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Neuroendocrine Study of the Korean Native Cattle: Pulsatile LHRH Release from Hypothalamic Tissues Superfused *in vitro*

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Present study examined the endogenous release of luteinizing hormone releasing hormone (LHRH) from superfused hypothalamic slices derived from Korean native cattle (KNC). In addition, the *in vitro* secretory pattern of LHRH release in KNC was compared with that in imported cattle such as Holstein cow. The median eminences (ME) of hypothalamic tissues were dissected out, sliced and quickly placed in ice-cold superfusion chamber. Superfusion chambers containing ME slices were maintained in a constant temperature water-bath at 37°C. Effluents were collected on ice at 10 min intervals for a 4 hr superfusion period, and kept -20°C prior to LHRH radioimmunoassay. LHRH release was analyzed by the PULSAR algorithm. The spontaneous release of LHRH from both cows was episodic during a 4 hr superfusion period. The mean LHRH release, pulse amplitude and pulse interval in KNC were 11.08 ± 1.50 pg/min/mg $\times 10^{-2}$, 21.43 ± 7.28 pg/mg $\times 10^{-2}$, and 39.42 ± 3.08 min, which were quite similar to those observed in Holstein cows.

The basic characteristics of the LHRH pulse generator of KNC appears important for a better understanding about the endocrine function of KNC.

KEY WORDS: Korean native cattle, LHRH release *in vitro*, Hypothalamus

It has been well documented that serum level of luteinizing hormone (LH) markedly fluctuates during a 24 hr period. The rhythmic, pulsatile nature of LH release is readily observed in gonadectomized animals (Anderson *et al.*, 1981; Carmel *et al.*, 1976; Clarke and Cummins, 1982). Since luteinizing hormone releasing hormone (LHRH) is the hypothalamic factor responsible for LH output from the anterior pituitary, it is reasonable to believe that the rhythmic, pulsatile LH release is the consequence of a pulatile LHRH release from the LHRH pulse generator (Kalra and Kalra, 1983; Pohl and Knobil, 1982). A pulsatile, ultradian mode of LHRH release has been inferred from the elegant experiments by Knobil and his coworkers (Knobil, 1980, 1981), in which rhesus monkeys were given replacement therapy with intermittent infusion of LHRH with a frequency of 1/hr was important for inducing LH release. Such data clearly compelled the conclusion that the rhythmic fluctuation of serum LH is a consequence of pulsatile LHRH release. Althogh the direct measurement of LHRH release has considerably hampered this notion, the pulsatile fluctuation of LHRH *in vivo* was clearly demonstrated by several techniques such as portal blood collection (Clarke *et al.*, 1987; Fink *et al.*, 1982) and push-pull perfusion (Ramirez and Dluzen, 1987). In the *in vitro* condition, the pulsatile release of LHRH was also evident (Kim and Ramirez, 1985, 1986; Meyer, 1987). Then, the rhythmic release of LHRH appears the most crucial component for the endocrine control of the hypothalamic-pituitary-ovarian axis responsible for the normal reproductive function.

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As far as Korean native cattle (KNC) is concerned, our knowledge about the endocrine function of KNC is not established: indeed, no attempt has been yet made to examine the neuroendocrine function of LHRH-LH axis. Therefore, it is primarily of interest to investigate the function of the LHRH pulse generator of KNC. Moreover, it has been known that the fecundity of KNC seems to be lower than the imported cattles (Shin, 1983). It is then of importance to elucidate whether the hypothalamic LHRH release of KNC may be related to the above notion. Within the scope of this proposal, we studied the endogenous release of LHRH from superfused hypothalamic fragments derived from KNC and Holstein cow.

MATERIALS AND METHODS

Tissue preparations

Fresh brains of KNC and Holstein cows were obtained from the slaughter house clustered in Seoul, Korea. The average age of both animals was about 3-year-old. The median eminence (ME) of hypothalamic tissues were dissected out, sliced and quickly placed in ice-cold superfusion chamber.

