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Effect of Intermittent Versus Continuous Infusion of Progesterone on LHRH Release *In Vivo* from the Rat Mediobasal Hypothalamus

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Present study examined the effect of intermittent versus continuous infusion of progesterone (P) on LHRH release *in vivo* from the mediobasal hypothalamus of ovariectomized, estradiol-primed adult rats bearing push-pull cannulae. Three experimental groups were studied: 1) control (perfusion medium only), 2) intermittent perfusion of P (10-min on, 20-min off), and 3) continuous perfusion of P. P (10 ng/ml) was directly infused into the MBH following a 3 hr basal collection. Perfusates were collected at 10 min intervals on ice and LHRH release was measured by LHRH radioimmunoassay. Cycle detector analysis revealed that the spontaneous LHRH output in the control group was pulsatile over a 7 hr push-pull perfusion period. The mean basal LHRH release, pulse amplitude and pulse period were 0.68 ± 0.03 pg/10 min, 1.15 ±0.08 pg and 60 ± 9 min, respectively. Intermittent perfusion of P clearly stimulated the mean LHRH release (pre-P vs post-P: 1.14 ± 0.18 vs 1.99 ± 0.53 pg) without changes in LHRH pulse frequency. In contrast to intermittent infusion of P, continuous administration of P failed to modify LHRH release, since the mean LHRH release and pulse amplitude between pre-P and post-P perfusion were similar.

The *in vivo* study clearly showed that intermittent, but not continuous administration of P is effective in stimulating LHRH release. Therefore, it appears that rhythmic secretion of P may be the effective signal for activating the neural LHRH apparatus.

KEY WORDS: LHRH release *in vivo*, Mediobasal hypothalamus, Progesterone, Push-pull perfusion

It is generally believed that rhythmic mode of hormone secretion is of fundamental importance in endocrine communication as shown in a variety of experimental models (Brinkley, 1981; Yates, 1981). In the hypothalamic-pituitary-ovarian axis, the phenomenon of desensitization (or refractoriness) after continuous stimulation by hormonal signal has been well documented either in the pituitary to LHRH (luteinizing hormone releasing hormone) (Belchetz *et al.*, 1978) or in the ovary to LH (luteinizing hormone) (Conti *et al.*, 1976). However, whether the hypothalamus also exhibits a similar response to ovarian steroid signal has not yet been elucidated.

Previously, we found that intermittent, but not continuous administration of *in vitro* progesterone (P) is effective in stimulating LHRH release from hypothalamic fragments superfused *in vitro* (Kim and Ramirez, 1982; 1985). Furthermore, the activation of the neural LHRH apparatus induced

by intermittent P pulses was followed by a refractory period during which this steroid was no longer effective. The cause of this particular response of the LHRH neural apparatus to intermittent P stimulation is unknown and remains to be elucidated. However, it seems likely that depending on the model of administration of P, the neural LHRH apparatus can be either activated or inhibited (Ramirez *et al.*, 1984). To explore the importance of the signal of P on LHRH release *in vivo*, two modes of P administration, either intermittent or continuous, were examined in ovariectomized, estradiol-primed adult rats bearing push-pull canulae.

Materials and Methods

Animals

Female Holtzman rats (200-300 g of B.W.) were individually caged in a temperature-controlled room with lights on from 0500 to 1900 hr. Water and food were available *ad libitum*. Each animal was bilaterally ovariectomized under light ether anesthesia.

Implantation of push-pull cannulae

About 7 days after ovariectomy, animals were implanted with push-pull cannulae (PPC). Details of PPC construction and implantation have been previously described (Levine and Ramirez, 1980; 1982). Briefly, following anesthetization with 10 mg/Kg of ketamine-acepromazine (10:1 ratio), a stainless outer cannulae constructed with 22 g syringe needle with a length of 10 mm, fitted with a removable stylette constructed with 28 g tubing which extended 1 mm beyond the outer cannulae was stereotaxically implanted into the MBH. Implantation coordinates consisted of 0.5 mm lateral to the superior sagittal sinus, 0.5 mm posterior to bregma, and ventrally to a depth of 9.8 mm from dura (de Groot, 1959). The cannula was secured in place with dental cement above both the cannulae hub and a set of three stainless steel screws fastened into the skull. Animals were allowed 1 week for surgical recovery. Two days prior to PPC, Silastic capsules filling with 235 μg/ml of 17-β estradiol (Ramirez *et al.*, 1980; Kim and Ramirez, 1982) were implanted into the neck region of OVX animals bearing PPC.

