dynorphin acting at the central nervous system modulate the release of PRL induced by footshock stress, since the stress-induced release of PRL is attenuated by intraventricular administration of antisera against ß-endorphin or dynorphin.

SUMMARY

The secretion of PRL is generally controlled by hypothalamic PIF/PRF. Mainly, it is tonically inhibited by PIF. Dopamine may be a primary hypothalamic PIF in control of PRL secretion. GnRH-associated peptide may also be a potent inhibitor of PRL secretion, as a PIF. It is believed that TRF, VIP and PHI are the most likely

PRF candidates in modulation of PRL secretion. It has been found that immunoreactive VIP neurons and fibers are localized within the SCN. But the physiological role of the VIP in the regulation of PRL secretion is unknown. It is apparent that gonadal steroids, various neurotransmitters, and stresses, are also involved in regulation of PRL secretion, possibly via affecting the dynamic changes of PIF and PRF. However, the precise mechanism of the control of PRL secretion still remains largely unknown. Particularly, the specific site of the control center in the hypothalamus and the site of gonadal steroids in the regulation of PRL secretion have not been fully understood.

MATERIAL AND METHODS

Animals

Female Sprague-Dawley rats (30 day old) were purchased from Charles Rivers Laboratories (Boston, Mass, USA). Animals were housed two per cage within a BioClean Duo-Flo Unit (Hazelton Systems, Aberdeen MD) in order to prevent post surgery infection. Light schedule (12 L : 12 D, lights on at 0230 h) and temperature (25 °C ± 1 °C) were controlled. Animals were allowed free access to lab chow and water and given 2-3 weeks to adjust to the light schedule. Estrous cycles were established by daily inspection of vaginal smears. Only 180-250 g animals showing at least two consecutive normal 4-day estrous cycles, (two days diestrus, one day proestrus and one day estrus), were selected for the experiment.

Experimental procedures (Figure 1)

Lesions

Lesion electrode preparations: The lesion electrodes were prepared as follows: Glass micropipettes (2 mm OD) were pulled on a Narishige Electrode Puller. The empty glass micropipettes were then filled with molten Wood's metal[197] and a silver wire was inserted and bordered to the Wood's metal. Electrodes with effective tip length 10-13 mm were checked under the microscope, the tip diameter was measured (60-75 μm), and the electrodes were tested for leakage and conductance.

Lesion procedures: The animals were anesthetized using freshly prepared 2.5% tribromoethanol in sterile saline (25 mg/100 g body weight, IP; Sigma, St. Louis, Mo), and placed in a stereotaxic apparatus. The surgical procedure was performed under the WILD MS-C microdissecting scope. A dental drill (EMESCO DENTAL CO. New York), was used to open a small square on the skull (about 3 x 3 mm, on the midline and rostral to the bregma approximately 0.5 mm), After surgical exposure of the cortex, a Wood's metal electrode (tip size 60-75 μm; 1-2 MΩ resistance) was lowered first to the rostral and next to the caudal part of the SCN or to the dorsal and ventral part of the AMPO[177], and bilateral electrolytic lesions were produced by passing 5-10 μA of anodal current for 2.5-3 min, using a Grass S9 stimulator (GRASS INSTRUMENT CO.). Sham lesioned animals were produced by lowering the electrode into the SCN or AMPO without passing the current. Vaginal smears were monitored daily after the lesions.

Cannulation

Cannula preparation: The blood sampling cannula was made of silastic tubing (Dow Corning, Medical-Grade Tubing No. 602-135). The length of the cannula was approximately 13 cm long. A 0.7 x 0.7 cm square of silastic sheeting (Dow Corning, Medical-Grade Tubing No. 602-135) was attached to the tubing 3-4 cm from one end of the cannula, using Dow Corning Medical Adhesive Silicone Type A. The prepared cannulae were autoclaved before use.

Cannulation procedures: The methods used were slight modification of Harms and Ojeda's cannulation procedure[91]. Approximately 4 weeks after lesion, both sham and lesioned rats were cannulated. Five days prior to blood sampling, the lesioned animals showing persistent estrus and partial lesioned animals showing either persistent diestrus or irregular estrous cycles and sham lesioned animals showing normal estrous cycles, were anesthetized with tribromoethanol as previously described and implanted with an intra-atrial cannula. The procedure for catheterization follows: The right jugular vein was exposed at the anterior side. A bow-shaped needle attached to the short end of the cannula (2.8-3 cm long), was inserted into the vein, then passed out of the vein under and through the brachiocephalic muscle. The needle was detached from the cannula, and the cannula was slowly pulled back into the vein and then pushed into the right atrium. The silastic sheeting was sutured on the adjacent brachiocephalic muscle. The other end of cannula was passed subcutaneously towards the nape of the neck and plugged with a gold pin. Immediately after the cannulation, animals were given 0.1 ml of cefazolin sodium (200 mg/ml) intravenously to prevent infection. The cannulation was done during late proestrus or early estrus, since the cannulation at these stages usually caused no change of the estrus cycling pattern. Catheter patency was maintained by daily flushing with 0.2-0.3 ml heparinized saline (5 IU/ml).

Blood sampling

Twelve 0.4 ml blood samples were drawn from each animal each day for 3 or 4 days. All blood sampling commenced at 09:00 h and ceased at 15:30 h. The time intervals for sampling were as follows: samples 1-3, 45 min; samples 4-10, 30 min; samples 11-12, 45 min. After the samples were drawn, they were centrifuged with 10 μl of heparinized saline (50 IU/ml). The plasma was then transferred to vials containing 5 μl of 13% EDTA to prevent clotting of the plasma during storage at -20 °C. The red blood cells were resuspended in 5% human plasma fraction (Plasmanate, Cutter Lab.) and reinfused following each subsequent sample.

Experimental protocol

Adaptation: 3 days before the first day of bleeding, the cannulated animals were transferred to a bleeding chamber (polypropylene cylinder 30 x 30 cm, one animal in one chamber), to become familiar with the experimental environment. On each experimental day, animals were transferred to the bleeding chambers 2 hours prior to the first blood sample. A 30 cm tubing extension was connected to the exposed indwelling catheter at the nape of the neck and extended outside the bleeding chamber.

Experimental day 1: Four main groups of animals were identified in this experiment: (1) Lesioned animals, which demonstrated persistent estrus (PE); (2) Partial-lesioned animals, which demonstrated persistent diestrus (PD), referred to as PL1; (3) Partial-lesioned animals, which demonstrated an irregular cycle, referred to as PL2; and (4) Sham-lesioned animals which continued to cycle normally. Blood samples were withdrawn from freely moving lesioned animals in PE, and sham lesioned animals during diestrus, proestrus and estrus. Partial lesioned PD animals were sampled during diestrus and partial lesioned irregular cycling animals were sampled during estrus.

Experimental day 2: All lesioned animals were injected with estradiol benzoate (EB, 25 μg/animal, SC) at 0800 h. Sham lesioned animals, were bled in the next consecutive cycle stage, as compared with day 1.

Experimental day 3: All lesioned animals were bled starting 25 h post EB injection. Sham lesioned animals were bled at the final consecutive cycle stage, as compared with day 2.

Experimental day 4: All lesioned animals were injected with progesterone (P, 2.5 mg/animal, SC) at 0800 h. The first blood sample was obtained 1 h post progesterone and 50 h post EB injection.

All the blood samples were drawn at the same time (± 5 min) on each of the bleeding days. Following the last blood sample of each day, each animal was given 20 mg antibiotics (cefazolin sodium, 0.1 ml of 200 mg/ml, IV).

Sacrifice: At least 10 days following the sampling, animals were decapitated from 0830 h to 0930 h. Lesioned animals were sacrificed on estrus and sham lesions on proestrus. Partial lesioned animals, were sacrificed on either diestrus or estrus. The brains were rapidly removed and frozen on dry ice then stored in liquid nitrogen.

Microdissection: The frozen brains were sectioned (300 μm) from the frontal plane at -12 °C (± 2 °C) using a cryostat. A modification of the Palkovits[173] microdissecting method was used to obtain preoptic area (POA) and basal hypothalamus (BH) tissues. The tissues were kept frozen in 1.5 ml polystyrene tubes until extraction (over night).

Tissue extraction: 1.2 ml 2 N acetic acid (90 °C) was added to each tube containing the punched tissue and allowed to equilibrate in a 90 °C water bath for 10 min. The tissue was sonicated and then centrifuged at 4 °C in an ultracentrifuge for 20 min at 13,000 g. The supernant was transferred into a polystyrene culture tube, the pH was adjusted to 3.1 and applied to Sep-Pak C_{18} cartridges. Sep-Pak C_{18} cartridges were activated with methanol, H_2O and 2 volumes of Triethylamine/ Formic acid (TF; TF solution: 10.5 ml formic acid, 20 ml triethylamine, and 969.5 ml H_2O) at 1.5 ml/min flow rate, followed by a 3 ml TF rinse of the sample tube. The Sep-Pak was then eluted with 4 ml of acetonitrile/TF (60:40, v:v) into polystyrene culture tubes coated with 0.1% bovine serum albumin (BSA). The samples were concentrated by a Savant Speed Vac for 10-15 hours and then frozen at -20 °C.

Radioimmunoassays (RIAs):

PRL, LH, proGnRH and GnRH were iodinated by using the chloramine-T method[87].

RIA for PRL and LH: Plasma PRL was determined in duplicate 20 μl samples by a heterologous double-antibody RIA using NIADDK antirPRL-S-9 antisera, NIADDK-rPRL-RP-3 reference preparation and NIADDK-rPRL-I-5 for iodination. The sensitivity of the assay was 0.01 ng/tube with an ED_{50} of 0.18 ng PRL/tube. The concentration of PRL was expressed in terms of ng/ml. Plasma LH was determinated in duplicate 50 μl samples by a heterologous double antibody RIA using GDN-15 antiovine LH antisera, NIADDK-LH-RP-1 reference preparation and LER 1374 A ovine LH for iodination. The sensitivity of the assay was 0.10 ng/tube with an ED_{50} of 32.68 ng LH/tube. The concentration of LH was expressed in terms of ng/ml.

Steroid determinations: Plasma 17ß-estradiol and progesterone were determinated by specific RIAs in 10 to 150 μl aliquots after ether extraction and chromatography on LH-20 Sephadex columns[191,192]. 17ß-Estradiol was measured in a nonequilibrium assay as described by Goodman[81]. Solvent blanks, percent recovery and interassay coefficient of variation were 2.4 pg, 72.4% and 7.6%, respectively, for estradiol, and 12.4 pg, 82.4% and 12.3% for progesterone. The steroid determinations were performed by Dr. Hess at the Oregon Regional Primate Research Center.

RIA for GnRH and proGnRH: GnRH (Peninsula Lab.) was used as reference preparation and iodination. Synthetic fragment preproGnRH (6-16 amino acids) was used as a reference preparation and synthetic preproGnRH (5-16 amino acids) for iodination. The extracts were resuspended by sonication in phosphate buffered saline and aliquoted for duplicate determinations. GnRH was measured by using a conformational El-14 antiserum[60], which does not recognize any form of proGnRH[196]. A highly specific ARK-2 antiserum was used to determine rat proGnRH[122].

Statistical Evaluation:

Statistical evaluation of differences between different groups was performed by using a two-way analysis of variance (Statpak statistics computer program) for repeated measures of PRL and LH determinations, which were adapted to unequal numbers of animals within groups[262]. A one-way analysis of variance and a Newman-Keuls test (Statpak statistics computer program) were performed to determine the differences between groups of GnRH and proGnRH.

Immunohistochemistry:

Rats which were lesioned in the SCN or AMPO (N=5), were perfused after 2-3 days to examine the lesion placement. In addition, lesioned, irregular cycling animals were sacrificed after 3-4 weeks. The animals were deeply anesthetized with pentobarbital (Nembutal) and perfused intracardially with 150 ml of 0.1 M phosphate buffer (PB; pH 7.2), containing 15,000 U of heparin followed by 650 ml of 4% parafomaldehyde in PB. The brains were postfixed in the same fixative for 1-2 h and rinsed for 2-3 days in PB. Thereafter, the brains were cut on a vibratome into 50-μm coronal sections, collected in vials containing 34% ethylene glycol and 20% sucrose in PB and stored at -20°C for 1-60 days. For immunohistochemistry, various sections from the different brains (SCN and AMPO lesions) were processed using the avidin-biotin (ABC) method for GnRH. A few sections were also reacted by immunohistochemistry using primary antisera to vasoactive intestinal peptide (VIP), neurophysin (NPH) and vasopressin (VP). This was done to check for completeness of SCN and AMPO lesions. The sections were rinsed well in PB, and incubated overnight at 4°C in the GnRH antiserum (EL-14) diluted 1:10,000 in 0.05 M Tris (hydroxymethyl aminomethane-phosphate buffer (Tris-PB) containing 3% bovine serum albumin (BSA), 0.7% seaweed gelatin, 0.4% Triton X-100, and 5% dimethylsulfoxide (DMSO). Antisera to VIP, NPH and VP were used at a 1:4,000 dilution. The following steps in the reaction procedure were performed sequentially at room temperature: biotinylated goat anti-rabbit gammaglobulin [biotin-ARGG] (Vector Laboratories) was diluted 1:200 and applied for 60 min; Peroxidase conjugated strepavidin was diluted 1:120 and applied for an additional 60 min, and 3,3'-diaminobenzidine (DAB) for 5-15 min.

Cells and fibers containing immunoreactive GnRH were observed and photographed through a Leitz-Dialux 20 microscope.

RESULTS

Lesion placement

The lesion procedure in this study was modified from previous studies[197,198] in order to lesion the AMPO or the SCN with minor damage to surrounding tissues (see methods) (figure 2, 3, 4). Placement of the lesions were tested in preliminary experiments and best results were obtained when small lesions were placed first in the rostral and next in the caudal part of the SCN, or in the ventral followed by the dorsal part of the AMPO. Moreover, the use of stereotaxic equipment, surgical microscope and rigid glass-insulated microelectrodes that did not bend during penetration to the base of the brain, further aided the precise placement of SCN or AMPO lesions.

Complete lesions of the AMPO or the SCN have been found to produce PE, whereas partial lesions produce irregular cycles or persistent diestrus (PD) (figure 5). Therefore, the vaginal smear data were used to evaluate the lesion placements. Moreover, during cryostat sectioning and microdissecting procedures, the lesion placements could be approximately defined, although the exact borders of the lesions could not be determined. Another criterion used to evaluate the lesion placements was the ability of steroid injections to elicit LH and PRL surges. Animals with complete AMPO or SCN lesions have been found not to elicit a LH surge in response to estrogen and progesterone[120,204,243,257].

Anesthesia and sham lesions caused 3-5 days of diestrous vaginal smears before the return of regular estrous cycles (figure 6. Sham-L). Lesions of the AMPO caused initial 8-10 days of diestrous smears, followed by 0-2 irregular cycles before the occurrence of PE (N=6) (figure 6. AMPO-L). Two AMPO-lesioned animals had PD. However, we were not able to induce a LH surge in these animals therefore, they were included as complete AMPO lesioned rats. Inspection during cryostat sectioning revealed dorsally-extending AMPO lesions. SCN lesions caused initial 8-12 days of diestrous smears, followed by 1-7 irregular cycles before the occurrence of PE (N=6) (figure 6. SCN-L). One SCN lesioned animal exhibited PD. Again, we were not able to induce a LH surge and only an attenuated PRL surge with estrogen and progesterone injections. This animal was perfused and the brain sectioned on a vibratome. Immunohistochemical staining for vasoactive intestinal polypeptide (VIP) which is localized in SCN cells, revealed a complete SCN lesion that extended lateral on one side. Another group of animals (N=11) that received AMPO or SCN lesions did not go into a PE state, but exhibited irregular cycles with prolonged estrous smears (PL2; N=6) (figure 6. PL-2), or PD (PL1; N=5) (figure 6. PL-2). Moreover, an LH and PRL surge could be induced by steroid treatment in these animals. One animal from each group was perfused, sectioned on a vibratome and stained immunohistochemically for GnRH. This analysis revealed that animals with irregular cycles had incomplete AMPO or SCN lesions. Animals in PD had lesions placed in the dorsal POA. As described earlier[197,198], immunoreactive GnRH cells and fibers surrounded the lesioned area.

Plasma steroids levels

Plasma estradiol levels as measured in pooled samples (exp. day 1) from the AMPO lesioned PE animals were 21.3 ± 2.4 pg/ml and the levels (exp. day 1) in the SCN lesioned PE animals were 27.8 ± 5.0 pg/ml (table 1). These levels were not significantly different as compared to the plasma estradiol levels in pooled samples from sham lesioned proestrous animals, which were 25.0 ± 6.5 pg/ml. In persistent diestrous (PL1) animals, the plasma estradiol levels (exp. day 1) of 14.5 ± 2.9 pg/ml were lower as compared to sham lesions, although the difference was not statistically significant. PL2 animals had plasma estradiol levels (exp. day 1) of 37.6 ± 2.6 pg/ml, which were not significantly higher vs. sham lesions (table 1).

Plasma progesterone levels (exp. day 1) were 5.6 ± 1.6 ng/ml and 5.1 ± 1.4 ng/ml in AMPO and SCN lesioned PE animals, respectively. These levels were significantly lower ($P<0.01$) as compared to plasma progesterone levels (20.4 ± 2.8 ng/ml) in the sham lesioned proestrous rats (table 1). In the PL1 group, progesterone levels (exp. day 1) of 37.4 ± 3.5 ng/ml were significantly higher ($P<0.05$), while in PL2 animals progesterone levels of 6.2 ± 1.4 ng/ml were significantly lower ($P<0.01$) as compared to sham lesioned proestrous rats (table 1).

Animals injected with estradiol benzoate had highly elevated plasma levels of estradiol in pooled samples obtained the following day (table 2). Also, progesterone injections caused highly elevated plasma progesterone concentrations (table 3).

Plasma LH and PRL levels

In sham lesioned animals (N=6), plasma PRL levels were characterized by low levels during diestrus and elevated levels during estrus (figure 7. top panel). An analysis of variance revealed that plasma levels of PRL during estrus were significantly ($P<0.05$) higher than the levels during diestrus. In contrast, plasma LH levels were low during diestrus and estrus, and no significant differences were found between the two stages of the estrous cycle (figure 7. bottom panel).

During proestrus, sham operated animals exhibited PRL and LH preovulatory surges (figure 7. proestrus). The surge of plasma PRL preceded the surge of plasma LH by approximately 2 h, and plasma PRL remained elevated throughout the sampling period.

In AMPO (N=6) (figure 8. top panel) or SCN (N=6) (figure 9. top panel) lesioned PE animals, the basal levels of plasma PRL were significantly lower as compared to sham lesioned animals during estrus ($P<0.01$, $P<0.05$, respectively). However, no significant differences were found between plasma PRL in the lesioned PE animals and sham lesioned animals during diestrus.

In partial lesioned animals, including PL1 (N=5) (figure 10. top panel) and PL2 (N=6) (figure 11. top panel), the basal levels of plasma PRL also were significantly lower ($P<0.05$) than the basal levels of plasma PRL in sham lesioned animals during estrus.

However, the basal levels of plasma PRL were not significantly different as compared to the basal levels of plasma PRL in sham lesioned rats during diestrus.

The basal levels of plasma LH in AMPO, SCN, PL1 or PL2 animals were not significantly different as compared to plasma LH in sham lesioned estrous or diestrous animals (figures 8,9,10,11. bottom panels). Finally, no significant differences were found in basal levels of plasma PRL or LH among PL1, PL2 animals, AMPO and SCN lesioned animals.