in vitro superfusion procedure

The superfusion system used in this study was slightly modified (Kim and Ramirez, 1985; Ramirez *et al.*, 1980). Briefly, the superfusion chambers were made of 10 ml-disposable plastic syringe barrels and fitted on the bottom with a 18 g needle. Superfusion medium was delivered to superfusion chambers through inflow tubing linked to needle at a flow rate of 90-100 μl/min. The superfusion medium consists of Krebs-Ringer-Phosphate, pH 7.40 with 0.1% bovine serum albumin (BSA) and 10 mM glucose. Superfusion chambers were sealed on the top with a rubber stopper allowing an inflow tubing for compressed air gas entry, and an outflow tubing for superfusates. Superfusion chambers containing ME slices (one ME per chamber) were maintained in a constant temperature water-bath at 37℃. ME slices were allowed to adjust to the *in vitro* environment for one hr. Effluents obtained from ME slices were then acidified by adding 1 N HCl to reach a final concentration of 0.1 N HCl. Fractions were kept -20℃ prior to radioimmunoassay for LHRH. Upon completion of the experiments, the post-superfusion ME slices were removed from the chambers and weighed. The mean tissue weights (means $\pm$ SE, n = 6) of ME slices in a supefusion chamber was 672 $\pm$ 61 and 695 $\pm$ 56 mg in KNC and Holstein cows, respectively.

Radioimmunoassay (RIA) for LHRH

LHRH concentration was assayed with aliquotes (300 μl) of superfusates in duplicate by LHRH RIA procedure (Kim and Ramirez, 1985; 1986). Samples were neutralized to pH 6.5–7.5 with 0.1 N phosphate buffer and 2 N NaOH before LHRH RIA. LHRH antiserum (CRR–11–B–72) was kindly provided by Dr. V. D. Ramirez (University of Illinois) and used at a final concentration of 1:200,000. Synthetic LHRH (Sigma) was served as the reference and for radioiodination. Using a logit–log regression, the assay values were linear between 20–90%. The intra– and inter-assay coefficient of variation for a 5 pg dose of LHRH was about 5.8 and 6.6%, respectively.

Statistical analysis

The pulsatile LHRH release *in vitro* was analyzed using the PULSAR algorithm adapted for use on the IBM–PC computer (Marriam and Wachter, 1982; Gitzen and Ramirez, 1986). The mean release, pulse amplitude and pulse frequency were determined using a 10% error for the calculation of the G values according to a normal distribution probability. Statistical differences between groups were determined with Student's t–test.

RESULTS AND DISCUSSION

Fig. 1 depicts the individual profiles of LHRH release from ME slices derived from KNC. LHRH release *in vitro* was clearly fluctuated with variable pulse amplitudes throughout a 4 hr superfusion period. Pulses of LHRH release were generally characterized by a rapid increase followed by a slow decline. In spite of a wide variation, the rhythmic, pulsatile LHRH release ensued when individual

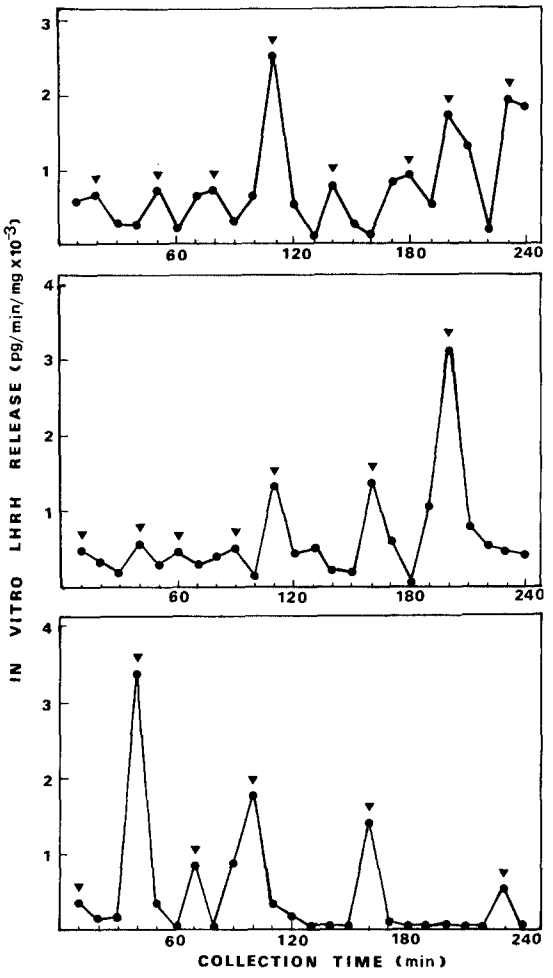

Fig. 1. Representative examples of the spontaneous LHRH release from superfused ME slices derived from Korean native cattles. Distinct pulses (*) were identified by the PULSAR program.