Push-pull perfusion experiment

On the day of perfusion, animal was lightly anesthetized with ether, the inner stylette was removed and an inner cannulae assembly was inserted into the outer cannulae, with its tip only extending 0.5 mm beyond the outer cannulae. The perfusion assembly consited of 31 g inflow (push) and 28 g outflow (pull) tubings connected via polyethylene tubing (PE-20, Becton Dickinson and Co., Parsippany, NJ) to two identically equillibrated pumps (Gilson Medical Electronics Inc., Middleton, WI). An artificial cerebrospinal fluid (CSF) medium consisted of 123 mM NaCl, 4.8 mM KCl, 0.78 mM $CaCl_2$ and 1.2 mM sodium phosphate, pH 7.4 was infused through push tubing and collected from the pull cannulae at a flow rate of 18-20 μl/min (Levine and Ramirez, 1980; 1982). Push-pull perfusion (PPP) was typically conducted for 7 hr from 1000-1700 hr. Ten-min samples were collected continuously on ice and acidified to pH 1.5-2.0 with 1 N HCl and stored at -20°C until LHRH assay.

Three experimental groups were studied: 1) control perfusion with CSF medium only, 2) intermittent perfusion of P (10 min on, 20 min off), and 3) continuous perfusion of P. Progesterone (10 ng/ml) was locally infused into the MBH following a 3 hr basal period. Each experiment was repeated by five times.

Radioimmunoassay (RIA) of LHRH

LHRH concentration in perfusates was measured by RIA procedure previously described (Kim and Ramirez, 1982: 1985). Chen-Ramirez LHRH antiserum (CRR-11-B-72) was used at a final dilution of 1:250,000. Synthetic LHRH was used for iodination and served as the reference standard. The sensitivity of LHRH RIA was 0.2 -0.5 pg/tube at 90 % binding. Samples with undetectable levels of LHRH were assigned to the values equal to the sensitivity of the assay. The intra- and inter-assay coefficient of variation were 4-5 % and 7-8 %, respectively.

Histology

Upon completion of PPP experiment, the rats were perfused with 0.9 % saline followed by 10 %

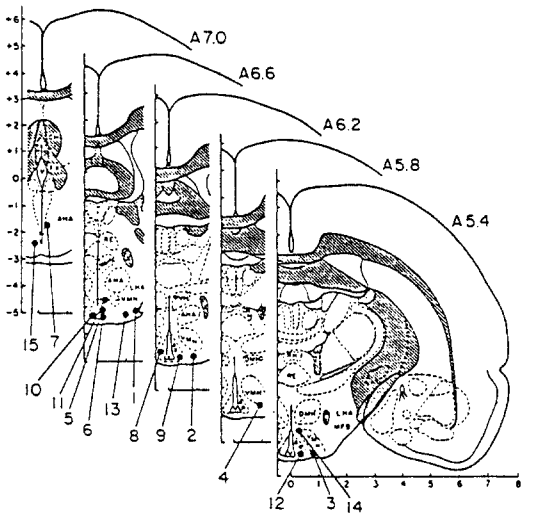

Fig. 1. Schematic diagram of placements of push-pull cannulae. Diagrams are based on the coordinate of de Groot's rat brain atlas. Abbreviation used: AHA, Anterior Hypothalamic Area; AR, Arcuate Nucleus; CH, Commisure Hippocampus; DMH, Dorsomedial Hypothalamus; FX, Fornix; LHA, Lateral Hypothalmic Area; MFB, Medial Forebrain Bundle; MT, Mamillothalamic Tractus; PVH, Paraventric Hypothalamic Nucleus; RE, Reuniens Thalamic a Nucleus; RH, Rhomboideus Thalamic Nulceus; TS, Triangularis septum; V, Ventricle III; VMH, Ventromedial Hypothalamic Nucleus.

formal in formalin-saline solution. The brain was removed and soaked in 10 % formal in formalin--saline for at least one week. Frozen serial sections (40 um) were cut and stained with crysyl violet. Examination of sequential tissue sections revealed that most of tip placements were restricted to the MBH and are schematically represented in Fig. 1.