Effects of EB and progesterone

In order to examine the effects of gonadal steroids on plasma PRL and LH in lesioned animals, EB and progesterone were injected into AMPO, SCN, PL1 and PL2 animals.

In AMPO lesioned PE animals, plasma LH levels during experimental day 3, were not significantly elevated after injection of 25 μg EB, as compared to the basal levels during experimental day 1 (figure 8. bottom panel). Similarly, plasma LH levels during experimental day 4, were not significantly elevated following injection of 2.5 mg progesterone (figure 8. bottom panel). The plasma PRL levels during experimental day 3, were significantly ($P<0.001$) elevated after injection of 25 μg EB, compared to the basal levels during experimental day 1 (figure 8. top panel); The plasma PRL levels during experimental day 4 following injection of 2.5 mg progesterone, were significantly elevated as compared to experimental day 1 ($P<0.0001$) and experimental day 3 ($P<0.001$)

(figure 8. top panel). However, plasma PRL levels during experimental day 3 and experimental day 4, were still significantly ($P<0.001$ and $P<0.01$, respectively) lower than the plasma PRL levels during proestrus in sham lesioned rats (figure 7).

Almost identical profiles of plasma PRL and LH were observed in SCN lesioned animals as compared to AMPO lesioned animals (figure 9). Moreover, the responses to EB and progesterone were similar in both SCN and AMPO lesioned animals.

In partial lesioned animals (N=5) which exhibited persistent diestrus (PL1), estradiol benzoate injection induced preovulatory-type surges of both LH and PRL (figure 10), which were similar to the surges seen in sham lesioned animals during proestrus. On the following day progesterone injection did not induce an additional surge of plasma LH. Therefore, plasma LH levels on day 4 were not significantly different as compared to basal levels. The plasma PRL levels following progesterone injection (experimental day 4) were significantly ($P<0.05$) elevated compared to the basal levels of PRL during experimental day 1, although the levels were significantly lower than plasma PRL levels following estradiol benzoate injection (figure 10. top panel).

Another group of partial lesioned animals (N=6) which exhibited irregular estrous cycles (PL2), showed no significant elevation in plasma PRL or LH following estradiol benzoate injections (figure 11). However, during experimental day 4, injection of progesterone induced preovulatory-type surges of both PRL and LH in PL2 animals, although the amplitude of the LH surge was significantly lower than that observed in sham lesioned proestrous animals (figure 7). In contrast, the amplitude of the PRL surge was significantly elevated as compared to plasma levels observed in sham lesioned proestrous rats.

Hypothalamic proGnRH levels

In microdissected tissues from the POA of sham operated proestrous rats (N=6), proGnRH levels were approximately 4 times higher as compared to proGnRH levels in the BH (figure 12). In AMPO- or SCN-lesioned rats, the levels of proGnRH in the POA and the BH were similar, and at least 4 times lower than the levels found in the POA of sham operated proestrous rats ($P<0.01$) (figure 12). In the BH proGnRH levels were similar in lesioned PE animals as compared to proestrous controls.

Also in partial lesioned PL1 (N=5) and PL2 (N=6) animals, the proGnRH levels observed in the POA were similar to that of the BH. However, proGnRH levels were significantly ($P<0.01$) lower in the POA, as compared to proestrous controls (figure 12). In the BH, there were no significant differences among the different groups.

Hypothalamic GnRH levels

In the sham lesioned proestrous animals, GnRH levels were approximately 6 times higher in the BH as compared to the POA (figure 13). Also, in the AMPO- or SCN-lesioned rats GnRH levels in the BH were significantly elevated as compared to levels in the POA. No significant differences were found in the GnRH levels in the POA or the BH in the AMPO or SCN lesioned rats as compared to sham lesioned animals (figure 13).

In the PL1 animals GnRH levels in the POA were slightly (not significantly) lower as compared to sham-lesioned, SCN- or AMPO-lesioned PE animals (fig 13. POA). In the BH, the GnRH levels were not significantly different in the PL1 rats as compared to the other experimental groups, although the levels were slightly (not significantly) higher than the others (figure 13. BH)

Similarly, PL2-lesioned irregularly cycling rats had slightly but not significantly reduced GnRH levels in the POA, as compared to sham-lesioned, AMPO- or SCN-lesioned rats (figure 13. POA). In the BH, GnRH levels in the PL2 rats were not significantly different from that observed in the other experimental groups (figure 13. BH)

DISCUSSION

Plasma LH and PRL

In this study, lesions of the AMPO or the SCN produced PE in the majority of animals. However, a small percentage of lesioned anovulatory rats were found to exhibit PD. It has been known for some time that lesions of the POA can produce PE or PD[53,54,88]. More recently it has been demonstrated that the PE is due to destruction of the SCN[26,27,197,198,256,258] or the AMPO[197,198,256,258], whereas PD is due to lesion of the dorsal POA[258] or the area between the AMPO and the SCN[256].

In the present study, sham-lesioned rats exhibited plasma LH and PRL profiles which were similar to well-described hormone profiles[76,133,198,231], indicating that sham lesions did not effect the secretory pattern of LH or PRL. Thus, plasma PRL levels during diestrus were significantly lower than plasma PRL levels during estrus, while plasma LH levels during diestrus were higher than the levels during estrus, although the difference was not statistically significant. Previously, Gallo[76] has demonstrated that the plasma levels of LH during the first day of diestrus are significantly higher than that observed during estrus, primarily due to increased frequency and amplitude of LH pulses. The difference between Gallo's results and those in the present study is probably due to differences in blood sampling frequency. Gallo et al.[76] sampled every 5 min, whereas we sampled every 30-45 min in this study.

During the afternoon of proestrus, all sham-lesioned rats exhibited preovulatory surges of LH and PRL, and the plasma PRL surge preceded the surge of LH by approximately 1 to 2 h. The timing of the LH and PRL surges has been found to vary considerably between animals[18,260]. However, within individual rats the timing of the LH[39,198] and the PRL[198] surges on the afternoon of proestrus is very consistent over successive cycles. This would indicate that the proestrous surges of LH and PRL may be controlled by a common clock mechanism[198].

In AMPO- or SCN-lesioned PE rats, the basal levels of PRL were significantly lower than the levels of PRL in sham-lesioned rats during estrus. These results are consistent with previous findings[198] which have demonstrated that the basal levels of PRL in AMPO-, SCN- or combined AMPO- and SCN-lesioned PE rats are significantly lower as compared to the levels of PRL in sham-lesioned rats during estrus. In contrast, Dunn et al.[57] have observed considerably higher basal levels of PRL 45 days after the isolation or lesion of the SCN. This could be due to different size and placement of the lesions, or could be due to different blood sampling procedures. Prolactin release is extremely sensitive to stressful stimuli[89,161,168]. Therefore, the stress of serial blood sampling obtained by reopening a tail vein incision[57] might have caused elevated plasma PRL. However, Blake et al.[21] have also reported that blood samples obtained by decapitation revealed significantly elevated levels of basal PRL, 3-5 weeks following anterior hypothalamic deafferentation that caused PE. They hypothesized that the main factor to cause elevated levels of PRL was high plasma levels of estrogen in the PE animals. Estrogen has been thought to stimulate PRL secretion or production, because administration of estrogen causes elevated basal plasma PRL[14]. Moreover, in vitro studies have demonstrated that estrogen stimulates PRL production by direct action on PRL gene transcription[222].

In our experiments, the basal levels of PRL in the AMPO- or SCN- lesioned, PE rats were significantly lower than that described previous[21,57], although the plasma estrogen levels were similar to the levels of sham-lesioned, proestrous rats. This would suggest that lesions of the AMPO or SCN result in lower basal levels of PRL possibly due to destruction of estrogen sensitive neurons. The lack of the effect of estrogen in SCN lesioned rats has been demonstrated by different investigators in experiments where injections of estrogen were unable to induce a PRL surge[120,121,176]. Another possibility is that lesions of the AMPO or SCN may effect a putative prolactin releasing factor (PRF) input to the pituitary. For instance, vasoactive intestinal polypeptide (VIP) has been found to stimulate PRL release[1]; and VIP immunoreactive cell bodies are found in the SCN[246,247], and a few VIP immunoreactive cell bodies are found in the AMPO[225-227]. Our SCN or AMPO lesions would destroy the respective VIP cell bodies.

In AMPO- or SCN-lesioned PE rats, injections of EB caused a significant elevation of basal PRL levels. Injections of progesterone to these animals further elevated basal PRL titers.

However, the levels were significantly lower than plasma PRL in sham-lesioned animals during proestrus or in partial-lesioned animals that received estrogen and progesterone sequentially. An experimental model which is similar to ours has been used previously by Bishop et al.[16]. They found that lesions of the SCN which induced persistent estrus in the female rats failed to prevent the surge of PRL after injection of EB. However, the results from their experiments can not be compared to ours due to repeated sampling under ether anesthesia in their study versus the non-stressed blood sampling from freely-moving animals in our experiments. It is well established that pituitary hormones, and in particular PRL, are extremely sensitive to the stress of handling and anesthesia[89,161,168].

Most investigators have used ovariectomized animals to explore the effects of EB and progesterone treatment on plasma levels of LH and PRL. Thus, Kawakami et al.[121] and Pan et al.[176] have demonstrated that the preovulatory surge of PRL induced by injection of estrogen is blocked in SCN-lesioned, ovariectomized rats. Also, in ovariectomized animals a single injection of EB does not increase basal PRL levels[121,176]. These results are different from ours. We found that in animals with intact ovaries, estrogen injection caused elevated basal PRL. It appears that estrogen priming is needed, so that a second injections of estrogen in the above cited studies[121,176] might have given results similar to ours. Kawakami et al.[120] further demonstrated that lesions of the medial basal part of the suprachiasmatic area had no effect on the or frequency, which are more accurate estimates of basal secretion. Blake et al.[22] have previously found that LH levels in anterior-deafferentated PE rats are significantly elevated as compared to LH levels during estrus in normal cycling rats. The discrepancy could be due to the fact that these investigators measured LH levels only at a single time point during the afternoon. Again, due to the pulsatile nature of basal LH secretion, a single sample might not be sufficient to characterize basal secretion.

In the present studies, estrogen and progesterone were administered to the lesioned animals in order to evaluate the completeness of the AMPO or SCN lesions. We found that a LH surge could be induced in lesioned, persistent diestrous or irregular cycling animals, which were judged to have incomplete or misplaced lesions. An LH surge could not be induced in lesioned, persistent estrous animals. Previously, Samson and McCann[204] demonstrated that complete SCN lesions prevented the progesterone-induced LH surge in ovariectomized, estrogen-primed rats. Also, Kawakami et al.[121] reported that lesions restricted to the SCN abolished the estrogen-induced LH surges in ovariectomized rats. However, Wiegand and Terasawa[256] and Wiegand et al.[257,258] have provided evidence that AMPO but not SCN lesions can eliminate the progesterone-induced LH surge in estrogen-primed ovariectomized rats. These discrepancies may be mainly due to different placement and size of the lesions.

Animals with lesion placement in the dorsal part of the POA which demonstrated persistent diestrus (PL1), had basal levels of

LH and PRL which were similar to the basal hormone levels in the AMPO- or SCN-lesioned PE rats. However, in contrast to AMPO- or SCN-lesioned PE rats, PL1 rats exhibited preovulatory type surges of LH and PRL induced by EB injection. As expected, progesterone injection caused no significant elevation of basal LH when injected the day after the estrogen-induced LH surge. In this case the injection of progesterone, in addition to endogenous secreted progesterone from the corpus luteum, would participate in the inhibition of LH secretion[35,265]. Interestingly, the persistent diestrous rats exhibited significantly higher levels of progesterone as compared to sham-lesioned rats, SCN- or AMPO-lesioned PE rats. This would indicate that lesions of the dorsal part of the POA resulted in repeated pseudopregnancy which is characterized by maintenance of progesterone secretion from corpora lutea due to an increase in serum PRL levels[70]. Previous, Clemens et al.[44] have found that large lesions of the preoptic area would induce persistent diestrus (11-14 days) and significantly elevate plasma PRL levels at 1400 h, as compared to the cycling diestrous controls. Further, they injected a dopamine agonist (lergotrile mesylate) into the persistent diestrous rats, which resulted in restoring the normal estrous cycles. Thus, they proposed that the repeated pseudopregnancies are due to the combination of increased plasma prolactin levels and the destruction of a dopaminergic pathway which is responsible for the regulation of the estrous cycle. Also in our study, lesions of the dorsal part of the POA produced persistent diestrus (10-20 days) with significantly elevated progesterone levels. However, plasma PRL levels were low, similar to that of sham lesioned controls during diestrus, suggesting that pseudopregnancy can be maintained in the presence of low PRL levels. The reasons for the different results are unclear.

Of the PL2 rats, two animals had small lateroventral lesions of the AMPO, and the other three had small medioventral lesions of the SCN. These animals exhibited irregular and prolonged estrous cycles characterized by 3-5 days of estrus mixed with 2-4 days of diestrus. The basal levels of LH and PRL in this group were also similar to those in complete AMPO-, SCN- and PL1- lesioned rats. In contrast to PL1 rats, however, PL2 rats did not elicit LH or PRL surges in response to EB but showed afternoon surges of LH and PRL in response to progesterone. It is interesting to note that plasma PRL but not LH, was slightly but significantly elevated following EB injections. Plasma estradiol levels were higher (not significantly). But progesterone levels were significantly lower in PL2 rats as compared to sham lesioned proestrous animals in this study. These results indicate that the steroid-induced surges of LH and PRL might be dependent on the original basal steroid levels, which are dependent on the lesion placement.

The sites of actions of estrogen and progesterone to induce LH and PRL surges remain unknown. Immunohistochemical evidence indicates that estrogen does not act directly on GnRH neurons to cause the release of GnRH, because estrogen concentrating cells have not been identified to contain GnRH-immunoreactivity[221]. In vitro studies by Kim and Ramirez[126] have provided evidence that progesterone can act directly on the MBH to stimulate GnRH release. Moreover, they have suggested that progesterone can act directly on the GnRH nerve terminals to cause GnRH release[126]. Our results in the AMPO- and SCN-lesioned animals do not support the latter hypothesis. We found that GnRH was present in the ME nerve terminals[197], progesterone injections however, were not able to produce a LH surge. It has been found that estrogen and progesterone concentrating neurons are present in the AMPO[237,255]. Thus, it is reasonable to speculate that estrogen and progesterone act at least in part on the AMPO to induce LH and PRL surges. Our data also suggests that the SCN could be a site of action involved in estrogen and progesterone induction of LH and PRL surges. The SCN, however, has been found not to contain estrogen or progesterone receptors[237,255]. It is therefore apparent that the AMPO and the SCN might be functionally coupled, although to date no direct anatomical connection has been found between the AMPO and the SCN[224,227].

GnRH

Experimental evidence indicates that hypothalamic GnRH levels are elevated prior to the LH surge on proestrus[263]. Therefore, we chose that time to compare GnRH levels in the lesioned rats to that of controls. Hypothalamic lesions or deafferentation that cause PE have been found to cause reduced levels of GnRH in the MBH. Thus, Samson and McCann[204,205] have found that GnRH content, as measured by RIA, was significantly reduced in the ME region within 7 days following both OVLT and SCN lesions. Kalra[99] has reported that anterior hypothalamic deafferentation (behind the SCN), which induced persistent estrus, also reduced the content of GnRH in the MBH when measured by RIA 30 days later. More recently, Köves et al.[134] have further demonstrated that extended retrochiasmatic frontal cut just behind the optic chiasm, or a cut in front of the SCN resulted in PE syndrome, which they attributed to a 60-70% reduction of GnRH content in the ME. Therefore, it has been hypothesized that a significant reduction in the MBH GnRH levels is the main cause of PE[134,204,212].

Recent immunohistochemical data has indicated that PE can be induced by lesions of the AMPO or the SCN without apparent reduction in immunoreactive GnRH[170,197,242]. Using a more quantitative experimental approach, we have presently demonstrated that neither AMPO- nor SCN-lesioned, PE animals exhibit reduced levels of GnRH in the POA or BH. Thus, this apparent conflict between immunohistochemical and RIA results have been resolved in the present study. Our lesions were primarily confined to areas containing no or few GnRH cells and fiber tracts. In contrast, Samson and McCann's lesions were produced with larger electrodes which in addition to the SCN, probably lesioned GnRH fibers passing to the ME in the area caudal to the SCN. Likewise, anterior deafferentation of the MBH which severs the POA-BH GnRH fiber tract, causes PE animals although the AMPO and the SCN remain intact[99,134], and in these PE animals the GnRH content in the MBH is significantly reduced. In contrast, AMPO or SCN lesions cause PE without a reduction in GnRH levels. These results would indicate that in addition to SCN or AMPO lesions, a significant reduction of GnRH content in the MBH is an additional factor effective to induce persistent estrus and prevent the preovulatory release of LH. It is also possible that the deafferentations might disconnect input from the AMPO/SCN onto the GnRH neurons and that this rather than the reduction in MBH levels of GnRH, is the reason for PE in the deafferentated animals. In this respect, it is important to note that arcuate neurons containing ß-endorphin, project rostrally and synapse on GnRH neurons[41], and ß-endorphin is thought to be involved in regulating LH secretion[43].

In the present results, GnRH levels in the BH of SCN or AMPO lesioned rats were similar to the GnRH levels in proestrous animals prior to the preovulatory surge. Whereas a LH surge would occur in the controls, LH levels remained low in the lesioned, PE animals. This would indicate that in the PE rats GnRH was not being released in a manner so as to induce a LH surge. The blockage or alteration of the release mechanism might be caused by removal of an essential neural input from the AMPO or the SCN which directly or indirectly control the GnRH neurons. Further, continued high levels of estrogen in lesioned PE animals might be another factor acting to inhibit the release of GnRH. Estrogen has been found to inhibit the electrical activity of GnRH neurons[123] which presumably would also inhibit the release of GnRH. The mechanism by which GnRH release in reduced or altered in PD animals remains unknown.

proGnRH

Recently, Seeburg and Adelman[215] have demonstrated that GnRH arises from the post-translational processing of a larger precursor molecule, preproGnRH which consists of 92 amino acids. The GnRH decapeptide is preceded by a signal peptide of 23 amino acids and followed by a Gly-Lys-Arg sequence necessary for proteolytic processing and carboxy terminal amidation of GnRH[215]. The C-terminal end of preproGnRH consists of a 56 amino acid peptide termed gonadotropin-releasing hormone-associated peptide (GAP) [169]. Originally, GAP was found to have potent prolactin inhibitory activity *in vitro*[169]. However, a physiological role for GAP has not yet been demonstrated.

In sham-lesioned proestrous rats, proGnRH levels in the POA are approximately 6 times higher than those in the BH whereas GnRH levels are approximately 6 times higher in the BH as compared to the POA. Immunohistochemical data has demonstrated that the number of cell bodies containing proGnRH are equal to the number of cell bodies containing GnRH, and these cells are located primarily in the POA[122,196,199]. It is therefore, apparent from our work as well as that of others[122] that processing of proGnRH to GnRH occurs in the cell soma. In addition, the data indicates that processing of proGnRH continues during transport to the nerve terminal region as suggested previously[127].