values were pooled. Computer–aid pulse analysis revealed that LHRH pulses occurred at periodic intervals (Fig. 2). The mean LHRH release, pulse amplitude and pulse intervals in KNC were 11.08 ± 1.50 pg/min/mg × 10^{-2}, 21.43 ± 7.28 pg/mg × 10^{-2}, and 39.42 ± 3.08 min, respectively as shown in Table 1. *In vitro* LHRH release from ME slices of Holstein cows was also pulsatile throughout the superfusion period (Table 1). Pulse analysis data also revealed that the mean LHRH release, pulse amplitude and pulse intervals observed in Holstein cows were quite comparable to those of KNC (Figs. 3 and 4.).

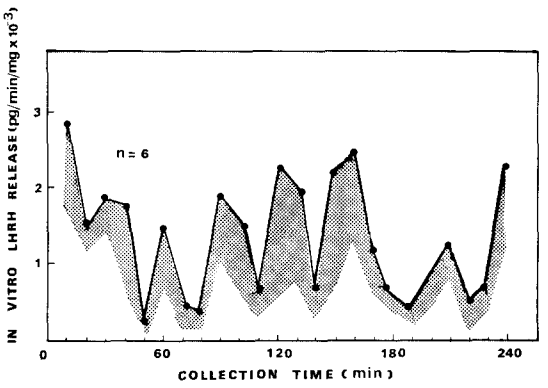

Fig. 2. Mean LHRH release *in vitro* from superfused ME slices derived from Korean native cattles. Each point represents the mean ± SE (shaded area). Experiments were repeated by six times (n = 6).

First of all, it should be pointed out that the isolated ME slices superfused are capable of retaining a remarkable rhythmic release of LHRH, although the ME slices obtained from 3–year–old animals were randomly assigned and the detailed description of reproductive state of those animals was not identified. A substantial evidence indicates that the LHRH–LH axis was clearly dependent upon many factors such as pubertal stages, the period of the estrous cycle, and endocrine manipulations (Amann *et al.*, 1986; de Silva and Reeves, 1988; Rahe *et al.*, 1980). It appears then the LHRH pulse generator may reside within such units and is still functional in the *in vitro* condition as shown

Table 1. Summary of the pattern of LHRH release from ME slices derived from Korean native cattles and Holstein cows

Experimental Group	Mean LHRH release (pg/min/mg × 10^{-2})	Pulse Amplitude (pg/mg × 10^{-2})	Pulse Interval (min)
Korean native cattle	11.08 ± 1.50	21.43 ± 7.28	39.42 ± 3.08
Holstein cow	13.00 ± 1.55	21.02 ± 0.41	40.35 ± 3.83

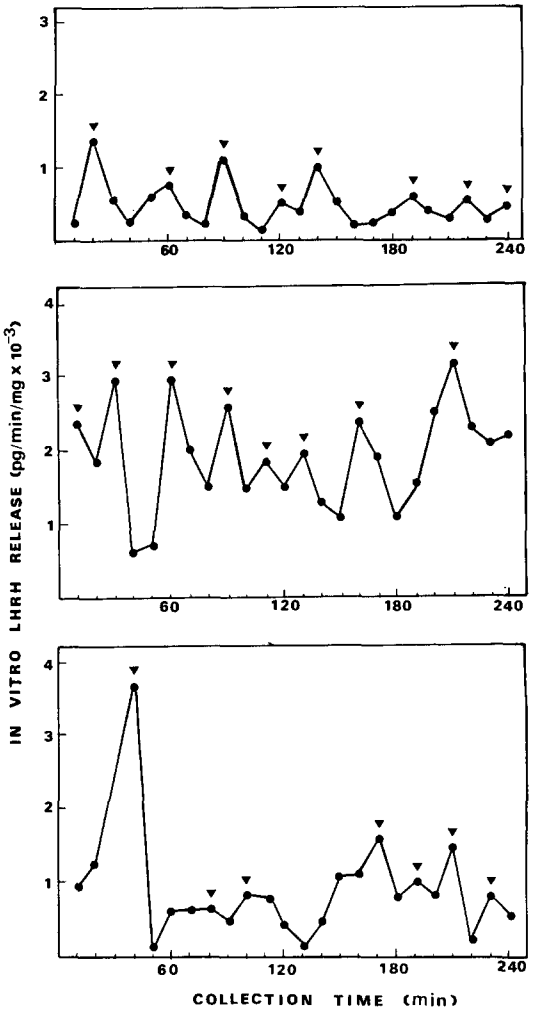

Fig. 3. Representative examples of the spontaneous LHRH release from superfused ME slices obtained from Holstein cows.