Statistical analysis

LHRH release data from individual rats were subjected to cycle detector analysis (Clifton and Steiner, 1983). Three parameters such as the mean LHRH release, pulse amplitude and cycle period were obtained. In addition, the mean LHRH release over 1-hr block intervals for each experiment was calculated. One-way analysis of variance (ANOVA) with one repeated measure for independent groups was employed to assess differences in LHRH release within and between groups, respectively. Post-hoc comparison between the mean values at 1 hr block intervals was made using Fisher's least significant difference (LSD) test with $p < 0.05$ for statistical significance.

Results

Figs. 2-4 depict the individual profiles of LHRH release *in vivo* from the MBH for five animals in each of three conditions. In the control group per-

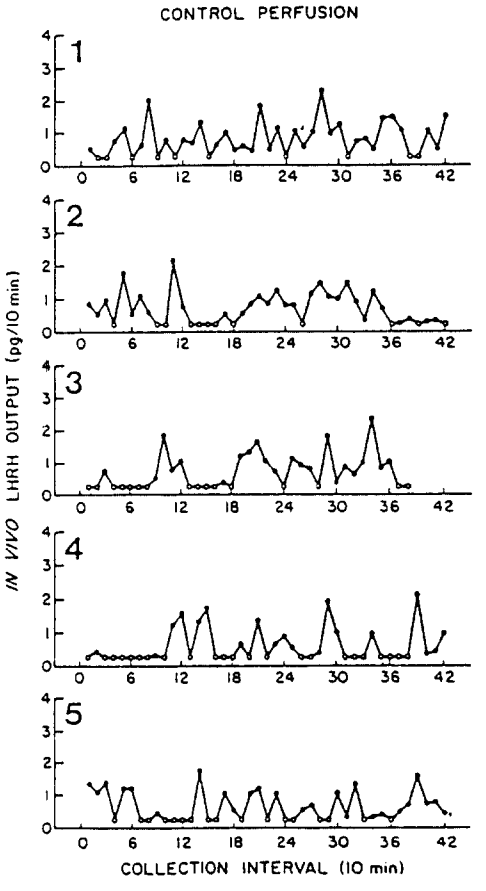

Fig. 2. *In vivo* LHRH release as determined with PPP in five individual OVX + E primed rats. Experiments were typically conducted from 1000 to 1700 hr, and LHRH output levels were determined in consecutive 10 min collections by LHRH radioimmunoassay. Open circles in this and succeeding figures denote undetectable LHRH values.

Table 1. Summary of the mean release, pulse amplitude, and period of *in vivo* LHRH release as analyzed by cycle detector analysis.

Group[a]	No of Exp	*In vivo* LHRH release					
		3-hr control period			4-hr progesterone (or vehicle) period		
		Release (pg/10 min)	Amplitude (pg)	Period (min)	Release (pg/10 min)	Amplitude (pg)	Period (min)
Control infusion	5	0.61 ± 0.50[b]	1.11 ± 0.17	60 ± 11	0.76 ± 0.06	1.04 ± 0.07	52 ± 9
Intermittent P infusion	5	0.72 ± 0.08	1.14 ± 0.18	48 ± 6	1.12 ± 0.23*	1.99 ± 0.35**	42 ± 5
Continuous P infusion	5	0.57 ± 0.11	1.22 ± 0.18	51 ± 5	0.59 ± 0.07	1.02 ± 0.16	63 ± 19

[a]Three groups were studied: 1) control infusion (perfusion medium only), 2) intermittent P infusion (10 min-on, 20 min-off), 3) continuous P infusion. Following 3-hr control period, P (10 ng/ml) was locally infused in the MBH of OVX + primed adult rats bearing push-pull cannulae. [b]Mean ± SEM.