A most interesting new finding in our study is the data showing that proGnRH levels in the POA were significantly reduced in the lesioned PE animals as compared to sham lesioned animals sacrificed prior to the preovulatory surge of LH. This would suggest that the synthetic activity was reduced or the turnover of proGnRH was increased in the lesioned PE rats. The latter is not likely because GnRH levels were similar in lesioned animals as compared to controls. Moreover, LH levels were low in the lesioned animals indicating that excessive GnRH was not being released. To date, the mechanism of control of GnRH synthesis remains unknown. The most recent findings by Kelly et al.[122] have shown that ovariectomy has no effect on GnRH mRNA levels, while proGnRH and GnRH levels are significantly reduced in ovariectomized female rats as compared to proestrous controls, indicating that ovarian steroid(s) affects post-translational processing of proGnRH mRNA. It is therefore tempting to speculate that continued high levels of estrogen in PE and high levels of progesterone in PD animals could disrupt post-translational processing of proGnRH mRNA. It is also possible that accumulation of GnRH in the nerve fibers and terminals of the lesioned animals have caused intracellular feedback inhibition of continued GnRH synthesis. This needs to be further elucidated.

SUMMARY AND CONCLUSIONS

The present studies have demonstrated that lesions of the AMPO or SCN produce persistent estrus and abolish preovulatory surges of LH and PRL. Moreover, estrogen- and progesterone-induced surges of LH are eliminated and the PRL surges are significantly attenuated in the AMPO- or SCN-lesioned animals. GnRH data have demonstrated that the induction of persistent estrus by AMPO or SCN lesions is not due to a reduction of the GnRH content in the BH as compared to proestrous controls. In contrast, proGnRH levels in the POA are significantly reduced in all lesioned animals as compared to sham operated rats during proestrus.

Therefore, these data suggest that the AMPO and the SCN participate in the control of the estrous cycle and are necessary for the preovulatory surges of PRL and LH to occur. The mechanism by which these nuclei control the PRL surge is unknown. However, the AMPO or the SCN participate in the control of the LH surge by regulating GnRH synthesis and release.

REFERENCES:

1 Abe, H.; Engier, D.; Molitch, M.E.; Bollinger-Gruber, J.: Vasoactive intestinal peptide is a physiological mediator of prolactin release in the rat. Endocrinology 116:1383-1390 (1985).

2 Adler, B.A.; Johnson, M.D.; Lynch, C.O.; Crowley, W.R.: Evidence that norepinephrine and epinephrine systems mediate the stimulatory effects of ovarian hormones on luteinizing hormone and luteinizing hormone-releasing hormone. Endocrinology 113:1431-1438 (1983).

3 Aiyer, M.S.; Chiappa, S.A.; Fink, G.: A priming efect of luteinizing hormone-releasing factor on the anterior pituitary gland in the female rat. J. Endocr. 62:573-588 (1974).

4 Alexander, M.J.; Clifton, D.K.; Steiner, R.A.: Vasoactive intestinal polypeptide effects a central inhibition of pulsatile luteinizing hormone secretion in ovariectomized rats. Endocrinology 117:2134-2139 (1985).

5 Alleva, J.J.: The Biological Clock and Pineal Gland: How They Control Seasonal Fertility in the Golden Hamster. In: Pineal Research Reviews, Reiter, R.J., editor,pp. 95-127 (Alan R. Liss, Inc., New York 1987).

6 Antunes-Rodrigues, J.; McCann, S.M.: Effect of suprachiasmatic lesions on the regulation of luteinizing hormone secretion in the female rat. Endocrinology 81:666-670 (1967).

7 Arimura, A.; Schally, A.V.: Prolactin release inhibiting and stimulating factors in the hypothalamus. In: Hypothalamic Peptide Hormones and Pituitary Regulation, Porter, J.C., editor,pp. 237-252 (Raven Press, New York 1977).

8 Azmitia, E.: The serotonin-producing neurons of the midbrain and dorsal raphe nuclei. In: Handbook of Psychopharmacology, Iversen, E.; Snyder, S., editors, pp. 233-314 (Plenum Press, New York 1978).

9 Baba, Y.; Matsuo, H.; Schally, A.V.: Structure of the porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analyses. Biochem. Biophys. Res. Commun. 44:459-463 (1971).

10 Barden, N.; Merand, Y.; Rouleau, D.; Garon, M.; dupont, A.: changes in the β-endorphin content of discrete hypothalamic nuclei during the estrous cycle in the rat. Brain Research 204:441-445 (1981).

11 Ben-Jonathan, N.; Olivaer, C.; Weiner, H.J.; Mical, R.S.; Porter, J.C.: Dopamine in hypophysial portal plasma of the rat during the estrous cycle and throughout pregnancy. Endocrinology 100:452-458 (1977).

12 Bethea, C.L.; Neill, J.D.: Prolactin secretion after cervical stimulation of rats maintained in constant dark or constant light. Endocrinology 104:870-876 (1979).

13 Bethea, C.L.; Sprangers, S.A.; West, N.B.; Brenner, R.M.: The effect of simultaneous versus sequential estradiol and

progesterone treatments on prolactin production in monkey pituitary cell cultures. Endocrinology 122:1786-1800 (1988).

14 Bethea, C.L.; Weiner, R.I.: Inhibition of prolactin secretion delays extinction of circadian LH surges in ovariectomized rats bearing estradiol implants. Proceedings of the Society for Experimental Biology and Medicine 172:65-69 (1983).

15 Bhattacharya, A.N.; Dierschke, D.J.; Yamaji, T.; Knobil, E.: The pharmacologic blockade of the circhoral mode of LH secretion in the ovariectomized rhesus monkey. Endocrinology 90:778-786 (1972).

16 Bishop, W.; Kalra, P.S.; Fawcett, C.P.; Krulich, L.; McCann, S.M.: The effects of hypothalamic lesions on the release of gonadotropins and prolactin in response to estrogen and progesterone treatment in female rats. Endocrinology 91:1404-1410 (1972).

17 Blake, C.: Stimulation of pituitary prolactin and TSH release in lactating and proestrous rats. Endocrinology 94:503-508 (1974).

18 Blake, C.A.: A detailed characterization of the proestrous luteinizing hormone surge. Endocrinology 98:445-450 (1976).

19 Blake, C.A.; Norman, R.L.; Sawyer, C.H.: Localization of the inhibitory actions of estrogen and nicotine on release of luteinizing hormone in rats. Neuroendocrinology 16:22-35 (1974).

20 Blake, C.A.; Sawyer, C.H.: Effects of hypothalamic deafferentation on the pulsatile rhythm in plasma concentrations of luteinizing hormone in ovariectomized rats. Endocrinology 94:730-736 (1974).

21 Blake, C.A.; Weiner, R.I.: Pituitary prolactin secretion in female rats made persistently estrous or diestrous by hypothalamic deafferentation. Endocrinology 90:862-866 (1972).

22 Blake, C.A.; Weiner, R.I.; Gorski, R.A.; Sawyer, C.H.: Secretion of pituitary luteinizing hormone and follicle stimulating hormone in female rats made persistently estrous or diestrous by hypothalamic deafferentation. Endocrinology 90:855-861 (1972).

23 Bloom, F.E.; Battenberg, E.L.F.; Rivier, J.; Vale, W.: Corticotropin-releasing factor (CRF): immunoreactive neurons and fibers in rat hypothalamus. Regulatory Peptides 4:43-48 (1982).

24 Bosler, O.; Beaudet, A.: VIP neurons as prime synaptic targets for serotonin afferents in rat suprachiasmatic nucleus: a combined radioautographic and immunocytochemical study. J. Neurocytol. 14:749-763 (1985).

25 Bowers, C.Y.; Friesen, H.G.; Hwang, P.; Guyda, H.J.; Folkers, K.: Prolactin and thyrotropin release in man by synthetic pyroglutamyl-histidyl-prolinamide. Biochem. Biophys. Res. Commun. 45:1033-1041 (1971).

26 Brown-Grant, K.; Murray, M.A.F.; Raisman, G.; Sood, M.C.: Reproductive function in male and female rats following extra- and intra-hypothalamic lesions. Proc.R.Soc.Lond.B. 198:267-278 (1977).

27 Brown-Grant, K.; Raisman, G.: Abnormalities in reproductive function associated with the destruction of the suprachiasmatic nuclei in female rats. Proc.R.Soc.Lond.B. 198:279-296 (1977).

28 Brownstein, M.J.; Arimura, A.; Schally, A.V.; Palkovits, M.; Kizer, J.S.: The effect of surgical isolation of the hypothalamus on its luteinizing hormone content. Endocrinology 98:662-665 (1976).

29 Bruhn, T.O.; Plotsky, P.M.; Vale, W.: Effect of paraventricular lesions on corticotropin-releasing factor (CRF)-like immunoreactivity in the stalk-median eminence: studies on the adrenocorticotropin response to ether stress and exogenous CRF. Endocrinology 114:57-62 (1984).

30 Bruni, J.F.; Van Vugt, D.; Marshall, S.; Meites, J.: Effects of naloxone, morphine and methionine enkephalin on serum prolactin, luteinizing hormone,follicle stimulating hormone,thyroid stimulating hormone and growth hormone. Life Sciences 21:461-466 (1977).

31 Burgus, R.; Butcher, M.; Amoss, M.; Ling, N.; Monahan, M.; Rivier, J.; Fellows, R.; Blackwell, R.; Vale, W.; Guillemin, R.: Primary structure of the ovine hypothalamic luteinizing

hormone-releasing factor (LRF). Proc. Nat. Acad. Sci. 69:278-282 (1972).

32 Butcher, R.L.; Fugo, N.W.; Collins, W.E.: Semicircadian rhythm in plasma levels of prolactin during early gestation in the rat. Endocrinology 90:1125-1127 (1972).

33 Caceres, A.; Taleisnik, S.: Pathways by which stimuli originating in the cingulate cortex inhibiting LH secretion reach the hypothalamus. Neuroendocrinology 32:317-324 (1981).

34 Calabro, M.A.; MacLeod, R.M.: Binding of dopamine to bovine anterior pituitary gland membranes. Neuroendocrinology 25:32-46 (1978).

35 Caligaris, L.; Astrada, J.J.; Taleisnik, S.: Biphasic effect of progesterone on the release of gonadotropin in rats. Endocrinology 89:331-337 (1971).

36 Campbell, C.S.; Schwartz, N.B.: The impact of constant light on the estrous cycle of the rat. The Physiologist 21:16 (1978).(Abstract)

37 Campbell, C.S.; Schwartz, N.B.; Firlit, M.G.: The role of adrenal and ovarian steroids in the control of serum LH an FSH. Endocrinology 101:162-172 (1977).

38 Card, J.P.; Moore, R.Y.: The suprachiasmatic nucleus of the golden hamster: immunohistochemical analysis of cell and fiber distribution. Neuroscience 13:415-431 (1984).

39 Carrillo, A.J.; Sawyer, C.H.: Observations on successive pro-oestrous LH surges in individual 4-day cycling and

post-pseudopregnant rats. Acta Endocrinologica 88:274-284 (1978).

40 Chen, C.L.; Amenomori, Y.; Lu, K.H.; Voogh, J.L.; Meites, J.: Serum prolactin levels in rat with pituitary transplants or hypothalamic lesions. Neuroendocrinology 6:220 (1970).

41 Chen, W.P.; Within, J.W.; Silverman, A.J.: Beta-endorphin (B-E) and GnRH innervation of GnRH neurons. Neuroscience 14(1):439 (1988). (Abstract)

42 Ching, M.: Correlative surges of LHRH, LH and FSH in pituitary stalk plasma and systemic plasma of rat during proestrus. Neuroendocrinology 34:279-285 (1982).

43 Ching, M.: Morphine suppresses the proestrous surge of GnRH in pituitary portal plasma of rats. Endocrinology 112:2209-2211 (1983).

44 Clemens, J.A.; Smalstig, E.B.; Sawyer, B.D.: Studies on the role of the preoptic area in the control of reproductive function in the rat. Endocrinology 99:728-735 (1976).

45 Clifton, D.K.; Steiner, R.A.; Resko, J.A.; Spies, H.G.: Estrogen-induced gonadotropin release in ovariectomized rhesus monkeys and its advancement by progesterone. Biology of Reproduction 13:190-194 (1975).

46 Coen, C.W.; Coombs, M.C.; Wilson, P.M.J.; Clement, E.M.; MacKinnon, P.C.B.: Possible resolution of a paradox concerning the use of p-chlorophenylalanine and 5-hydroxytryptophan: evidence for a mode of action involving adrenaline in

manipulating the surge of luteinizing hormone in rats. Neuroscience 8:583-591 (1983).

47 Conti, A.; Togni, E.; Travaglini, P.; Muratori, M.; Faglia, G.: Vasoactive intestinal polypeptide and dopamine: effect on prolactin secretion in normal women and patients with microprolactinomas. Neuroendocrinology 46:241-245 (1987).

48 Cox, B.B.: Endogenous opioid peptides: a guide to structures and terminology. Life Sciences 31:1645-1659 (1982).

49 Crowley, W.R.; Terry, L.C.: Effects of an epinephrine synthesis inhibitor, SKF64139, on the secretion of luteinizing hormone in ovariectomized female rats. Brain Research 204:231-235 (1981).

50 Cummings, S.; Elde, R.; Ells, J.; Lindall, A.: Corticotropin-releasing factor immunoreactivity is widely distributed within the central nervous system of the rat: an immunohistochemical study. J. Neurosci. 3:1355-1368 (1983).

51 Dannies, P.S.; Gautvik, K.M.; Tashjian, A.H.; Jr.: A possible role of cyclic AMP in mediating the effects of thyrotropin-releasing hormone on prolactin release and on prolactin and growth hormone synthesis in pituitary cells in culture. Endocrinology 98:1147-1159 (1976).

52 DeKoning, J.; van Dieten, J.A.M.J.; van Rees, G.P.: Inhibitory and augmentative effects of oestradiol on LH-RH-induced release of LH by anterior pituitary glands from intact female rats in vitro. Mol. Cell. Endocrinol. 5:321-326 (1976).

53 Dey, F.L.: Evidence of hypothalamic control of hypophyseal gonadotropic functions in the female guinea pig. Endocrinology 33:75-82 (1943).

54 Dey, F.L.; Leininger, C.R.; Ranson, S.W.: The effect of hypophysial lesions on mating behavior in female guinea pigs. Endocrinology 30:323-326 (1942).

55 Deyo, S.N.; Miller, R.J.: The role of endogenous opioids in the stress-and estrogen-induced activation of prolactin release. Life Sciences 31:2171-2175 (1982).

56 Drouva, S.V.; Gallo, R.V.: Further evidence for inhibition of episodic luteinizing hormone release in ovariectomized rats by stimulation of dopamine receptors. Endocrinology 100:792-798 (1977).

57 Dunn, J.D.; Johnson, D.C.; Castro, A.J.; Swenson, R.: Twenty-four hour pattern of prolactin levels in female rats subjected to transection of the mesencephalic raphe or ablation of the suprachiasmatic nuclei. Neuroendocrinology 31:85-91 (1980).

58 Dykshoorn, M.A.; Ronnekleiv, O.K.; Kelly, M.J.: Ventral medial preoptic nucleus (vMPN) or suprachiasmatic nucleus (SCN) lesions combined with estrogen, progesterone treatment, effects on prolactin (PRL) and luteinizing hormone (LH) in the cycling female rat. Neuroscience (1985).(Abstract)

59 Eichler, V.B.; Moore, R.Y.: The primary and accessory optic systems in the golden hamster, mesocricetus auratus. Acta

Anat. 89:359-371 (1974).

60 Ellinwood, W.E.; Ronnekleiv, O.K.; Kelly, M.J.; Resko, J.A.: A new antiserum with conformational specificity for LHRH:usefulness for radioimmunoassay and immunocytochemistry. Peptides 6:45-52 (1985).

61 Epelbaum, J.: Somatostain in the central nervous system: physiology and pathological modifications. Prog. Neurobilo. 27:63-100 (1986).

62 Estes, K.S.; Simpkins, J.W.; Kalra, S.P.: Resumption with clonidine of pulsatile LH release following acute norepinephrine depletion in ovariectomized rats. Neuroendocrinology 35:56-62 (1982).

63 Evans, G.A.; David, D.N.; Rosenfeld, M.G.: Regulation of prolactin and somatotropin mRNAs by thyroliberin. Proc. Natl. Acad. Sci. 75:1294-1298 (1978).

64 Everett, J.W.; Sawyer, C.H.: A 24-hour periodicity in the "LH-release apparatus" of female rats, disclosed by barbiturate sedation. Endocrinology 47:198-218 (1950).

65 Everett, J.W.; Sawyer, C.H.; Markee, J.E.: A neurogenic timing factor in control of the ovulatory discharge of luteinizing hormone in the cyclic rat. Endocrinology 44:234-250 (1949).

66 Everitt, B.J.; Hökfelt, T.; Terenius, L.; Tatemoto, K.; Mutt, V.; Goldstein, M.: Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. Neuroscience 11:443-462

(1984).

67 Fawley, L.S.; Neill, J.D.: Stimulation of prolactin secretion in rhesus monkeys by vasoactive intestinal polypeptide. Neuroendocrinology 33:79-83 (1981).

68 Fink, G.; Henderson, S.R.: Steroids and pituitary responsiveness in female, androgenized female and male rats. J. Endocr. 73:157-164 (1977).

69 Finley, J.W.; Maderdrut, J.; Petrusz, P.: The immunocytochemical localization of enkephalin in the central nervous system of the rat. J. Comp. Neurol. 198:541-565 (1981).

70 Freeman, M.E.; Neill, J.D.: The pattern of prolactin secretion during pseudopregnancy in the rat: a daily nocturnal surge. Endocrinology 90:1292-1294 (1972).

71 Füxe, K.; Hökfelt, T.: Catecholamines in the hypothalamus and the pituitary gland. In: Frontiers in Neuroendocrinology, Ganong, W.; Martini, L., editors, pp. 47-96 (Oxford University Press, New York 1969).

72 Füxe, K.; Hökfelt, T.; Said, S.I.; Mutt, V.: VIP and the nervous system: immunohistochemical evidence for localization in central and peripheral nerves, particularly intracortical neurons of cerebral cortex. Neuroscience Letters 5:241-246 (1977).

73 Gabriel, S.M.; Simpkins, J.W.; Kalra, S.P.: Modulation of endogenous opioid influence on luteinizing hormone secretion by progesterone and estrogen. Endocrinology 113:1806-1811

(1983).

74 Gallo, R.V.: The effect of blockade of dopamine receptors on the inhibition of episodic luteinizing hormone release during electrical stimulation of the arcuate nucleus in ovariectomized rats. Endocrinology 102:1026-1035 (1978).

75 Gallo, R.V.: Neuroendocrine regulation of pulsatile luteinizing hormone release in the rat. Neuroendocrinology 30:122-131 (1980).

76 Gallo, R.V.: Pulsatile LH release during periods of low level LH secretion in the rat estrous cycle. Biology of Reproduction 24:771-777 (1981).

77 Gallo, R.V.: Further studies on dopamine-induced suppression of pulsatile LH release in ovariectomized rats. Neuroendocrinology 32:187-192 (1981).

78 Gallo, R.V.; Drouva, S.V.: Effects of intraventricular infusion of catecholamines on luteinizing hormone release in ovariectomized and ovariectomized steroid primed rats. Neuroendocrinology 29:149-162 (1979).

79 Gibbs, D.M.; Neill, J.D.: Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion in vivo. Endocrinology 102:1895-1900 (1978).

80 Gnodde, H.P.; Schuiling, G.A.: Involvement of catecholaminergic and cholinergic mechanisms in the pulsatile release of LH in the long-term ovariectomized rat. Neuroendocrinology 20:212-223 (1976).

81 Goodman, R.L.: A quantitative analysis of the physiological role of estradiol and progesterone in the control of tonic and surge secretion of luteinizing hormone in the rat. Endocrinology 102:142-150 (1978).

82 Goodman, R.L.: The site of the positive feedback action of estradiol in the rat. Endocrinology 102:151-159 (1978).

83 Goodman, R.L.; Knobil, E.: The sites of action of ovarian steroids in the regulation of LH secretion. Neuroendocrinology 32:57-63 (1981).