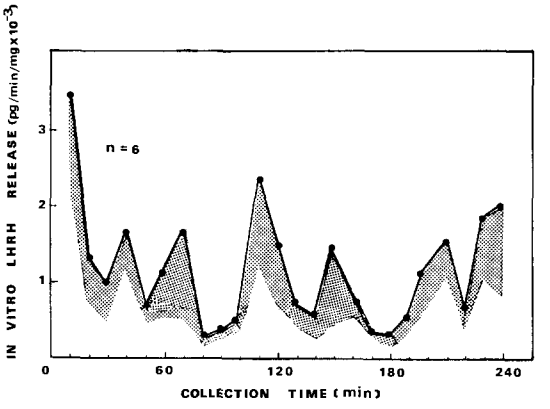

Fig. 4. Mean LHRH release *in vitro* from superfused ME slices derived from Holstein cows. Each point represents the mean ± SE (shaded area). Experiments were repeated by six times (n = 6).

previously. It remains, however, to be elucidated whether the neural oscillator for the pulsatile release of LHRH resides within LHRH neurons or is composed of complex neural circuitry involving catecholaminergic neurons and others in the vicinity of LHRH neurons (Barraclough and Wise, 1982;Kalra and Kalra, 1983; Ramirez *et al.*, 1984). Moreover, it should be noticed that the neuroendocrine function of the LHRH pulse generator is heavily influenced by catecholaminergic neurotransmission and/or feedback signal of steroid hormones. Previously, it has been shown that catecholaminergic neurotransmission and/or steroid hormones may alter the frequency and/or amplitude of pulsatile LHRH release.

In the present study, LH release from anterior pituitary was not determined together with LHRH output. Therefore, we are not able to address one of the important question of which each pulse of LHRH may result in a pulse of LH secretion. Many studies, however, indicate that LH pulses are a direct reflection of LHRH pulses when the two hormones (LHRH and LH) are measured simultaneously in sheep (Clarke and Commins, 1985; Clarke *et al.*, 1987; Levine *et al.*, 1982) and rat as well (Levine and Ramirez, 1982). On some occasions, LHRH pulses seems to be missed. Although these missing of LH pulses may be attributable to a mere experimental artifact, a more interesting explanation would be that LHRH pulses may occur with a regular periodicity, but that the transduction of LHRH signals into LH responses is liable to interference or modification. Moreover, other factors including fluctuations in anterior pituitary sensitivity or responsiveness to LHRH that may be brought about by manipulation of catecholamines and steroid hormones. Since there are catecholamine receptors and steroid receptors in anterior pituitary, it appears that catecholamine and steroid can act at the level of the anterior pituitary to influence the pulsatile release of LH (Barraclough and Wise, 1982; Ramirez *et al.*, 1984).

Another interesting point is that the improvement of reproductive performance of KNC is one of the

most important national task now we confront, since a recent dramatic trend in meat demand appears to continue, and consequently KNC became unable to respond with our rapid increasing demand for beef consumption (Shin, 1983). Unfortunately, the endocrine function of KNC is not yet established, although a number of research avenues such as genetic improvement, nuclear transplantation, efficient methods for the estrous synchronization and fertility are being under investigation (Shin, 1983). Therefore, it is of importance to get a better understanding about the endocrine function of LHRH–LH axis in KNC. In summary, the present study examined the endogenous release of LHRH from either KNC or Holstein cow. No difference in the mean LHRH release, pulse amplitude and pulse intervals was evident in KNC and Holstein cow. Therefore, it is tempting to postulate that the cause of the low fecundity of KNC may be not due to the function of the hypothalamic LHRH pulse generator, but perhaps due to some unidentifed factor(s) at the level of the pituitary and/or ovary. The basic characteristic of LHRH pulse generator of KNC as revealed in this *in vitro* study appears important for the further understanding of the LHRH pulse generator *in vivo*.