*Mean LHRH release during a 4-hr P period: intermittent P infusion > control infusion and continuous P infusion ($p < 0.05$). **Pulse amplitude of intermittent P infusion: control vs P-infused ($p < 0.05$).

fused with CSF medium only (Fig. 2), the spontaneous LHRH release was characterized by a series of irregular episodes with an interpulse interval of about 60 min. The mean LHRH release and amplitude over 7 hr period were 0.68 ± 0.03 pg/10 min and 1.15 ± 0.08 pg, respectively. No difference in these two parameters between the first 3 hr and the following 4 hr perifusion was observed (Table 1). Intermittent infusion of P clearly stimulated the LHRH pulse generator (Fig. 3). The mean LHRH release and pulse amplitude during the post-P perfusion period were significantly higher (55-75 %, $p < 0.05$) than those observed during the pre-P period (Table 1). In contrast to intermittent infusion of P, continuous administration of this steroid failed to modify LHRH release (Fig. 4). The mean LHRH release and cycle amplitude during the continuous P infusion period were similar to those obtained during the pre-P period and those seen in the control group (Table 1). Figure 5 represents the mean LHRH release *in vivo* when the individual values in the five replicates were pooled. Intermittent P infusion stimulated the LHRH pulse generator during the 2nd and 3rd hr after initiation of P administration. Analysis of data corresponding to the 3rd hr after the initiation of P pulses revealed that the mean LHRH release was signigicantly higher ($p < 0.05$) than that observed during the same period of the two other experimental groups (1.36 ± 0.24 vs 0.73 ± 0.13 and 0.57 ± 0.15 pg/10 min), whereas in the continuous P mode, the release of LHRH was maintained at a relatively stable without large pulsatile fluctuations.

Discussion

The present *in vivo* study clearly demonstrated that intermittent, but not continuous administration of P stimulated LHRH release from the MBH of OVX + E primed rats bearing PPC. It takes approximately one hr to observe this effect of P on the neural LHRH apparatus, confirming the previous *in vitro* data (Kim and Ramirez, 1982; 1985). A similar latency has been reported for the capacity of P to increase LHRH content in the MBH (Kalra and Kalra, 1979; Simpkins *et al.*, 1980) in a similar animal model following systemic injection of this steroid.

The finding that continuous P infusion failed to stimulate LHRH release as opposed to intermittent P infusion led us to believe that *in vivo* pattern of P administration rather than the amount of P delivered to the MBH is the most likely physiological effective signal. The effectiveness of this rhythmic

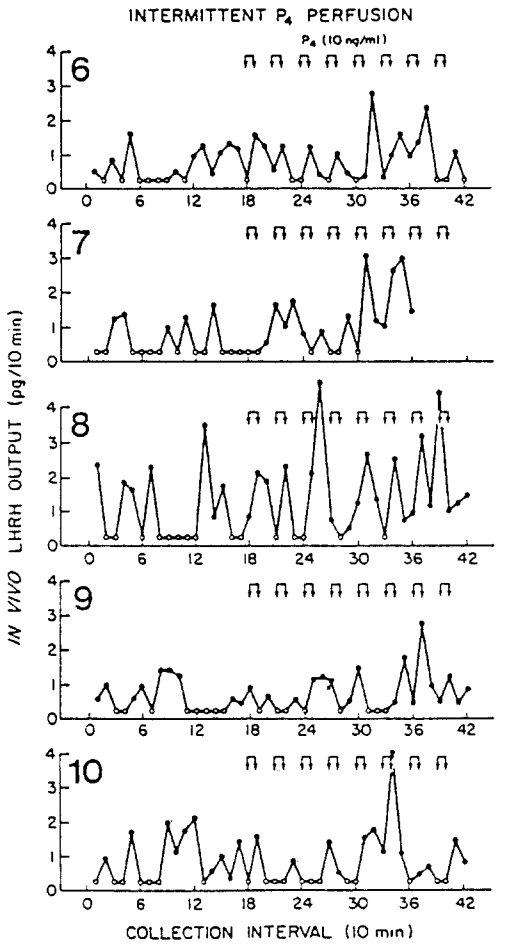

Fig. 3. Effect of intermittent perfusion of progesterone on LHRH release *in vivo* for five individual OVX + E primed rats. Following 3 hr control period in which the basal LHRH release was established, pulses of progesterone (P, 10 ng/ml, 10 min on 20 min off) were locally infused into the MBH.