84 Gorski, J.; Shull, J.; Weber, J.; Durrin, L.: Estrogen regulation of prolactin gene transcription and chromatin structure. In: Prolactin Basic and clinical correlates, MacLeod, R.M.; Thorner, M.O.; Scapagnini, U., editors, pp. 259-269 (Liviana Press, Padova 1985).

85 Grandison, L.; Guidotti, A.: Regulation of prolactin release by endogenous opiates. Nature 270:357-359 (1977).

86 Gray, G.D.; Sodersten, P.; Tallentire, D.; Davidson, J.M.: Effects of lesions in various structures of the suprachiasmatic-preoptic region on LH regulation and sexual behavior in female rats. Neuroendocrinology 25:174-191 (1978).

87 Greenwood, F.C.; Hunter, W.M.; Glover, J.S.: The preparation of 125I-labelled human growth hormone of high specific activity. Biochem J 89:114-123 (1963).

88 Greer, M.A.: Evidence of hypothalamic control of the pituitary release of thyrotropin. Proc. Soc. Exp. Biol. Med. 77:603-608

(1951).

89 Grosvenor, C.E.; McCann, S.M.; Nallar, R.: Inhibition of nursing-induced and stress-induced fall in pituitary prolactin concentration in lactating rats by injection of acid extracts of bovine hypothalamus. Endocrinology 76:883-889 (1965).

90 Grosvenor, C.E.; Whitworth, N.: Evidence for a steady rate of secretion of prolactin following suckling in the rat. J. Dairy Sci. 57:900-904 (1974).

91 Harms, P.G.; Ojeda, S.R.: A rapid and simple procedure for chronic cannulation of the rat jugular vein. Journal of Applied Physiology 36:391-392 (1974).

92 Hisano, S.; Chikamori-Aoyama, M.; Katoh, S.; Maegawa, M.; daikoku, S.: Immunohistochemical evidence of serotoninergic regulation of vasoactive intestinal polypeptide (VIP) in the rat suprachiasmatic nucleus. Histochemistry 86:573-578 (1987).

93 Hitoshi, Kawano; Shigeo, Daikoku: Immunohistochemical demonstration of LHRH neurons and their pathways in the rat hypothalamus. Neuroendocrinology 32:179-186 (1981).

94 Héry, M.; Laplante, E.; Kordon, C.: Participation of serotonin in the phasic release of LH. I. Evidence from pharmacological experiments. Endocrinology 99:496-503 (1976).

95 Hökfelt, T.; Fahrenkrug, J.; Tatemoto, K.; Mutt, V.; Werner, W.; Hulting, A.L.; Terenius, L.; Chang, K.J.: The PHI (PHI-27)/corticotropin-releasing factor/enkephalin immunoreactive hypothalamic neuron: Possible morphological

basis for integrated control of prolactin, corticotropin, and growth hormone secretion. Proc. Natl. Acad. Sci. 80:895-898 (1983).

96 Ibata, Y.; Watanabe, K.; Kinoshita, H.; Kubo, S.; Sano, Y.: The location of LHRH neurons in the rat hypothalamus and their pathways to the median eminence. Cell Tissue Res. 198:381-395 (1979).

97 Jennes, L.; Beckman, w.; Stumpf, W.E.; Grzanna, R.: Anatomical relationships of serotoninergic and noradrenergic projections with the GnRH system in septum and hypothalamus. Exp. Brain Res. 46:331-338 (1982).

98 Kaji, H.; Chihara, K.; Kita, T.; Kashio, Y.; Okimura, Y.: Administration of antisera to vasoactive intestinal polypeptide and peptide histidine isoleucine attenuates ether-induced prolactin secretion in rats. Neuroendocrinology 41:529-531 (1985).

99 Kalra, P.S.: Tissue levels of luteinizing hormone-releasing hormone in the preoptic area and hypothalamus, and serum concentrations of gonadotropins following anterior hypothalamic deafferentation and estrogen treatment of the female rat. Endocrinology 99:101-107 (1976).

100 Kalra, P.S.; Fawcett, C.P.; Krulich, L.; McCann, S.M.: The effects of gonadal steroids on plasma gonadotropins and prolactin in the rat. Endocrinology 92:1256-1268 (1973).

101 Kalra, P.S.; Kalra, S.P.: Temporal changes in the hypothalamic and serum luteinizing hormone-releasing hormone (LHRH) levels and the circulating ovarian steroids during the rat oestrous cycle. Acta Endocrinol. 85:449-455 (1977).

102 Kalra, P.S.; Kalra, S.P.: Modulation of hypothalamic luteinizing hormone-releasing hormone levels by intracranial and subcutaneous implants of gonadal steroids in castrated rats: Effects of androgen and estrogen antagonists. Endocrinology 106:390-397 (1980).

103 Kalra, P.S.; Kalra, S.P.: Discriminative effects of testosterone on hypothalamic luteinizing hormone-releasing hormone levels and luteinizing hormone secretion in castrated male rats: analyses of dose and duration characteristics. Endocrinology 111:24-29 (1982).

104 Kalra, P.S.; Kalra, S.P.: Control of gonadotropin secretion. In: The Pituitary Gland, Imura H., editor,pp. 189-220 (Raven Press, New York 1985).

105 Kalra, P.S.; Kalra, S.P.; Krulich, L.; Fawcett, C.P.; McCann, S.M.: Involvement of norepinephrine in transmission of the stimulatory influence of progesterone on gonadotropin release. Endocrinology 90:1168-1176 (1972).

106 Kalra, P.S.; McCann, S.M.: The stimulatory effect on gonadotropin release of implants of estradiol or progesterone in certain sites in the central nervous system. Neuroendocrinology 19:289-302 (1975).

107 Kalra, S.P.: Effect of estrogen on 3H-leucine uptake by the hypothalamus and pituitary: correlation with hypothalamic and serum LHRH and LH. J. Endocrinol. Invest. 2:131-135 (1978).

108 Kalra, S.P.: Neural circuitry involved in the control of LHRH secretion: a model for preovulatory LH release. In: Frontiers in Neuroendocrinology, Ganong W.F.; Martini L., editors, pp. 31-75 (Raven Press, New York 1986).

109 Kalra, S.P.; Crowley, W.R.: Norepinephrine-like effects of neuropeptide Y on LH release in the rat. Life Sciences 35:1173-1176 (1984).

110 Kalra, S.P.; Crowley, W.R.: Differential effects of pancreatic polypeptide on luteinizing hormone release in female rats. Neuroendocrinology 38:511-513 (1984).

111 Kalra, S.P.; Kalra, P.S.: Neural regulation of luteinizing hormone secretion in the rat. Endocrine reviews 4:311-351 (1983).

112 Kalra, S.P.; Simpkins, J.W.; Kalra, P.S.: Effects of pentobarbital on hypothalamic catecholamines and LRH activities. Acta Endocrinol. 96:1-6 (1980).

113 Kamberi, I.A.; Mical, R.S.; Porter, J.C.: Effect of anterior pituitary perfusion and intraventricular injection of catecholamines and indoleamines on LH release. Endocrinology 87:1-12 (1970).

114 Kamberi, I.A.; Mical, R.S.; Porter, J.C.: Effect of melatonin and serotonin on the release of FSH and prolactin.

Endocrinology 88:1288-1293 (1971).

115 Kant, G.J.; Mougey, E.H.; Meyerhoff, J.L.: Diurnal variation in neuroendocrine response to stress in rats: plasma ACTH, β-endorphin, β-LPH, corticosterone and prolactin and pituitary cyclic AMP responses. Neuroendocrinology 43:383-390 (1986).

116 Kant, G.J.; Mougey, E.H.; Pennington, L.L.; Meyerhoff, J.L.: Graded footshock stress elevates pituitary cyclic AMP and plasma β-endorphin, β-LPH, corticosterone and prolactin. Life Sciences 33:2657-2663 (1983).

117 Karsch, F.J.; Weick, R.F.; Hotchkiss, J.; Dierschke, D.J.; Knobil, E.: An analysis of the negative feedback control of gonadotropin secretion utilizing chronic implantation of ovarian steroids in ovariectomized rhesus monkeys. Endocrinology 93:478-486 (1973).

118 Kato, Y.; Iwasaki, Y.; Iwasaki, J.; Abe, H.; Yanaihara, N.; Imura, H.: Prolactin release by vasoactive intestinal polypeptide in rats. Endocrinology 103:554-558 (1978).

119 Kawadami, M.; Arita, J.; Yoshioka, E.: Loss of estrogen-induced daily surges of prolactin and gonadotropins by suprachiasmatic nucleus lesions in ovariectomized rats. Endocrinology 106:1087-1092 (1980).

120 Kawakami, M.; Arita, J.: Effects of lesions in the medial basal part of the suprachiasmatic area on prolactin and gonadotropin surges induced by estrogen and progesterone treatment in ovariectomized rats. Neuroendocrinology

32:242-247 (1981).

121 Kawakami, M.; Arita, J.; Yoshioka, E.: Loss of estrogen-induced daily surges of prolactin and gonadotropins by suprachiasmatic nucleus lesions in ovariectomized rats. Endocrinology 106:1087-1092 (1980).

122 Kelly, M.J.; Garrett, J.; Bosch, M.A.; Roselli, C.E.; Douglass, J.; Adelman, J.P.; Ronnekleiv, O.K.: Effects of ovariectomy on GnRH mRNA, proGnRH and GnRH levels in the preoptic-hypothalamus of the female rat. Neuroendocrinology (1988).(In Press)

123 Kelly, M.J.; Ronnekleiv, O.K.; Eskay, R.L.: Identification of estrogen-responsive LHRH neurons in the guinea pig hypothalamus. Brain Research Bulletin 12:399-407 (1984).

124 Khachaturian, H.; Lewis, M.E.; Schäfer, M.K.; Watson, S.J.: Anatomy of the CNS opioid systems. Tins March:111-119 (1985).

125 Kim, K.; Ramirez, V.D.: In vitro progesterone stimulates the release of luteinizing hormone-releasing hormone from superfused hypothalamic tissue from ovariectomized estradiol-primed prepubertal rats. Endocrinology 111:750-757 (1982).

126 Kim, K.; Ramirez, V.D.: In vitro luteinizing hormone-releasing hormone release from superfused rat hypothalami: site of action of progesterone and effect of estrogen priming. Endocrinology 116:252-258 (1985).

127 King, J.C.; Anthony, E.L.P.: Biosynthesis of LHRH: inferences from immunocytochemical studies. Peptides 4:963-970 (1983).

128 Kinoshita, F.; Nakai, Y.; Katakami, H.; Kato, Y.; Imura, H.: Role of α-adrenergic mechanism in regulating tonic luteinizing hormone release in conscious ovariectomized rats. Endocrinology 108:1272-1275 (1981).

129 Kinoshita, F.; Nakai, Y.; Katakami, H.; Kato, Y.; Yajima, H.; Imura, H.: Effect of β-endorphin on pulsatile luteinizing hormone release in conscious castrated rats. Life Sciences 27:843-846 (1980).

130 Koch, Y.; Goldhaber, G.; Fireman, I.; Zor, U.; Shani, J.; Tal, E.: suppression of prolactin and thyrotropin secretion in the rat by antiserum to thyrotropin-releasing hormone. Endocrinology 100:1476-1478 (1977).

131 Koves, K.; Halasz, B.: Location of the neural structures triggering ovulation in the rat. Neuroendocrinology 6:180-193 (1970).

132 Krieg, R.J.; Ching, M.C.H.: Stimulation of luteinizing hormone releasing hormone (LHRH) secretion by norepinephrine (NE) in steroid-primed or unprimed ovariectomized (OVX) rats. Fed. Proc. 42:976-(Abst.) (1982).

133 Krieg, R.J.; Salisbury, R.L.; MacLeod, R.M.: Timing of prolactin and luteinizing hormone surges in steroid-primed-ovariectomized and proestrous rats. In: The Anterior pituitary Gland, Bhatnagar A., editor,pp. 313-318

(Raven Press, New York 1983).

134 Köves, K.; Molnàr, J.: Effect of various hypothalamic deafferentations injuring different parts of the GnRH pathway on ovulation, GnRH content of the median eminence, and plasma LH and FSH levels. Neuroendocrinology 44:172-183 (1986).

135 Lagace, L.; Massicotte, J.; Labrie, F.: Acute stimulatory effects of progesterone on luteinizing hormone and follicle-stimulating hormone release in rat anterior pituitary cells in culture. Endocrinology 106:684-689 (1980).

136 Leadem, C.A.; Kalra, S.P.: Stimulation with estrogen and progesterone of luteinizing hormone (LH)-releasing hormone release from perifused adult female rat hypothalami: correlation with the LH surge. Endocrinology 114:51-56 (1984).

137 Leung, P.C.K.; Arendash, G.W.; Whitmoyer, D.I.; Gorski, R.A.; Sawyer, C.H.: Differential effects of central adrenoreceptor agonists on luteinizing hormone release. Neuroendocrinology 34:207-214 (1982).

138 Leung, P.C.K.; Whitmoyer, D.I.; Arendash, G.W.; Sawyer, C.H.: Effects of intraventricular norepinephrine on preoptic-anterior hypothalamic electrical activity in the freely-moving rat: modulation by ovarian steroid hormones. Brain Research 226:143-154 (1981).

139 Levine, J.E.; Ramirez, V.D.: In vivo release of luteinizing hormone-releasing hormone estimated with push-pull cannulae from the mediobasal hypothalami of ovariectomized,

steroid-primed rats. Endocrinology 107:1782-1790 (1980).

140 Levine, J.E.; Ramirez, V.D.: Luteinizing hormone-releasing hormone release during the rat estrous cycle and after ovariectomy, as estimated with push-pull cannulae. Endocrinology 111:1439-1448 (1982).

141 Libertun, C.; McCann, S.M.: The possible role of histamine in the control of prolactin and gonadotropin release. Neuroendocrinology 20:110-120 (1976).

142 Libertun, C.; Orias, R.; McCann, S.M.: Biphasic effect of estrogen on the sensitivity of the pituitary to luteinizing hormone-releasing factor (LRF). Endocrinology 94:1094-1100 (1974).

143 Lindvall, O.; Bjorklund, A.: Anatomy of the dopaminergic neuron systems in the rat brain. In: Advances in Biochemical Psychopharmacology, Roberts, P.J., editor,pp. 1-23 (Raven Press, New York 1978).

144 MacLeod, R.M.: Influence of norepinephrine and catecholamine-depleting agents on the synthesis and release of prolactin and growth hormone. Endocrinology 85:916-923 (1969).

145 MacLeod, R.M.: Regulation of Prolactin Secretion. In: Frontiers in Neuroendocrinology, Martini, L.; Ganong, W.F., editors, pp. 169-194 (Raven Press, New York 1976).

146 Maegawa, M.; Hisano, S.; Tsuruo, Y.; Katoh, S.; Nakanishi, J.; Chikamori-Aoyama, J.; daikoku, S.: Differential

immunolabeling for electron microscopy of diverse peptidergic neurons. J. Histochem. Cytochem. 31:251-255 (1987).

147 Malarkey, W.B.; D'Orisio, T.M.; Kennedy, M.; Cataland, S.: The influence of vasoactive intestinal polypeptide and cholecystokinin on prolactin release in rat and human monolayer culture. Life Sciences 28:2489-2495 (1981).

148 Matsuo, H.; Baba, Y.; Nair, R.M.G.; Arimura, A.; Schally, A.V.: Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem. Biophys. Res. Commun. 43:1334-1339 (1971).

149 Maurer, R.A.: Dopaminergic Inhibition of prolactin synthesis and prolactin messenger RNA accumulation in cultured pituitary cells. The Journal of Biological Chemistry 255:8092-8097 (1980).

150 McCann, S.M.: Regulation of secretion of follicle stimulating hormone and luteinizing hormone. In: Handbook of Physiology, Knobil, E.; Sawyer, W.H., editors, pp. 489-517 (American Physiology Society, Washington, D.C. 1974).

151 McCann, S.M.; Lumpkin, M.D.; Mizunuma, H.; Khorram, O.; Ottlecz, A.; Samson, W.K.: Peptidergic and dopaminergic control of prolactin release. Tins 127-131 (1984).

152 McCann, S.M.; Taleisnik, S.; Friedman, H.M.: LH-releasing activity in hypothalamic extracts. Proc. Soc. Exp. Biol. Med. 104:342-433 (1960).

153 McCann, S.M.; Vijayan, E.; Negro-Vilar, A.; Mizunuma, H.; Mangat, H.: Gamma aminobutyric acid (GABA), a modulator of anterior pituitary hormone secretion by hypothalamic and pituitary action. Psychoneuroendocrinology 9:97-106 (1984).

154 McEwen, B.S.: Neural gonadal steroid actions. Science 211:1303-1311 (1981).

155 McLean, B.K.; Chang, N.; Nikitovitch-Winer, M.B.: Ovarian steroids directly alter luteinizing hormone (LH) release by pituitary homografts. Endocrinology 96:196-201 (1975).

156 Meltzer, H.Y.; Fang, V.S.; Paul, S.M.; Kaluskar, R.: Effect of quipazine on rat plasma prolactin levels. Life Sciences 19:1073-1078 (1976).

157 Merchenthaler, L.; Vigh, S.; Petrusz, P.; Schally, A.V.: The paraventriculoinfundibular corticotropin releasing factor (CRF) pathway as revealed by immunocytochemistry in long-term hypophysectomized or adrenalectomized rats. Regulatory Peptides 5:295-305 (1983).

158 Meyerhoff, J.L.; Mougey, E.H.; Kant, G.J.: Paraventricular lesions abolish the stress-induced rise in pituitary cyclic adenosine monophosphate and attenuate the increases in plasma levels of proopiomelanocortin-derived peptides and prolactin. Neuroendocrinology 46:222-230 (1987).

159 Milmore, J.B.; Reece, R.P.: Effects of porcine hypothalamic extract on prolactin release in the rat. Endocrinology 96:732-738 (1975).

160 Negro-Vilar, A.; Ojeda, S.R.: Catecholaminergic and steroidal modification of LHRH and somatostatin (SRIF) release by median eminence in vitro. Endocrinology 102:459-(Abst.) (1978).

161 Neill, J.D.: Effect of "stress" on serum prolactin and luteinizing hormone levels during the estrous cycle of the rat. Endocrinology 87:1192-1197 (1970).

162 Neill, J.D.: Comparison of plasma prolactin levels in cannulated and decapitated rats. Endocrinology 90:568-572 (1972).

163 Neill, J.D.: Prolactin: Its secretion and control. In: Handbook of Physiology, pp. 469-488 (American Physiological Society, Washington, D.C. 1974).

164 Neill, J.D.; Freeman, M.E.; Tillson, S.A.: Control of the proestrus surge of prolactin and luteinizing hormone secretion by estrogens in the rat. Endocrinology 89:1448-1453 (1971).

165 Neill, J.D.; Reichert, L.E.; Jr.: Development of radioimmunoassay for rat prolactin and evaluation of the NIAMD rat prolactin radioimmunoassay. Endocrinology 88:548-555 (1971).

166 Nicoll, C.S.: Physiological actions of prolactin. In: Handbook of Physiology, pp. 253-292 (, 1974).

167 Nicoll, C.S.; Fiorindo, R.P.; McKennee, C.F.; Parsons, J.A.: Assay of hypothalamic factors which regulate prolactin secretion. In: Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry, Meites, J., editor,pp. 115-150 (Williams

& Wilkins, Baltimore 1970).

168 Nicoll, C.S.; Talwalker, P.K.; Meites, J.: Initiation of lactation in rats by nonspecific stresses. Am. J. Physiol. 198:1103-1106 (1960).