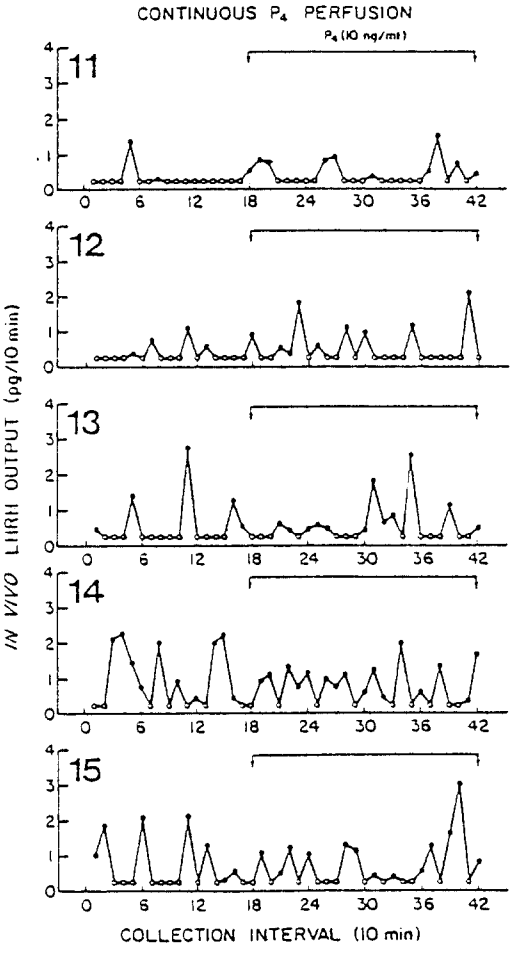

Fig. 4. Effect of continuous perfusion of progesterone on LHRH release *in vivo* in OVX + E primed rats. Progesterone (10 ng/ml) was continuously infused in the MBH followed by 3 hr basal collection.

regimen of P (10 min on, 20 min off) used in this study to stimulate the LHRH pulse generator suggests that a similar pulsatile secretion of P may be present in the natural condition. Blood P secretion of P has been shown to be pulsatile in several species including the rat (Ridley and Greenwald, 1975; Baird *et al.*, 1976; Hixon *et al.*, 1983; Donham *et al.*, 1984). As shown in several systems (Belchetz *et al.*, 1978; Connor and Hedge, 1980; Gallo, 1982), this mode of secretion appears to be the rule and not the exception in neuroendocrine communication, and to be the physiological signal required for modifying the secretory responses.

The cause of this particular response to the neural LHRH apparatus to intermittent P stimulation remains to be elucidated. There is substantial evidence that the stimulatory effect of P on LH release is mediated by increase in catecholaminergic (CA) neurotransmission, particularly, norepinephrine (NE) (Ramirez *et al.*, 1984; Kalra, 1986). The NE-stimulated LHRH release appears to be mediated through alpha-adrenergic receptors as indicated by *in vivo* and *in vitro* studies

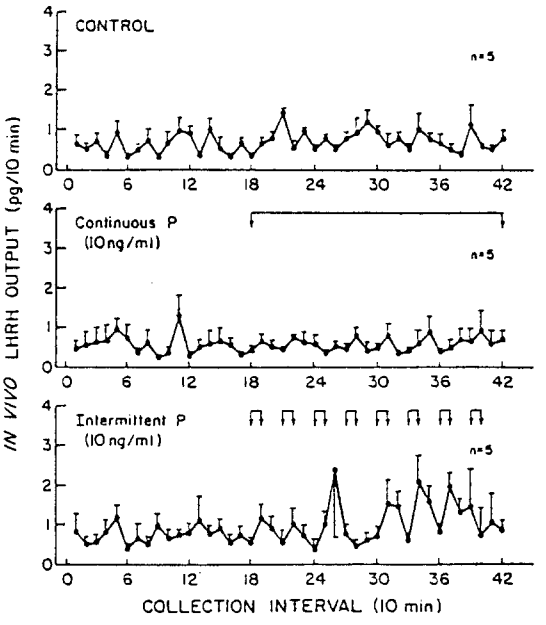

Fig. 5. The mean LHRH release *in vivo* was determined with PPP under three conditions: control and either intermittent or continuous infusion of progesterone. For details, refer to Figs. 2-4. Notice that intermittent infusion of P significantly increased the mean LHRH release and cycle amplitude, whereas continuous infusion of P failed to do so, although the spontaneous basal LHRH release values were very similar in those three groups.