169 Nikolics, K.; Mason, A.J.; Szonyi, E.; Seeberg, P.H.; Ramachandran, J.: A prolactin-inhibiting factor within the precursor for human gonadotropin-releasing hormone. Nature 316:511-542 (1985).

170 Nomura, K.; Takahashi, S.; Kawashima, S.: Maintenance of estrous cycle in female rats with anterior or posterior deafferentation of the suprachiasmatic nucleus. Neuroendocrinology 47:444-452 (1988).

171 Ohtsuka, S.O.; Miyake, A.; Nishizaki, T.; Tasaka, K.; Tanizawa, O.: Vasoactive intestinal peptide stimulates gonadotropin-releasing hormone release form rat hypothalamus in vitro. Acta Endocrinologica (Copenh) 117:399-402 (1988).

172 Palka, Y.; Coyer, D.; Critchlow, V.: Effects of isolation of medial basal hypothalamus on pituitary-adrenal and pituitary-ovarian functions. Neuroendocrinology 5:333-349 (1969).

173 Palkovits, M.: Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Research 59:449-450 (1973).

174 Palkovits, M.: Catecholamines in the hypothalamus: an anatomical review. Neuroendocrinology 33:123-128 (1981).

175 Palkovits, M.; Mezey, É.; Ambach, G.; Kivovics, P.: Neural and vascular connections between the organum vasculosum laminae terminalis and preoptic nuclei. In: Neural Hormones and Reproduction, Scott D.E.; Rochester; Kozlowski G.P.; Weindl A., editors, pp. 302-312 (Karger AG, Switzerland 1978).

176 Pan, J.T.; Gala, R.R.: Central nervous system regions involved in the estrogen-induced afternoon prolactin surge. I. lesion studies. Endocrinology 117:382-387 (1985).

177 Paxinos, G.; Watson, C.: The rat brain in stereotaxic coordinates. ed. 2, Academic Press, Sydney 1986).

178 Petraglia, F.; Vale, W.; Rivier, C.: Beta-endorphin and dynorphin participate in the stress-induced release of prolactin in the rat. Neuroendocrinology 45:338-342 (1987).

179 Pfaff, D.; Keiner, M.: Atlas of estradiol-concentrating cells in the central nervous system of the female rat. J. Comp. Neurol. 151:121-131 (1973).

180 Pfaff, D.W.: Gene expression in hypothalamic neurons: luteinizing hormone releasing hormone. Journal of Neuroscience Research 16:109-115 (1986).

181 Piacsek, E.B.; Schneider, T.C.; Gay, V.L.: Sequential study of luteinizing hormone (LH) and progestin secretion on the afternoon of proestrus in the rat. Endocrinology 89:39-45 (1971).

182 Piotte, M.; Beaudet, A.; Brawer, J.R.: Light and electron microscopic study of tyrosine hydroxylase-immunoreactive neurons within the developing rat arcuate nucleus. Brain Research 439:127-137 (1988).

183 Pohl, C.R.; Weiner, R.I.; Smith, M.S.: Relation between luteinizing hormone and prolactin pulses in ovariectomized rats with or without dopamine inhibition. Endocrinology 123:1591-1597 (1988).

184 Popolow, H.B.; King, J.C.; Gerall, A.A.: Rostral medial preoptic area lesions influence on female estrous processes and LHRH distribution. Physiology & Behavior 27:855-861 (1981).

185 Price, D.: Feedback control of gonadal and hypophyseal hormones: evolution of the concept. In: Pioneers in Neuroendocrinology, Meites, J.; Donovan, T.; McCann, S.M., editors, pp. 219-238 (Plenum Press, New York 1975).

186 Ragavan, V.V.; Frantz, A.G.: Opioid regulation of prolactin secretion: evidence for a specific role of β-endorphin. Endocrinology 109:1769-1771 (1981).

187 Raisman, G.; Brown-Grant, K.: The "suprachiasmatic syndrome": endocrine and behavioral abnormalities following lesions of the suprachiasmatic nuclei in the female rat. Proc. R. Soc. Lond. B. 198:297-314 (1977).

188 Ramirez, V.D.; Feder, H.H.; Sawyer, C.H.: The role of brain catecholamines in the regulation of LH secretion: A critical

inquiry. In: Frontiers in Neuroendocrinology (Vol 8), Martini, L.; Ganong, W.F., editors, pp. 27-84 (Raven Press, New York 1984).

189 Rasmussen, D.D.; Jacobs, W.; Kissinger, P.T.; Malven, P.V.: Plasma luteinizing hormone in ovariectomized rats following pharmacologic manipulation of endogenous brain serotonin. Brain Research 229:230-235 (1981).

190 Raymond, V.; Beaulieu, M.; Labrie, F.: Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. Science 200:1173-1175 (1978).

191 Resko, J.A.; Ellinwood, W.E.; Pasztor, L.M.; Buhl, A.E.: Sex steroids in the umbilical circulation of fetal rhesus monkeys from the time of gonadal differentiation. J. Clin. Endocrinol. Metab. 50:900-905 (1980).

192 Resko, J.A.; Ploem, J.G.; Stadelman, H.L.: Estrogens in fetal and maternal plasma of the rhesus monkey. Endocrinology 97:425-430 (1975).

193 Reynolds, L.R.; Rubel, A.M.; Nikitovitch-Winer, M.B.: Cimetidine inhibits the histamine induced prolactin release in male rats. Proc. Soc. Exp. Biol. Med. 163:322-325 (1980).

194 Riegle, G.D.; Meites, J.: The effect of stress on serum prolactin in the female rats. Proc. Soc. Exp. Biol. Med. 152:441-448 (1976).

195 Rodgers, C.H.; Schwartz, N.B.: Differentiation between neural and hormonal control of sexual behavior and gonadotropin

secretion in the female rat. Endocrinology 98:778-786 (1976).

196 Ronnekleiv, O.K.; Adelman, J.P.; Weber, E.; Herbert, E.; Kelly, M.J.: Immunohistochemical demonstration of proGnRH and GnRH in the preoptic-basal hypothalamus of the primate. Neuroendocrinology 45:518-521 (1987).

197 Ronnekleiv, O.K.; Kelly, M.J.: Luteinizing hormone-releasing hormone neuronal system during the estrous cycle of the female rat: effects of surgically induced persistent estrus. Neuroendocrinology 43:564-576 (1986).

198 Ronnekleiv, O.K.; Kelly, M.J.: Plasma prolactin and luteinizing hormone profiles during the estrous cycle of the female rat: Effects of surgically induced persistent estrus. Neuroendocrinology 47:133-141 (1988).

199 Ronnekleiv, O.K.; Naylor, B.R.; Bond, C.T.; Adelman, J.P.: GnRH, proGnRH and GnRH mRNA in rat brain. Molecular Endocrinology (1988).(In Press)

200 Ruberg, M.; Rotsztejn, W.H.; Arancibia, S.; Besson, J.; Enjalbert, A.: Stimulation of prolactin release by vasoactive intestinal peptide. Eur. J. Pharmacol. 51:319-320 (1978).

201 Rubinstein, L.; Sawyer, C.H.: Role of catecholamines in stimulating the release of pituitary ovulating hormone(s) in rats. Endocrinology 86:988-995 (1970).

202 Said, S.I.; Mutt, V.: Polypeptide with broad biological activity: isolation from small intestine. Science 169:1217-1218 (1970).

203 Samson, W.K.: Prolactin releasing activity of porcine intestinal peptide (PHI-27). Peptides 4:817-819 (1983).

204 Samson, W.K.; McCann, S.M.: Effects of suprachiasmatic nucleus lesions on hypothalamic LH-releasing hormone (LHRH) content and gonadotropin secretion in the ovariectomized (OVX) female rat. Brain Research Bulletin 4:783-788 (1979).

205 Samson, W.K.; McCann, S.M.: Effects of lesions in the organum vasculosum lamina terminalis on the hypothalamic distribution of luteinizing hormone-releasing hormone and gonadotropin secretion in the ovariectomized rat. Endocrinology 105:939-946 (1979).

206 Sarkar, D.K.; Chiappa, S.A.; Fink, G.; Sherwook, N.M.: Gonadotropin-releasing hormone surge in pro-oestrous rats. Nature 264:461-463 (1976).

207 Sarkar, D.K.; Fink, G.: Effects of gonadal steroids on output of luteinizing hormone releasing factor into pituitary stalk blood in the female rat. J. Endocr. 80:303-313 (1979).

208 Sarkar, D.K.; Fink, G.: Luteinizing hormone releasing factor in pituitary stalk plasma from long-term ovariectomized rats: effects of steroids. J. Endocr. 86:511-524 (1980).

209 Saunders, A.; Terry, L.C.; Audet, J.; Brazeau, P.; Martin, J.B.: Dynamic studies of growth hormone and prolactin secretion in the female rat. Neuroendocrinology 21:193-203 (1976).

210 Sawyer, C.H.: First Geoffrey Harris Memorial Lecture Some recent developments in brain-pituitary-ovarian physiology. Neuroendocrinology 17:97-124 (1975).

211 Schally, A.V.; Redding, T.W.; Arimura, A.; dupont, A.; Linthicum, G.L.: Isolation of gamma-amino butyric acid from pig hypothalami and demonstration of its prolactin release-inhibiting (PIF activity in vivo and in vitro. Endocrinology 100:681-691 (1977).

212 Schneider, H.P.G.; Crighton, D.B.; McCann, S.M.: Suprachiasmatic LH-releasing factor. Neuroendocrinology 5:271-280 (1969).

213 Schneider, H.P.G.; McCann, S.M.: Possible role of dopamine as transmitter to promote discharge of LH-releasing factor. Endocrinology 85:121-132 (1969).

214 Schwartz, N.B.: A model for the regulation of ovulation in the rat. Recent Prog. Horm. Res. 25:1-55 (1968).

215 Seeburg, P.H.; Adelman, J.P.: Characterization of cDNA for precursor of human luteinizing hormone releasing hormone. Nature 311:666-668 (1984).

216 Setalo, G.; Vigh, S.; Schally, A.V.; Arimura, A.; Flerko, B.: Immunohistological study of the origin of LHRH-containing nerve fibers of the rat hypothalamus. Brain Research 103:597-602 (1976).

217 Shaar, C.J.; Clemens, J.A.: The role of catecholamines in the release of anterior pituitary prolactin in vitro.

Endocrinology 95:1202-1212 (1974).

218 Shimatsu, A.: Peptide histidine isoleucine and vasoactive intestinal polypeptide-like immunoreactivity coexist in rat hypophysial portal blood. Neuroscience Letters 43:259-262 (1983).

219 Shimatsu, A.; Kato, Y.; Matsushita, N.; Katakami, H.; Ohta, H.; Yanaihara, N.; Imura, H.: Serotonin stimulates vasoactive intestinal polypeptide release from rat hypothalamus in vitro. Brain Research 264:148-151 (1983).

220 Shin, S.H.: Prolactin secretion in acute stree is controlled by prolactin releasing factor. Life Sciences 25:1829-1836 (1979).

221 Shivers, B.D.; Harlan, R.E.; Morrell, J.I.; Pfaff, D.W.: Absence of oestradiol concentration in cell nuclei of LHRH-immunoreactive neurones. Nature 304:345-347 (1983).

222 Shull, J.D.; Gorski, J.: Estrogen regulates the transcription of the rat prolactin gene in vivo through at least two independent mechanisms. Endocrinology 116:2456-2462 (1985).

223 Silverman, A.J.; Jhamandas, J.; Renaud, L.P.: Localization of luteinizing hormone-releasing hormone (LHRH) neurons that project to the median eminence. The Journal of Neuroscience 7:2312-2319 (1987).

224 Simerly, R.B.; Swanson, L.W.: The organization of neural inputs to the medial preoptic nucleus of the rat. The Journal of Comparative Neurology 246:312-342 (1986).

225 Simerly, R.B.; Swanson, L.W.: The distribution of neurotransmitter-specific cells and fibers in the anteroventral periventricular nucleus: implications for the control of gonadotropin secretion in the rat. Brain Research 400:11-34 (1987).

226 Simerly, R.B.; Swanson, L.W.; Gorski, R.A.: The distribution of monoaminergic cells and fibers in a periventricular nucleus involved in the control of gonadotropin release: immunohistochemical evidence for a dopaminergic sexual dimorphism. Brain Research 330:55-64 (1985).

227 Simerly, R.B.; Swanson, L.W.; Handa, R.J.; Gorski, R.A.: Influence of perinatal androgen on the sexually dimorphic distribution of tyrosine hydroxylase-immunoreactive cells and fibers in the anteroventral periventricular nucleus of the rat. Neuroendocrinology 40:501-510 (1985).

228 Simpkins, J.W.; Kalra, P.S.; Kalra, S.P.: Temporal alterations in luteinizing hormone-releasing hormone concentrations in several discrete brain regions: Effects of estrogen-progesterone and norepinephrine synthesis inhibition. Endocrinology 107:573-577 (1980).

229 Sims, K.B.; Hoffman, D.L.; Said, S.I.; Zimmerman, E.A.: Vasoactive intestinal polypeptide (VIP) in mouse and rat brain: an immunocytochemical study. Brain Research 186:165-183 (1980).

230 Smith, E.R.; Davidson, J.M.: Location of feedback receptors: effects of intracranially implanted steroids on plasma LH and LRF response. Endocrinology 95:1566-1573 (1974).

231 Smith, M.S.; Freeman, M.E.; Neill, J.D.: The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology 96:219-226 (1975).

232 Speigel, K.; Kourides, I.A.; Pasternack, G.W.: Porlactin and growth hormone release by morphine in the rat: different receptor mechanisms. Science 217:745-747 (1982).

233 Steinbusch, H.W.M.: Distribution of serotonin-immunoreactivity in the central nervous system of the rat - cell bodies and terminals. Neuroscience 6:557-618 (1981).

234 Stetson, M.H.: Nucleus suprachiasmaticus: the biological clock in the hamster?. Science 191:197-199 (1976).

235 Stone, R.T.; Maurer, R.A.; Gorski, J.: Effect of estradiol-17β on preprolactin messenger ribonucleic acid activity in the rat pituitary gland. Biochemistry 16:4915-4921 (1977).

236 Stumpf, W.E.: Estrogen-containing neurons and estrogen-neuron systems in the periventricular brain. Am. J. Anat. 129:207-214 (1970).

237 Stumpf, W.E.; Sar, M.; Keefer, D.A.: Atlas of estrogen target cells in the brain. In: Anatomical Neuroendocrinology, pp. 104-119 (Karger,Basel, 1975).

238 Takahara, J.; Arimura, A.; Schally, A.V.: Suppression of prolactin release by a purified porcine PIF preparation and actecholamines infused into a rat hypophysial portal vessel. Endocrinology 95:462-465 (1974).

239 Taleisnik, S.; McCann, S.M.: Effects of hypothalamic lesions on the secretion and storage of hypophysial luteinizing hormone. Endocrinology 68:263-272 (1961).

240 Tashjian, A.; Barowsky, N.; Jensen, D.: Thyrotropin releasing hormone: direct evidence for stimulation of prolactin production by pituitary cells in culture. Biochem. Biophys. Res. Commun. 43:516-523 (1971).

241 Tatemoto, K.; Carlquist, M.; Mutt, V.: Neuropeptide Y-a novel brain peptide with structural similarities to peptide Y and pancreatic polypeptide. Nature 296:659-660 (1982).

242 Terasawa, E.; Davis, G.A.: The LHRH neuronal system in female rats: relation to the medial preoptic nucleus. Endocrinol. Japon. 30:405-417 (1983).

243 Terasawa, E.; Wiegand, S.J.; Bridson, W.E.: A role for medial preoptic nucleus on afternoon of proestrus in female rats. Am. J. Physiol. 238:E533-E539 (1980).

244 Tindal, J.S.: Hypothalamic control of secretion and release of prolactin. J. Reprod. Fert. 39:437-461 (1974).

245 Valverde, C.; Chieffo, V.; Reichin, S.: Prolactin releasing factor in porcine and rat hypothalamic tissue. Endocrinology 91:982-993 (1972).

246 Van Den Pol, A.N.; Gorcs, T.: Synaptic relationships between neurons containing vasopressin, gastrin-releasing peptide, vasoactive intestinal polypeptide, and glutamate decarboxylase immunoreactivity in the suprachiasmatic nucleus: dual ultrastructural immunocytochemistry with gold-substituted silver peroxidase. The Journal of Comparative Neurology 252:507-521 (1986).

247 Van Den Pol, A.N.; Tsujimoto, K.L.: Neurotransmitters of the hypothalamic suprachiasmatic nucleus: immunocytochemical analysis of 25 neuronal antigens. Neuroscience 15:1049-1086 (1985).

248 Van Vugt, D.A.; Bruni, J.F.; Meites, J.: Naloxone inhibition of stress-induced increase in prolactin secretion. Life Sciences 22:85-90 (1977).

249 Velchez-Martinez, J.A.; Arimura, A.; Debeljuk, L.; Schally, A.V.: Biphasic effect of estradiol benzoate on the pituitary responsiveness to LH-RH. Endocrinology 94:1300-1303 (1974).

250 Vijayan, E.; McCann, S.M.: Re-evaluation of the role of catecholamines in control of gonadotropin and prolactin release. Neuroendocrinology 25:150-165 (1978).

251 Vijayan, E.; Samson, W.K.; Said, S.I.; McCann, S.M.: Vasoactive intestinal peptide (VIP): evidence for a hypothalamic site of action to release growth hormone, luteinizing hormone and prolactin in conscious ovariectomized rats. Endocrinology 104:53-57 (1979).

252 Walker, R.F.: Quantitative and temporal aspects of serotonin's facilitatory action on phasic secretion of luteinizing hormone in female rats. Neuroendocrinology 36:468-474 (1983).

253 Wardlaw, S.L.; Weherenberg, W.B.; Ferin, M.; Antunes, J.L.; Frantz, A.G.: Effect of sex steroids on β-endorphin in hypophyseal portal blood. J. Clin. Endocrinol. Metab. 55:877-881 (1982).

254 Weiner, R.I.; Patton, E.; Kerdelhue, B.; Kordon, C.: Differential effects of hypothalamic deafferentation upon luteinizing hormone-releasing hormone in the median eminence and organum vasculosum of the lamina terminalis. Endocrinology 7:1597-1600 (1975).

255 Wiegand, S.J.; Morrell, J.I.: The distribution of estradiol-concentrating neurons in relation to cytologically defined subdivisions of the preoptic area in the female rat. Biology of Reproduction (abstract) 38 (supplement):100 (1988).

256 Wiegand, S.J.; Terasawa, E.: Discrete lesions reveal functional heterogeneity of suprachiasmatic structures in regulation of gonadotropin secretion in the female rat. Neuroendocrinology 34:395-404 (1982).

257 Wiegand, S.J.; Terasawa, E.; Bridson, W.E.: Persistent estrus and blockade of progesterone-induced LH release follows lesions which do not damage the suprachiasmatic nucleus. Endocrinology 102:1645-1648 (1978).

258 Wiegand, S.J.; Terasawa, E.; Bridson, W.E.; Goy, R.W.: Effects of discrete lesions of preoptic and suprachiasmatic structures in the female rat Alterations in the feedback regulation of gonadotropin secretion. Neuroendocrinology 31:147-157 (1980).

259 Wiegand, S.J.; Watson, R.E.; Hoffman, G.E.; Tang, L.K.: Differential effects of lesions of the LHRH system or the medial preoptic nucleus (MPN) on phasic gonadotropin release in the rat. Biology of Reproduction 32:110-(abstract) (1985).