(Negro-Vilar and Ojeda, 1979; Krieg and Ching, 1982; Ojeda *et al.*, 1982). We also found that clonidine, an alpha-adrenergic receptor agonist, effectively induced LHRH release *in vitro*, whereas isoproterenol, a beta-adrenergic receptor agonist was unable to do so (Ramirez *et al.*, 1984). Gallo (1982) also demonstrated that intermittent infusion of NE into the third ventricle of OVX + E + P treated rats maintained a pulsatile release of LH with each pulse of NE followed by an elivation of LH levels. On the other hand, continuous infusion of NE for a prolonged period resulted in an initial single peak of LH with a subsequent return of LH to nearly low pre-infusion values. It seems then likely that the effective signal to stimulate the LHRH pulse generator may be episodic fluctuation of NE tone (Gallo, 1982). Moreover, the steroid internal *milieu* appears to be important parameter in determining stimulatory or inhibitory effects will be manifested on a particular occasion in terms of the interaction between CA-LHRH neurons (Sawyer and Clifton, 1980; Ramirez, *et al.*, 1984; Kalra, 1986). For instance, Gallo (1984) has shown that in OVX steroid-unprimed rats, continuous infusion of NE can suppress only LH pulse frequency, but not LH pulse amplitude presumably through changes in LHRH output. At the present time, whether the neural oscillator initiating the pulsatile release of LHRH resides predominantly within LHRH neuron or is composed of complex neural circultry involving CA neurons and endogeneous opioid neurons in the vicinitiy of LHRH neurons remains to be elucidated (Kalra and Kalra, 1984; Wilkes and Yen, 1981; Blank *et al.*, 1983; Ching, 1983). It is, however, tempting to presume that intermittent P signal can effectively activate the LHRH pulse generator through stimulatory action on NE neurons leading to pulsatile NE output. The continuous P signal, as opposed to the intermittent P mode, may be perceived by other neurons, for instance, endogenous opioid neurons which likely inhibit LHRH release through suppressing NE transmission on LHRH neurons (Ferin *et al.*, 1984; Kalra and Klara, 1984). Recently, we found that *in vitro* infusion of naloxone, a mu-opiate receptor antagonist, markedly stimulated LHRH release *in vitro* from MBH when P was chronically given 48 hr, whereas it was unable to do so in the rats received a single injection of P 6 hr prior to decapitation (Kim *et al.*, 1988), supporting the above notion. As an alternative, pulsatile NE release as result of pulsatile P administration can precisely tune and temporally coordinate both alpha- (mainly stimulatory) (Sawyer and Clifton, 1980) and beta-adrenergic receptor (mainly inhibitory) (Caceres and Taleisnik, 1980; Leung *et al.*, 1982) shifting the balance towards stimulation, whereas continuous NE output induced by continuous P administration may disrupt the coordinated action of these types of adrenergic receptors leading to suppression of the LHRH pulse generator by predominant action of beta-adrenergic receptor. The proof of the above hypotheses remains to be established.

References

Baird, D. T., I. Swanston, and R. J. Scharamuzzi, 1976. Pulsatile release of LH and secretion of ovaulation steroids in sheeps during the luteal phase of the estrous cycle. *Endocrinology* **98:**1490-1496.

Belchetz, P. E., T. M. Plant, Y. Nakai, E. J. Keohg, and E› Knobil, 1978. Hypophyseal response to continuous and intermittent delivery of hypothalamic gonadotropin-induced release of luteinizing hormone binds selectively to hypothalamic opiates receptors. *Brain Res.* **279:**153-158.

Brinkley, H. J., 1981. Endocrine signaling and female reproduction. *Biol. Reprod.* **24:**22-43.

Caceres, A. and S. Taleisnik, 1980. Inhibition of secretion of luteinizing hormone induced by electochemical stimulation of the anterior cingulate cortex mediated by a beta-adrenergic mechanism. *J. Endocrinol.* **87:**419-429.

Ching, M., 1983. Morphine suppress the proestrous surge of GnRH in pituitary portal plasma of the rats. *Endocrinology* **112:**2209-2211.

Clifton, D. K. and R. A. Steiner, 1983. Cycle detection: a technique for estimating the frequency and amplitude of episodic fluctuation in blood hormone and substrate concentration. *Endocrinology* **112:**1057-1064.

Connor, J. M. and G. A. Hedge, 1980. Feedback effectiveness of periodic versus constant triiodothyronine replacement. *Endocrinology* **106:**911-917.

Conti, M., J. P. Harwood, A. J. W. Heueh, and K. J. Catt, 1976. Gonadotropin-induced loss of hormonne receptors and desensitization of adenylate cyclase in the ovary. *J. Biol. Chem.* **251:**7729-7731.

Donham, R. S., M. N. DiDinto, and M. H. Stetson, 1984. Twenty four hour rhythm of gonadotropin release induces cyclic progesterone secretion by the ovary of prepubertal and adult golden hamster. *Endocrinology* **114:**821-826.

de Groot, J., 1959. The forebrain in Stereotaxic Coordinate. North Holland Amsterdan, pp 1-40.

Ferin, M., D. Van Vugt, and S. Waedlaw, 1984. The hypothalamic control of the menstrual cycle and the role of endogenous opioid peptides. *Recent Prog. Horm. Res.* **40:**441-480.

Gallo, R. V., 1982. Luteinizing hormone secretion during continuous or pulsatile infusion of norepinephrine: central nervous system desensitization to constant norepinephrine input. *Neuroendocrinology* **35:**380-387.

Gallo, R. V., 1984. Further studies on norepinephrine-induced suppression of pulsatile luteinizing hormone release in ovariectomized rats. *Neuroendocrinology* **39:**120-125.

Healy, D. L., R. S Schenker, A. Lynch, R. F. William, and G. D. Hodgen, 1984. Pulsatile progesterone secretion: its relevance to clinical evaluation of corpus luteum function. *Fertil. Steril.* **41:**114-121.

Hixon, J. E., G. J. Pijanowski, P. G. Weston, R. D. shanks, and W. B. Wagner, 1983. Evidence for an oscillator other than luteinizing hormone controlling the secretion of progesterone in cattle. *Biol. Reprod.* **9:**1155-1162.

Kalra, S. P., 1986. Neural circuitry involved in the control of LHRH secretion: a model for preovulatory LH release. *In* . Frontiers in Neuroendocrinology (W. F. Ganong and L. Martini eds.), Raven Press, New York, Vol. 9, pp. 31-75.

Kalra, S. P. and P. S. Kalra, 1979. Dynamic changes in hypothalamic LHRH level associated with the ovarian steroid induced gonadotropin surge. *Acta Endocrinol.* (Copenh.) **92:**1-7.

Kalra, S. P. and P. S. Kalra, 1984. Opioid-adrenergic-steroid connection in regulation in luteinizing hormone secretion in the rat. *Neuroendocrinology* **38:**418-426.

Kalra, S. P. and Simpkins, J. W., 1981. Evidence for noradrenergic mediation of opioid effects on luteinizing hormone secretion. *Endocrinology* **109:**776-782.

Kim, K., C. S. Lee, W. K. Cho, and V. D. Ramirez, 1988. Effect of chronic administration of progesterone on the naloxone-induced LHRH release from hypothalamic of ovariectomized, estradiolprimed prepubertal rats. *Life Sci.* **43:**609-613.

Kim, K. and V. D. Ramirez, 1982. *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.

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

Leung, P. C. K., D. I. Whitmoyer, K. E. Garland, and C. H. Sawyer, 1982. Beta-adrenergic suppression of progesterone-induced luteinizing hormone surge in ovariectomized, estrogen primed rats. *Proc. Soc. Exp. Biol. Med.* **169:**161-164.

Levine, J. E. and V. D. Ramirez, 1980. *In vivo* release of luteinizing hormone releasing hormone estimated with push-pull cannulae from the mediobasal hypothalamus of ovariectomized, steroid-primed rats. *Endocrinology* **107:**1782-1790.

Levine, J. E. and V. D. Ramirez, 1982. Luteinizing hormone releasing hormone release during the rat estrous

cycle and after ovariectomy as estimated with pushpull cannulae. *Endocrinology* **111**:1439-1448.

Negro-Vilar, A. and S. R. Ojeda, 1979. Catecholaminergic modulation of luteinizing hormone releasing hormone release by median eminences terminals *in vitro*. *Endocrinology* **104**:1749-1757.

Ojeda, S. R., A. Negro-Vilar, and S. M. McCann, 1982. Evidence for involvement of alpha-adrenergic receptors in norepinephrine-induced prostaglandin E_2 and luteinizing hormone releasing hormone release from the median eminence. *Endocrinology* **110**:409-412.