260 Wiersma, J.: A detailed characterization of prolactin secretion patterns during daylight in individual cycling and pseudopregnant rats. Neuroendocrinology 33:288-294 (1981).

261 Willoughby, J.O.; Menadue, M.F.; Liebelt, H.J.: Activation of 5-HT serotonin receptors in the medial basal hypothalamus stimulates prolactin secretion in the unanesthetized rat. Neuroendocrinology 47:83-87 (1988).

262 Winer, B.J.: Statistical principles in experimental design. McGraw-Hill, New York 1962).

263 Wise, P.M.; Rance, N.; Selmanoff, M.; Barraclough, C.A.: Changes in radioimmunoassayable luteinizing hormone-releasing hormone in discrete brain areas of the rat at various times on proestrus, diestrous day 1, and after phenobarbital administration. Endocrinology 108:2179-2185 (1981).

264 Wood, P.L.: Multiple opiate receptors: support for unique mu,delta and kappa sites. Neuropharmacology 21:487-497 (1982).

265 Zeilmaker, G.H.: The biphasic effect of progesterone on ovulation in the rat. Acta Endocrinologica 51:461-468 (1966).

266 Zoeller, R.T.; Seeburg, P.H.; Young, W.S.: In situ hybridization histochemistry for messenger ribonucleic acid encoding gonadotropin-releasing hormone: effect of estrogen on cellular levels of GnRH mRNA in female rat brain. Endocrinology (1988).(In Press)

Figure 1. A flow diagram of experimental protocol. For further elucidation, see material and methods.

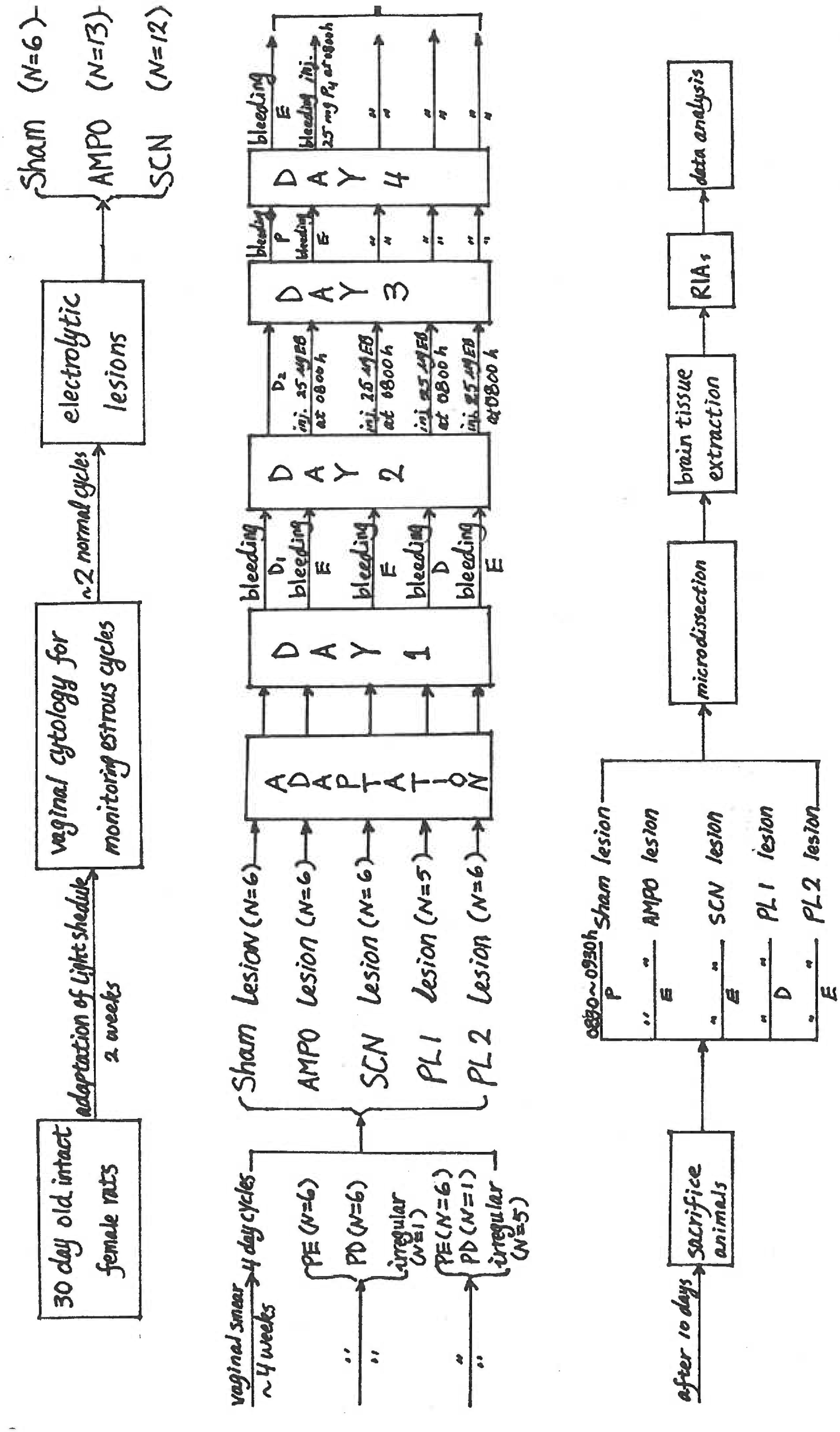
30 day old intact female rats
adaptation of light shedule 2 weeks
vaginal cytology for monitoring estrous cycles
~2 normal cycles
electrolytic lesions
Sham (N=6)
AMPO (N=13)
SCN (N=12)
vaginal smear ~4 weeks
4 day cycles
PE (N=6)
PD (N=6)
irregular (N=1)
PE (N=6)
PD (N=1)
irregular (N=5)
Sham
AMPO
SCN
PL 1
PL 2
Sham Lesion (N=6)
AMPO Lesion (N=6)
SCN Lesion (N=6)
PL 1 Lesion (N=5)
PL 2 Lesion (N=6)
ADAPTATION
DAY 1
bleeding
D₁
E
D
DAY 2
D₂
inj. 25 µg EB at 0800 h
DAY 3
P
DAY 4
25 mg P₄ at 0800 h
after 10 days
sacrifice animels
0830~0930h
Sham lesion
AMPO lesion
SCN lesion
PL 1 lesion
PL 2 lesion
microdissection
brain tissue extraction
RIAs
data analysis

Figure 2. Schematic drawing of sagittal view of rat brain illustrating the location of the AMPO and the SCN. The dotted area indicate the extent of a complete AMPO or SCN lesion.

AMPO = anterior medial preoptic nucleus
AC = anterior commissure
AH = anterior hypothalamus
AR = arcuate nucleus
DB = diagonal band
F = fornix
MPA = medial preoptic area
OC = optic chiasm
RCH = retrochiasmatic area
SCN = suprachiasmatic nucleus
VMH = ventromedial nucleus

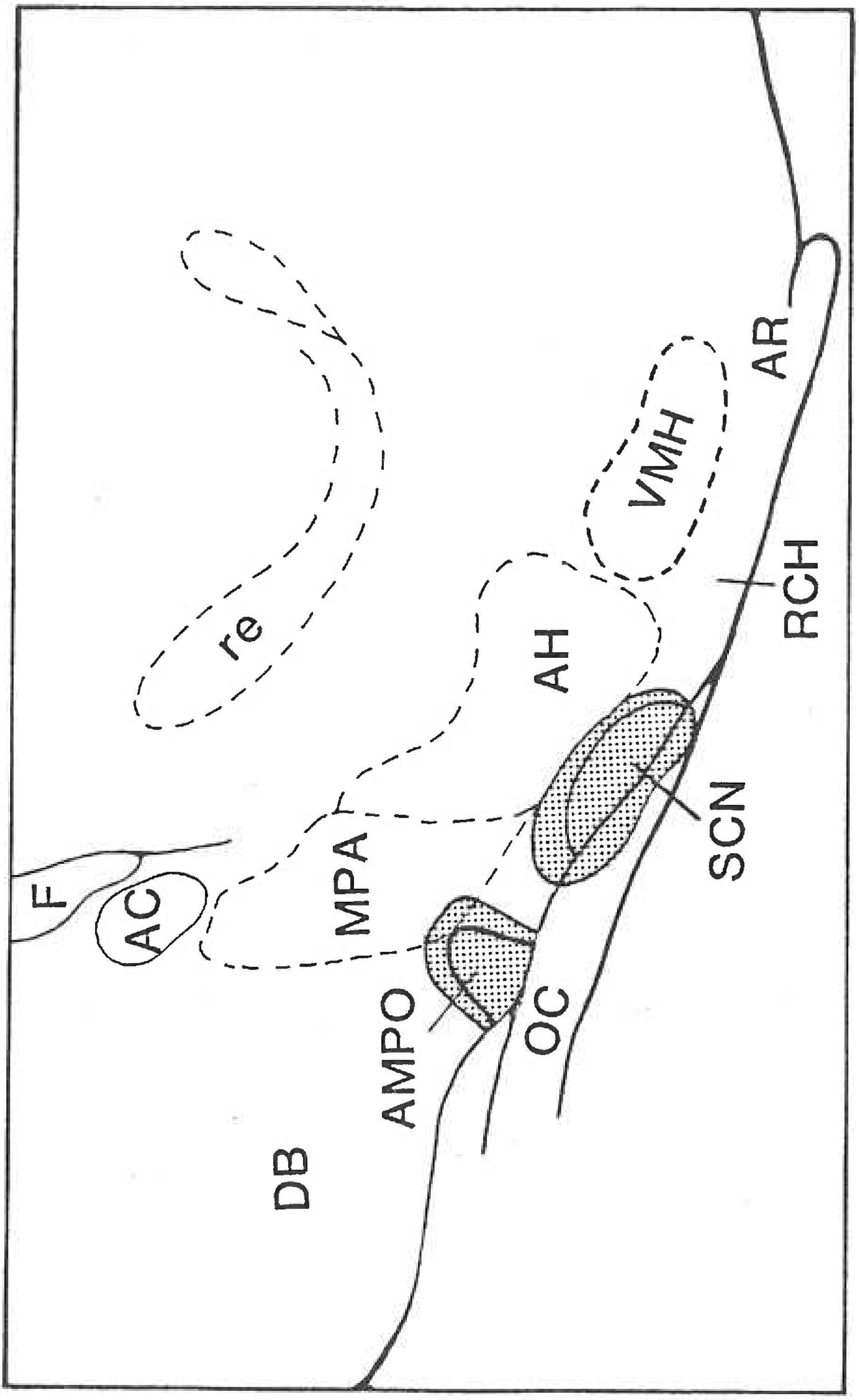
AMPO/SCN Lesions

Figure 3. Photograph of coronal section through the AMPO area illustrating the location of immunoreactive GnRH neurons surrounding the AMPO. The outline of a typical lesion which induced PE, is indicated.

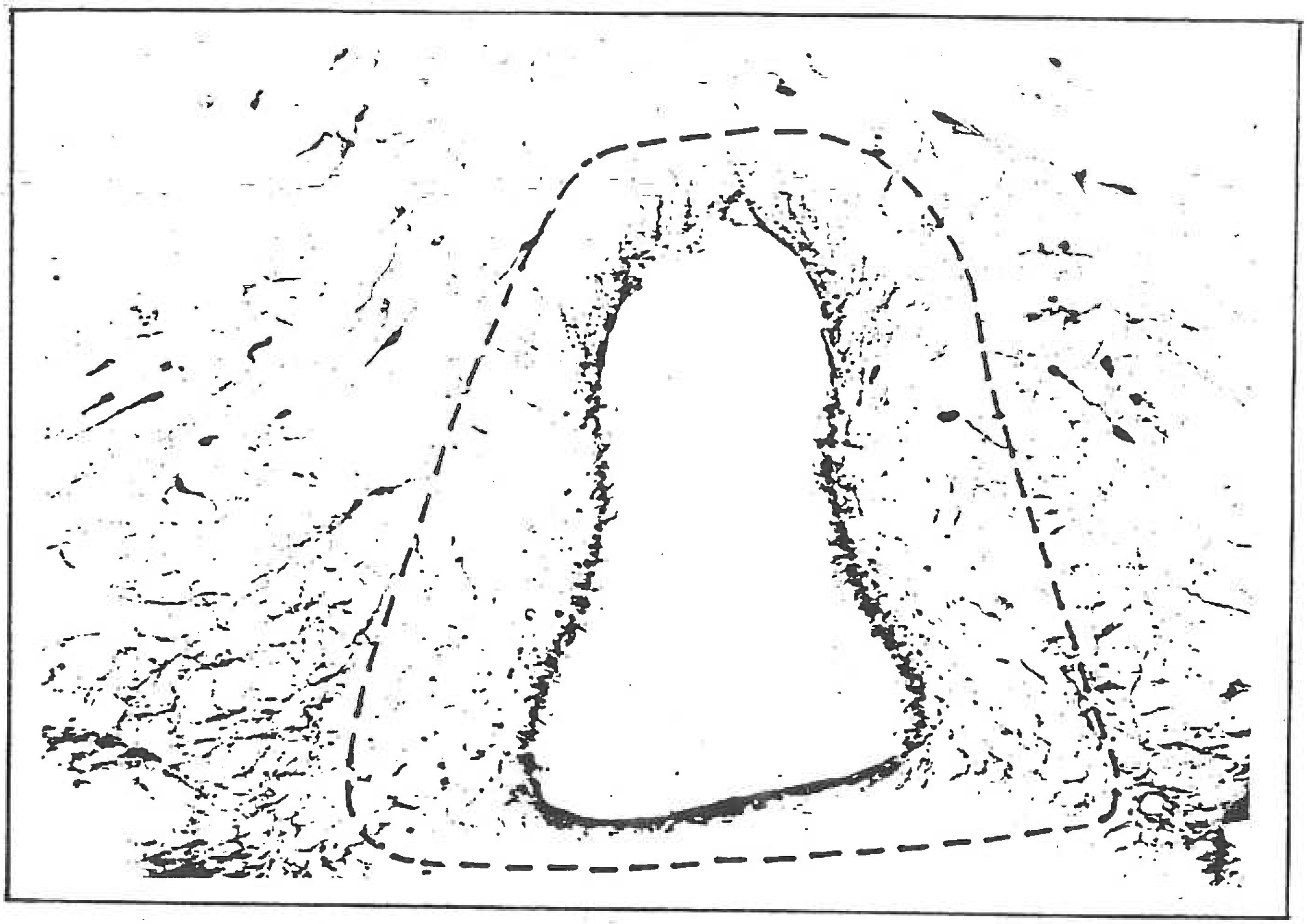

Figure 4. Photograph of coronal section through the SCN area illustrating the location of vasoactive intestinal peptide within the SCN. The outline of a typical lesion which induced PE, is indicated.

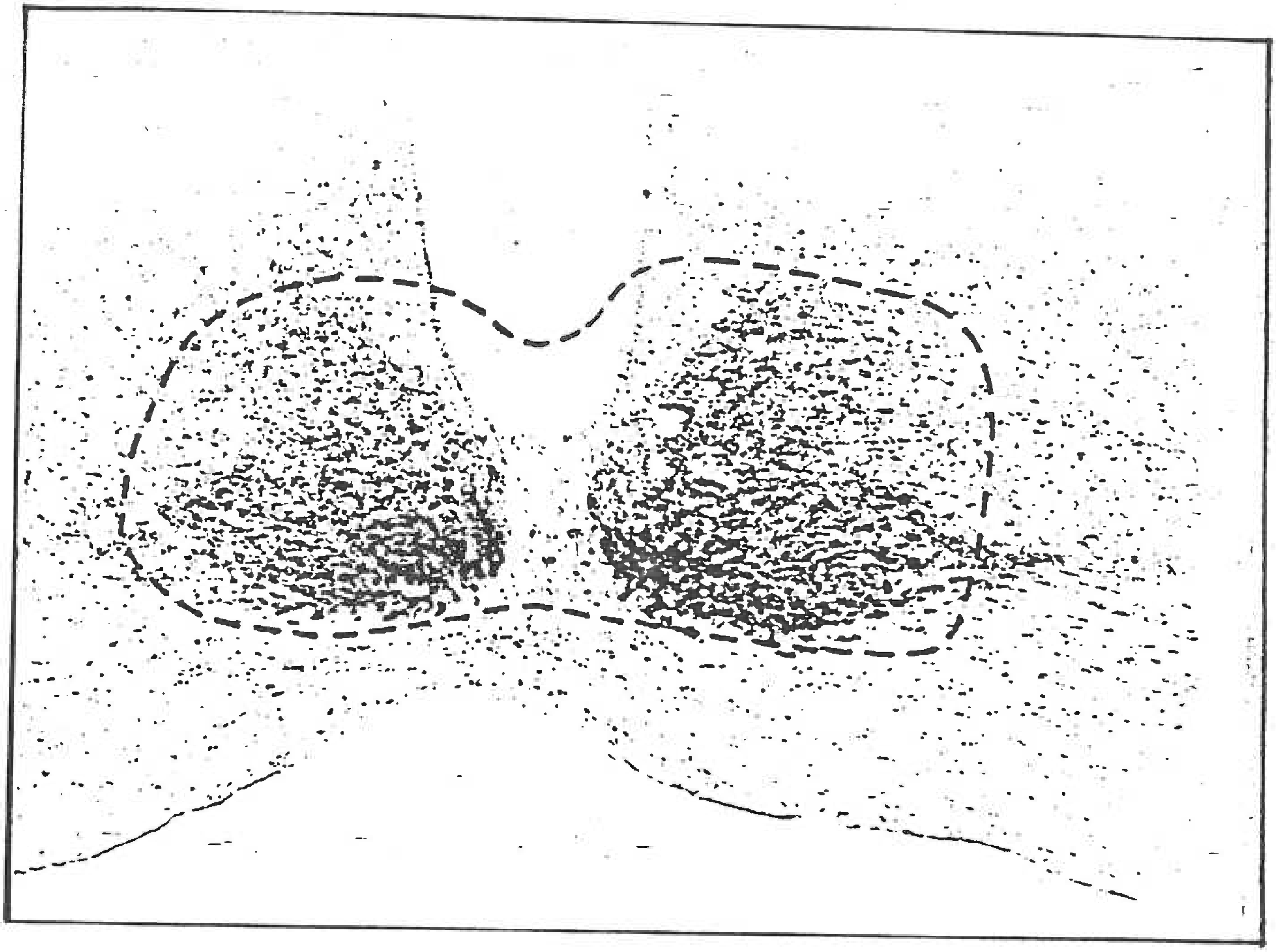

Figure 5. Schematic drawing of sagittal view of rat brain illustrating the location and extent of partial lesions. The PL1 lesion (dotted area) includes the dorsal part of the AMPO. The PL2 lesions (hatched area) include ventral parts of the AMPO or the SCN.