Park, O. K. and V. D. Ramirez, 1986. Plasma levels of Progesterone are pulsatile during the rat estrous cycle. *Biol. Reprod.* **34**:72 (Suppl.).

Ramirez, V. D., D. Dluzen, and D. Lin, 1980. Progesterone administration *in vivo* stimulates luteinizing hormone releasing hormone release *in vitro*. *Science* **208**:1037-1039.

Ramirez, V. D., H. H. Fader, and c. H. Sawyer, 1984. The role of brain catecholamines in the regulation of LH secretion: a critical inquiry. *In*: Frontiers in Neuroendocrinology (L. Martini and W. F. Ganong eds), Raven Press, New York, Vol. 8, pp 27-84.

Ramirez, V. D., K. Kim, and D. Dluzen, 1985. Progesterone action on the LHRH and nigrostriatal dopamine neuronal systems: *in vitro* and *in vivo* studies. *Recent Prog. Horm. Res.* **41**:421-472.

Ridley, K. and G. S. Greenwald, 1975. Progesterone levels measured every two hours in the cyclic hamster. *Proc. Soc. Exp. Biol. Med.* **149**:10-12.

Sawyer, C. H. and D. K. Clifton, 1980. Aminergic innervation of the hypothalamus. *Fed. Proc.* **39**:2889-2895.

Soules, M. R., R. A. Steiner, D. K. Clifton, N. L. Cohen, S. Aksel, and W. J. Bremner, 1984. Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. *J. Clin. Endocrinol. Metab.* **58**:378-383.

Simpkins, J. W., P. S. Kalra, and S. P. Kalra, 1980. Temporal alterations in luteinizing hormone relasing hormone concentration in several discrete brain regions: effects of estrogen, progesterone and norepinephrine synthesis inhibitor. *Endocrinolgy* **107**:573-577.

Wilkes, M. M. and S. S. C. Yen, 1981. Augmentation of naloxone of efflux of LRF from superfused medial basal hypothalamus. *Life Sci.* **28**:2359-2359.

Yates, Y., 1981. Analysis of endocrine signals: the engineering and physics of biochemical communication system. *Biol. Reprod.* **24**:73-94.

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흰쥐 시상하부 중앙기저부에서 *in vivo* LHRH 분비에 미치는 프로제스테론의 단속적 대 연속적 주입효과

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본 연구에서는 push-pull 캐눌러를 시상하부 중앙기저부에 부착한 흰쥐(난소 절제후 에스트로젠을 처리한)에서 *in vivo* LHRH 분비에 미치는 프로제스테론의 연속적 혹은 단속적 주입효과를 조사하였다. 대조군(perfusion medium만을 주입), 프로제스테론의 단속적(10분 주입후 20분 중단)주입군 그리고 프로제스테론의 연속적 주입군이 연구되었다. 세시간 동안 LHRH의 기저분비를 수거한 후, 프로제스테론(10 mg/ml)을 시상하부 중앙기저부에 직접 주입하였다. 분획은 10분 간격으로 수거하였으며 LHRH 방사면역측정법으로 LHRH 분비를 측정하였다. Push-pull perfusion기간(7시간) 동안, *in vivo* LHRH 분비는 맥동적이었으며, 대조군의 LHRH 평균 기저분비, 펄스진폭 그리고 펄스간격은 각각 0.68 ± 0.03 pg/10 min과 1.15 ± 0.08 pg/10 min 그리고 60 ± 9 min이었다. 단속적인 프로에서테론의 주입은 LHRH 분비와 펄스진폭을 촉진하였으나, 펄스빈도에는 영향이 없었다. 프로레스테론 처리전과 후의 LHRH 분비와 펄스진폭은 각각 0.72 ± 0.08 pg/10 min과 1.12 ± 0.23 pg/10 min 그리고 1.14 ± 0.18 pg과 1.99 ± 0.53 pg 이었다. 반면에 연속적인 프로제스테론의 주입은 LHRH 분비와 펄스진폭에 영향을 미치지 못하였다.

본 *in vivo*연구는 단속적인 프로제스테론의 처리가 LHRH 분비를 촉진하는데 효과적임을 보였으며, 이는 리드믹한 프로제스테론의 분비양상이 LHRH 신경세포를 활성화하는데 효과적임을 시사한다.