Partial Lesions

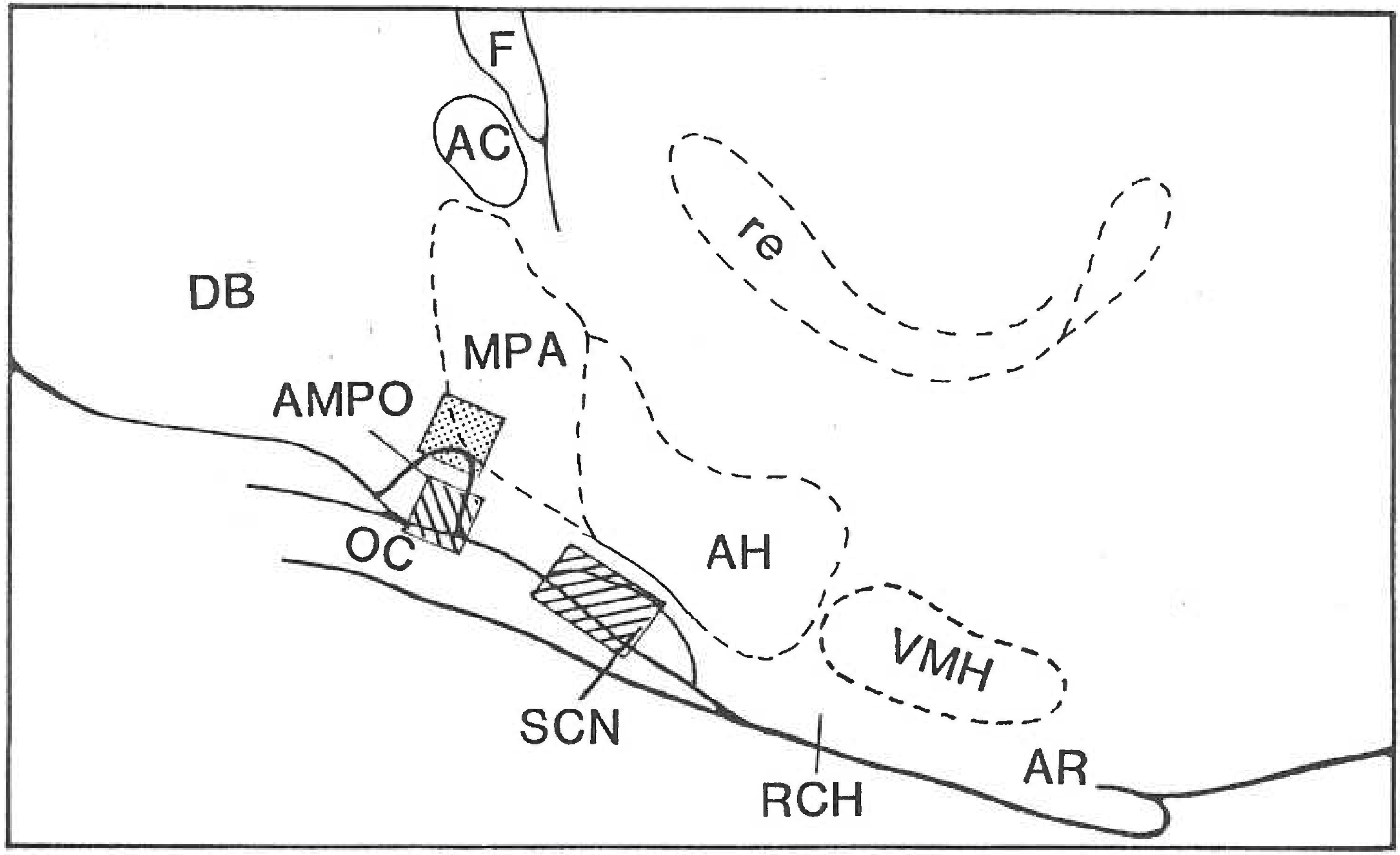

Figure 6. Vaginal smear patterns in each representative rat. Numbers below the bottom ordinate are the days after the lesions. D refers to diestrus, P refers to proestrus and E refers to estrus.

VAGINAL SMEAR PATTERNS

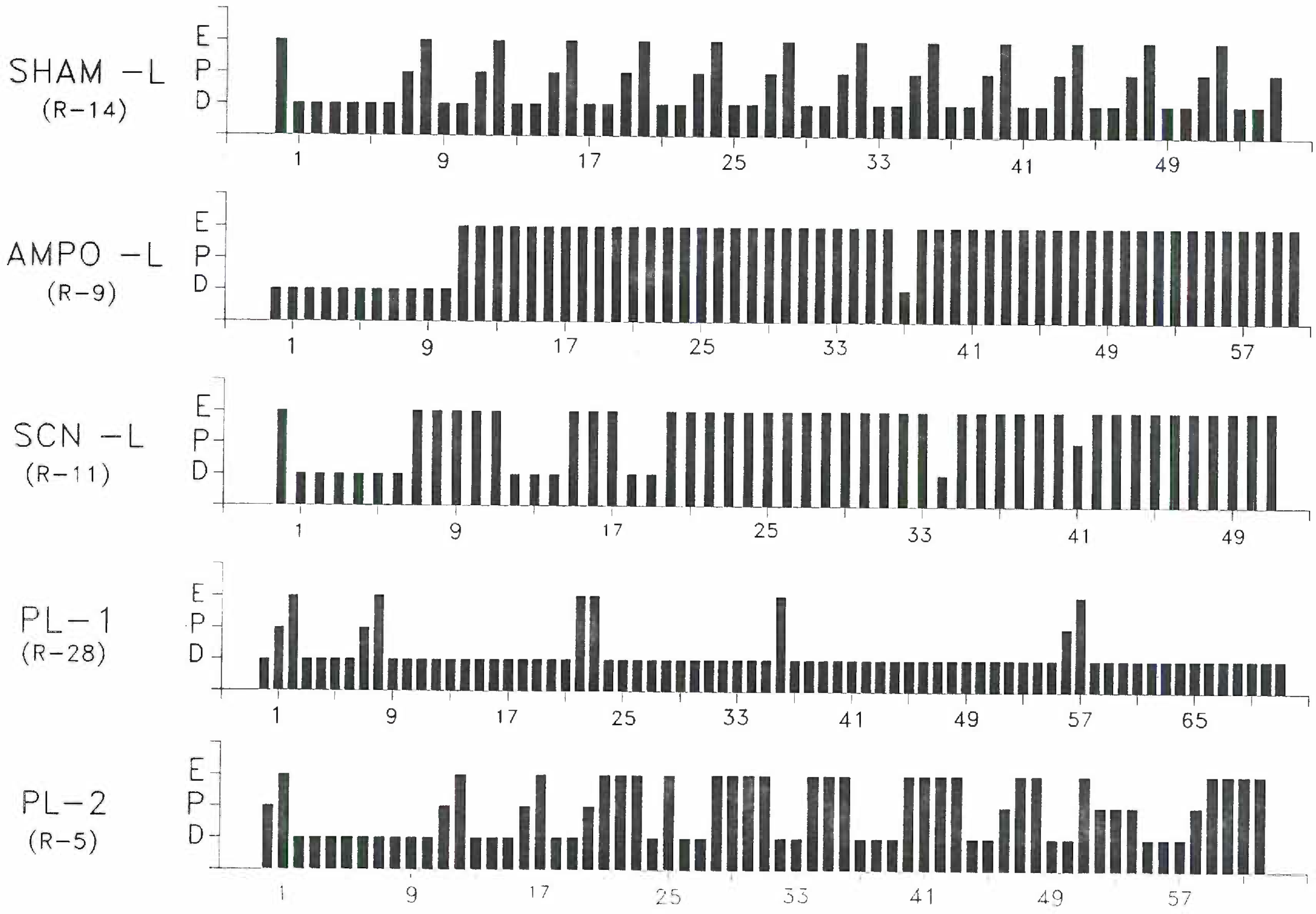

Figure 7. Mean levels of plasma PRL (top panel) and LH (bottom panel) during diestrus, proestrus and estrus of sham lesioned rats (n=6). Each point on the graph is the mean ± SE. The clock time is given along the abscissa. The black bar indicate part of the dark period of the 12:12 h light/dark regimen. Note that noon colony time is at 0830 h clock time.

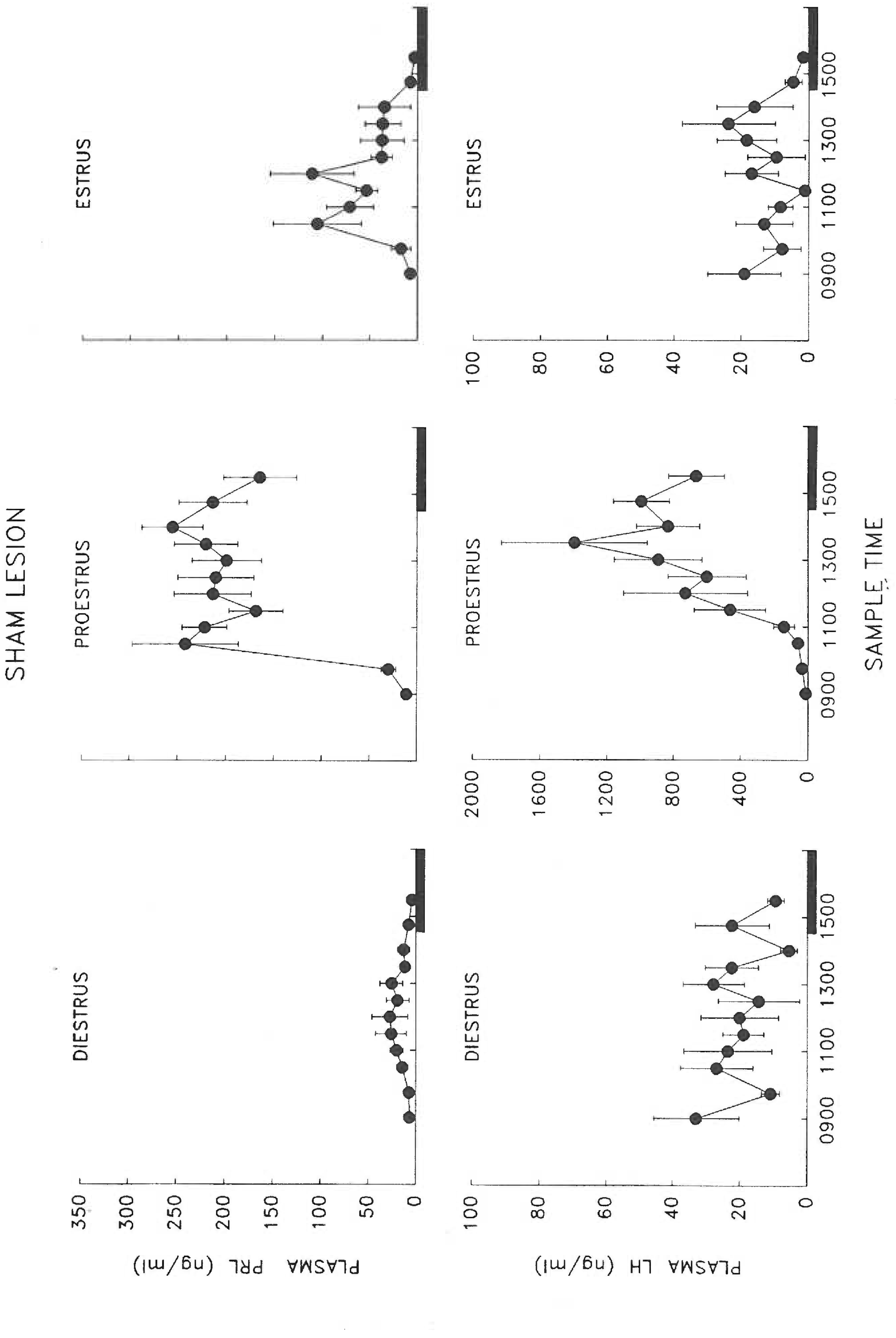
SHAM LESION
DIESTRUS
PROESTRUS
ESTRUS
PLASMA PRL (ng/ml)
350
300
250
200
150
100
50
0
PLASMA LH (ng/ml)
100
80
60
40
20
0
2000
1600
1200
800
400
0
0900
1100
1300
1500
SAMPLE TIME

Figure 8. Mean levels of plasma PRL (top panel) and LH (bottom panel) in AMPO lesioned rats (n=6). The left panel shows basal levels during persistent estrus. The middle panel illustrates the effects of EB injection. The right panel shows the effects of progesterone injection at 0800 h. See the legend to figure 6 for further text.

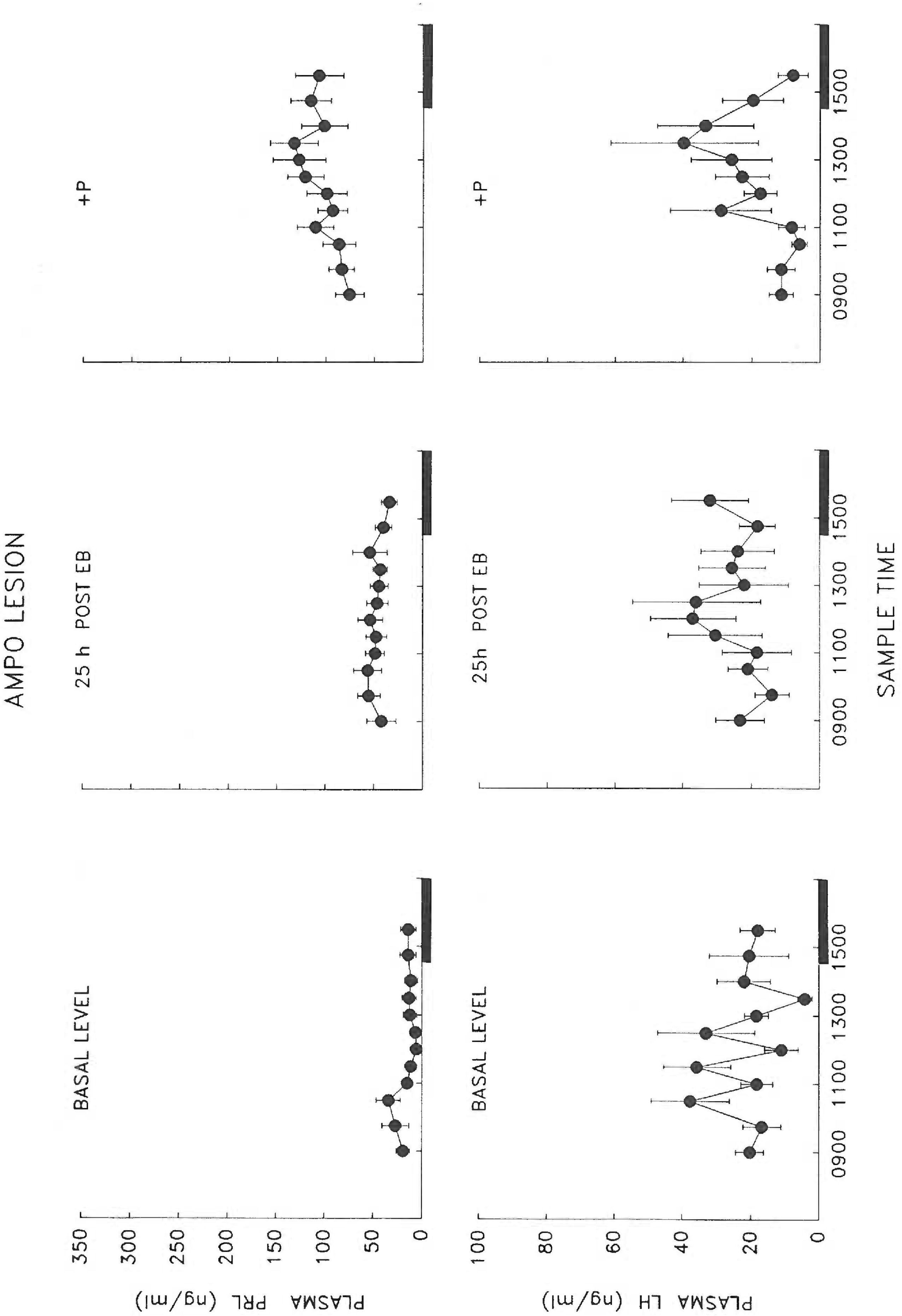
AMPO LESION
BASAL LEVEL
25 h POST EB
+P
BASAL LEVEL
25h POST EB
+P
PLASMA PRL (ng/ml)
350
300
250
200
150
100
50
0
PLASMA LH (ng/ml)
100
80
60
40
20
0
0900
1100
1300
1500
SAMPLE TIME

Figure 9. Mean levels of plasma PRL (top panel) and LH (bottom panel) in SCN lesioned PE rats (n=6). The left panel shows basal levels during persistent estrus. The middle panel illustrates the effects of EB injection. The right panel shows the effects of progesterone injection at 0800 h. See the legend to figure 6 for further text.

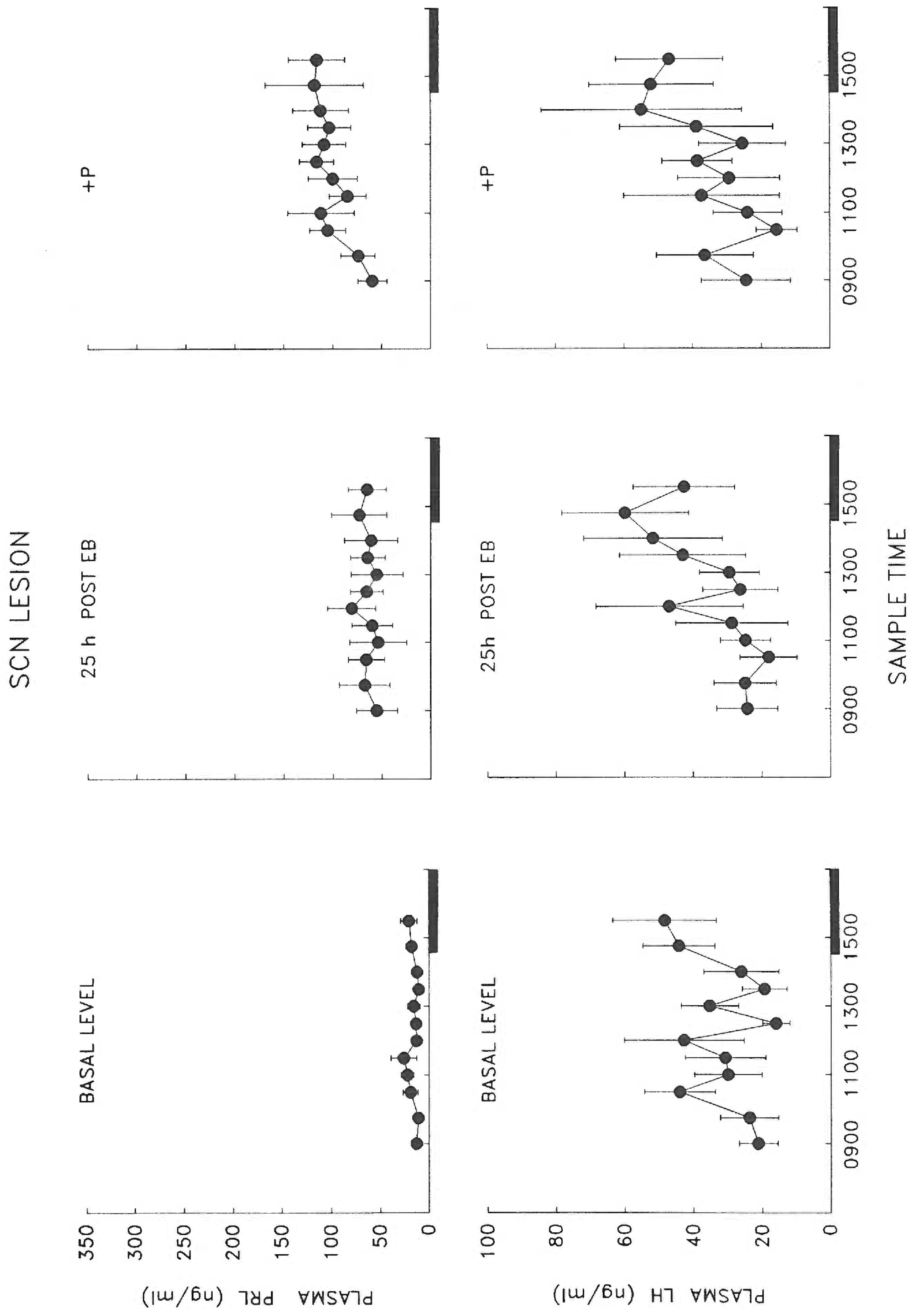
SCN LESION
BASAL LEVEL
25 h POST EB
+P
BASAL LEVEL
25h POST EB
+P
PLASMA PRL (ng/ml)
350
300
250
200
150
100
50
0
PLASMA LH (ng/ml)
100
80
60
40
20
0
0900
1100
1300
1500
SAMPLE TIME

Figure 10. Mean levels of plasma PRL (top panel) and LH (bottom panel) in LP1 PD rats(n=5). The left panel shows basal levels during persistent diestrus. The middle panel illustrates the effects of EB injection. The right panel shows the effects of progesterone injection at 0800 h. See the legend to figure 6 for further text.

PARTIAL LESION (PL1)

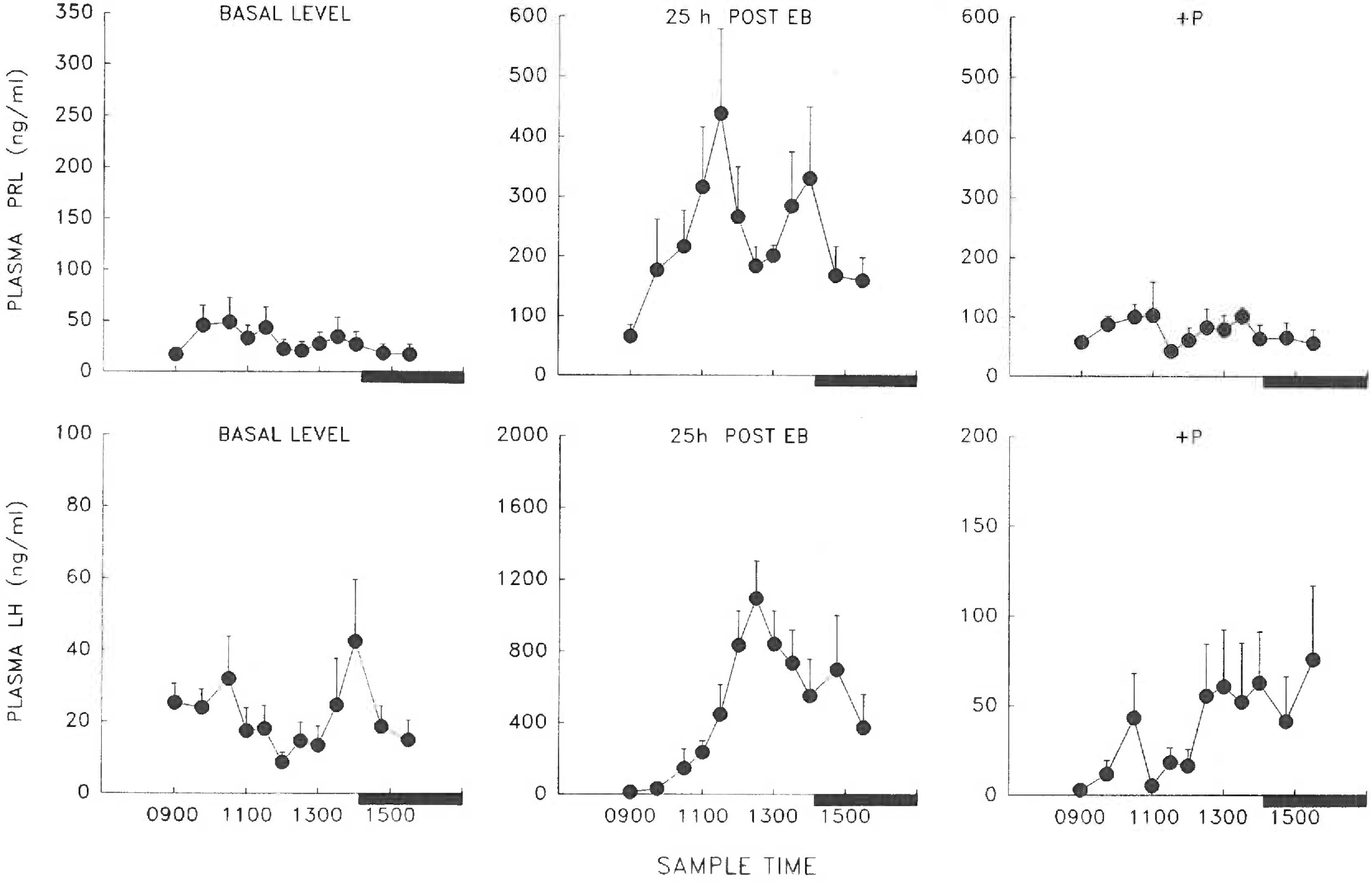

Figure 11. Mean levels of plasma PRL (top panel) and LH (bottom panel) in LP2 irregular cycling rats(n=6). The left panel shows basal levels during estrus. The middle panel illustrates the effects of EB injection. The right panel shows the effects of progesterone injection at 0800 h. See the legend to figure 6 for further text.

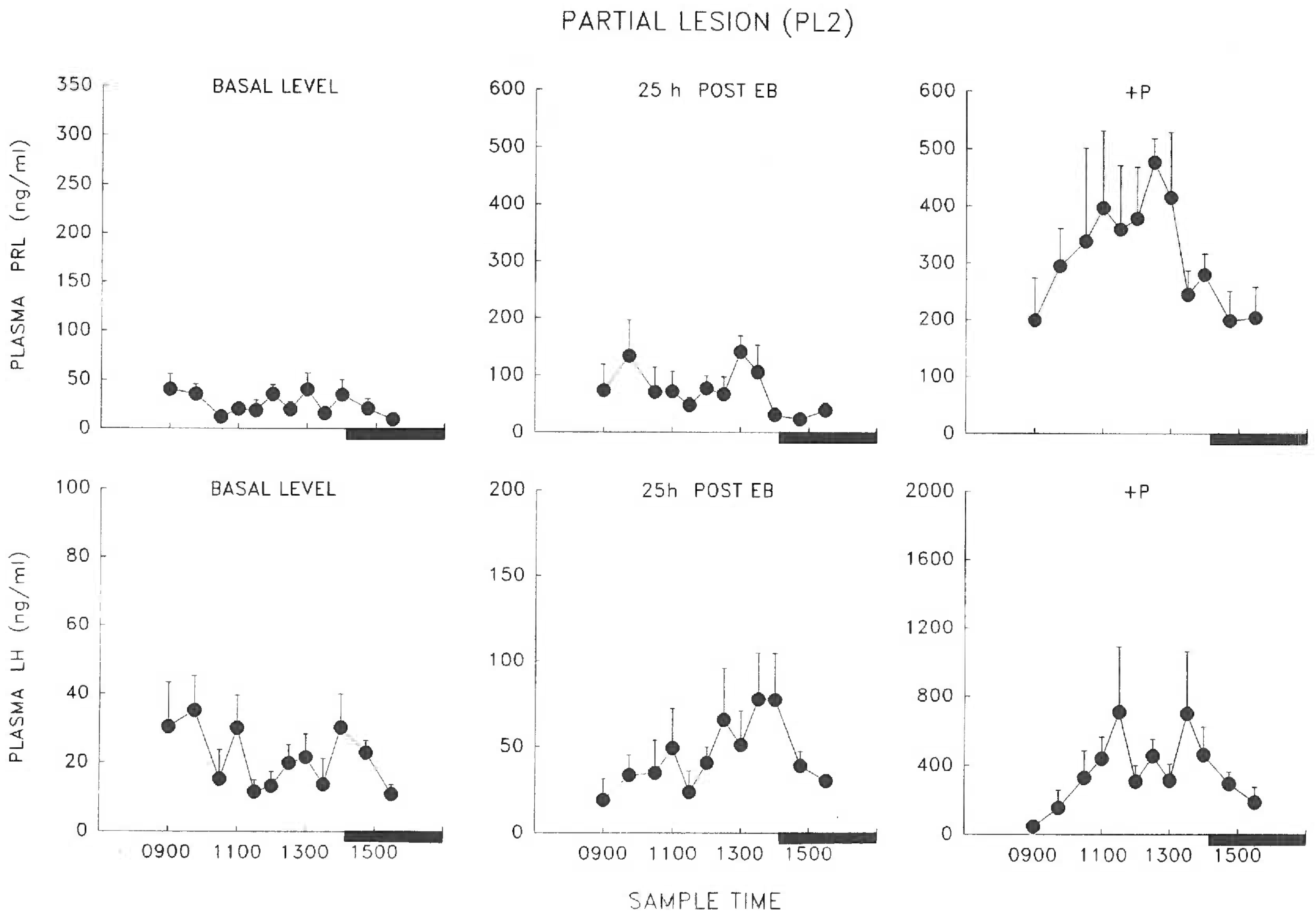
PARTIAL LESION (PL2)
BASAL LEVEL
25 h POST EB
+P
PLASMA PRL (ng/ml)
350
300
250
200
150
100
50
0
600
500
400
300
200
100
0
BASAL LEVEL
25h POST EB
+P
PLASMA LH (ng/ml)
100
80
60
40
20
0
200
150
100
50
0
2000
1600
1200
800
400
0
0900
1100
1300
1500
SAMPLE TIME

Figure 12. proGnRH levels (mean ± SE) of the POA and the BH in Sham (n=6), AMPO (n=6), SCN (n=6), PL1 (n=5) and PL2 (n=6) lesioned rats sacrificed between 0830 to 0930 h. POA = preoptic area. BH = basal hypothalamus.

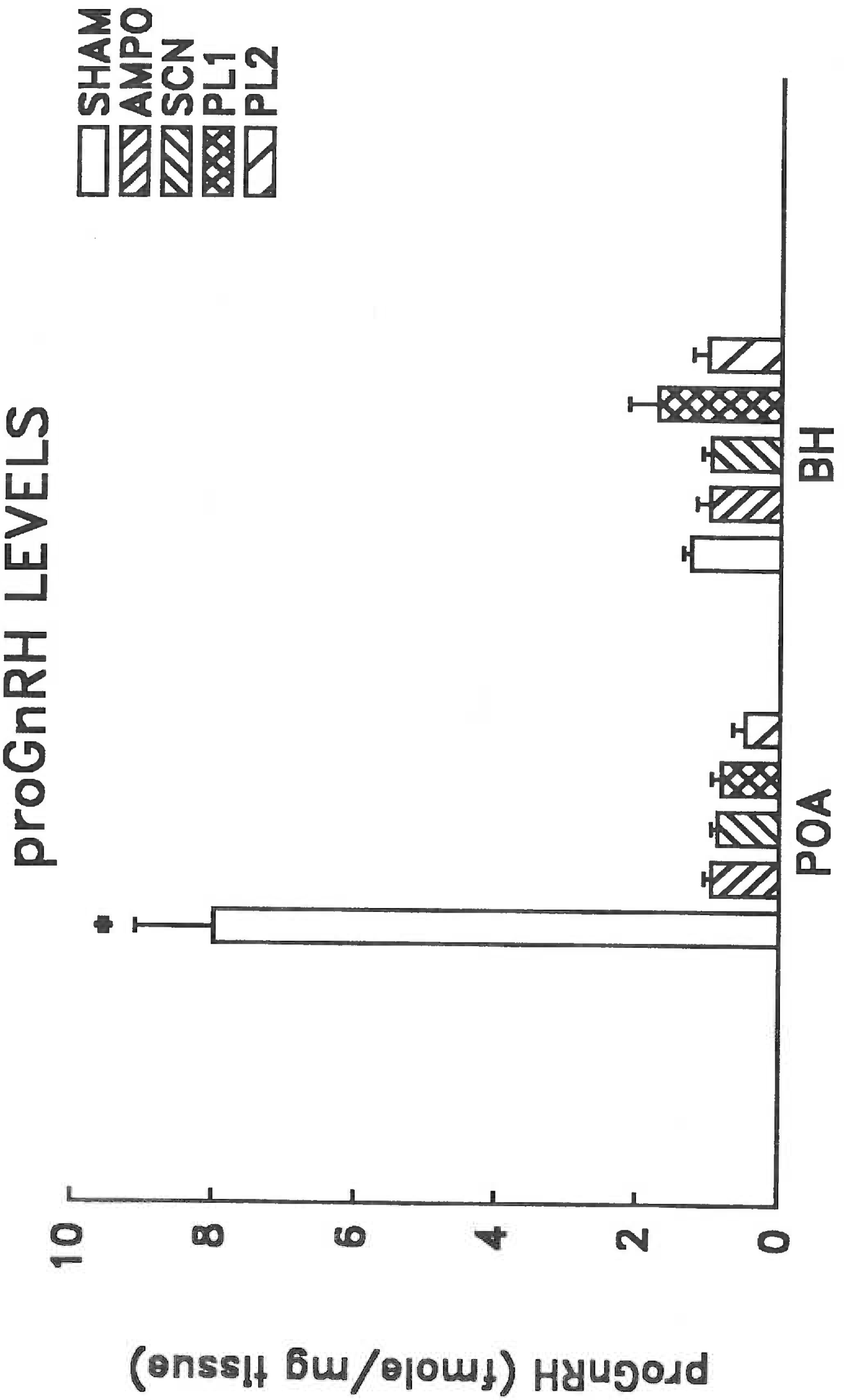
proGnRH LEVELS
SHAM
AMPO
SCN
PL1
PL2
proGnRH (fmole/mg tissue)
10
8
6
4
2
0
*
POA
BH

Figure 13. GnRH levels (mean ± SE) of the POA and the BH in Sham (n=6), AMPO (n=6), SCN (n=6), PL1 (n=5) and PL2 (n=6) lesioned rats sacrificed between 0830 to 0930 h. POA = preoptic area. BH = basal hypothalamus.

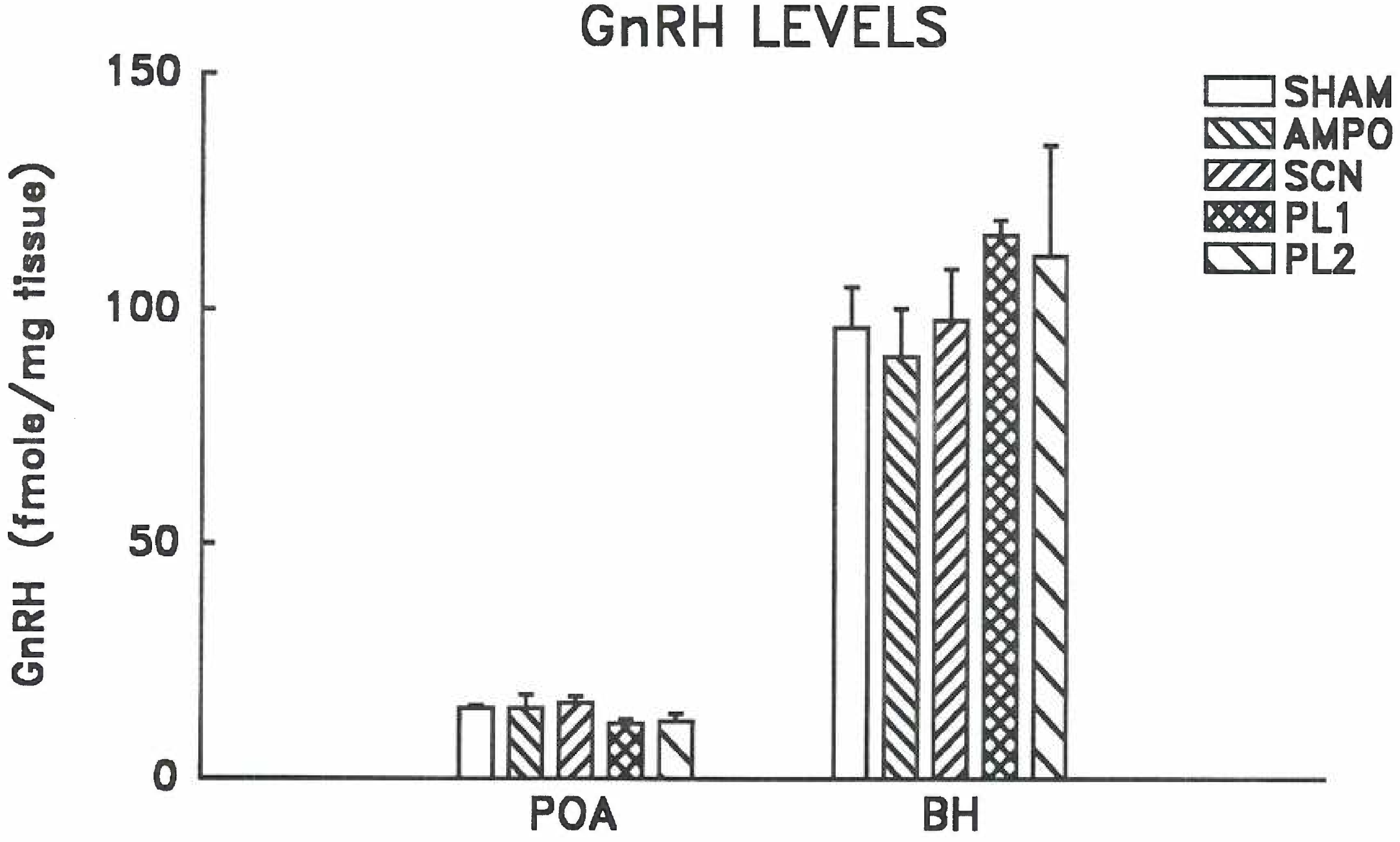
GnRH LEVELS
GnRH (fmole/mg tissue)
150
100
50
0
POA
BH
SHAM
AMPO
SCN
PL1
PL2

Table 1. Effect of lesions on plasma estradiol and progesterone concentrations in sham operated proestrus; AMPO and SCN lesioned PE rats; PL1 PD rats and PL2 irregular cycling rats. Asterisks indicate significant differences between lesioned group and sham operated controls. * = $P<0.05$, ** = $P<0.01$.

TABLE 1 STEROIDS LEVELS

	ESTRADIOL(PG/ML)	PROGESTERONE(NG/ML)	N
SHAM	25.0 ± 6.5	20.4 ± 2.8	6
AMPO	21.3 ± 2.4	5.6 ± 1.6**	6
SCN	27.8 ± 5.0	5.1 ± 1.4**	6
PL1	14.5 ± 2.9	37.4 ± 3.5*	5
PL2	37.6 ± 2.6	6.2 ± 1.4**	6

Table 2. Levels of plasma estradiol and progesterone 25 h after administration of estradiol benzoate (25 μg, SC) in AMPO, SCN, PL1 and PL2 rats.

TABLE 2 STEROIDS LEVELS

	ESTRADIOL (PG/ML)	PROGESTERONE (NG/ML)	N
AMPO	186 ± 13	5.5 ± 1.3	6
SCN	226 ± 21	6.3 ± 1.9	6
PL1	183 ± 38	31.6 ± 12.3	5
PL2	251 ± 46	6.4 ± 0.5	6

Table 3. Levels of plasma estradiol and progesterone 50 h after administration of estradiol benzoate (25 μg, SC) followed 1 h after administration of progesterone (2.5 mg, SC) in AMPO, SCN, PL1 and PL2 rats.

TABLE 3 STEROIDS LEVELS

	ESTRADIOL(PG/ML)	PROGESTERONE(NG/ML)	N
AMPO	87.9 ± 14.2	96.4 ± 12.6	6
SCN	70.7 ± 8.7	77.3 ± 8.4	6
PL1	83 ± 11	73.2 ± 8.9	5
PL2	81 ± 8.5	86.8 ± 12.9	6