REFERENCE

Amann, R. P., M. F. Wise, J. D. Glass, and T. M. Nett, 1986. Prepubertal changes in the hypothalamic–pituitary axis of Holstein bulls. *Biol. Reprod.* **34**:71–80.

Anderson, L. L., D. L. Hard, L. S. Carpenter, E. K. Awotwi, and M. A. Diekman, 1981. Neuroendocrine regulation of luteinizing hormone secretion in beef calves. *Biol. Reprod.* **24**:795–800.

Barraclough, C. A. and P. M. Wise, 1982. The role of catecholamines in the regulation of pituitary luteinizing hormone and follicle–stimulating hormone secretion. *Endocr. Rev.* **3**:91–119.

Carmel, P. W., S. Araki, and M. Ferin, 1976. Pituitary stalk portal blood collection in rhesus monkeys: evidence for pulsatile release of gonadotropin–releasing hormone (GnRH). *Endocrinology* **99**:243–248.

Clarke, I. J. and J. T. Cummins, 1982. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology* **111**:1737–1739.

Clarke, I. J. and J. T. Cummins, 1985. GnRH pulse frequency determines LH pulse amplitude by altering the amount of releasable LH in the pituitary gland. *J. Reprod. Fertil.* **73**:425–431.

Clake, I. J., G. B. Thomas, B. Yao, and J. T. Cummins, 1987. GnRH secretion throughout the ovine estrous cycle. *Neuroendocrinology* **46**:82–88.

de Silva, M. and J. J. Reeves, 1988. Hypothalamic–pituitary function in chronically cystic and regularly cycling daily cows. *Biol. Reprod.* **38**:264–269.

Fink, G., M. Aiyer, S. Chiappa, S. Henderson, M. Jamieson, V. Levy–Perez, A. Pickering, D. Sarkar, N. Sherwood, A. Speight, and A. Watts, 1982. Gonadotropin–releasing hormone. Release into hypophyseal portal blood and mechanism of action. In: Hormonally Active Brain Peptides (K. W. McKerns and V. Pantic eds), Plenum Press, New York, pp. 397–425.

Gallo, R. V., 1980. Neuroendocrine regulation of pulsatile luteinizing hormone release in the rat. *Endocrinology* **102**:112–131.

Gitzen, J. and V. D. Ramirez, 1986. PC–pulsar (pulsar analysis for the IMB–PC). *Life Sci.* **36**:17III.

Kalra, S. P. and P. S. Kalra, 1983. Neuroendocrine regulation of luteinizing hormone secretion in the rat. *Endocr. Rev.* **4**:331–355.

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 effects of estrogen–priming. *Endocrinology* **116**:252–258.

Kim, K. and V. D. Ramirez, 1986. *In vitro* LHRH release from superfused hypothalamus as a function of the rat estrous cycle: effect of progesterone. *Neuroendocrinology* **42**:392–398.

Knobil, E., 1980. Neuroendocrine control of the menstrual cycle. *Recent Prog. Horm. Res.* **60**:1-46.

Knobil, E., 1981. Patterns of hypophysiotropic signals and ganadotropin sectretion in the rhesus monkey. *Biol. Reprod.* **24**:44-49.

Levine, J. E., K. Y. F. Pau, V. D. Ramirez, and G. L. Jackson, 1982. Simultaneous measurement of luteinizing hormone releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology* **111**:1449-1455.

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

Marriam, G. R. and K. W. Wachter, 1982. Algorithms for the study of episodic hormone secretion. *Am. J. Physiol.* **243**:E310-318.

Meyer, D. C., 1987. *In vitro* luteinizing hormone releasing hormone output is dependent on hypothalamic region and the stage of the estrous cycle. *Biol. Re-*

prod. **37:**1207-1214.

Pohl, C. R. and E. Knobil, 1982. The role of the central nervous system in the control of ovarian function in higher primate. *Ann. Rev. Physiol.* **44:**583-593.

Rahe, C. H., R. E. Owens, J. L. Fleeger, H. J. Newton, and P. G. Harms, 1980. Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle. *Endocrinology* **107:**498-503.

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

Ramirez, V. D. and D. E. Dluzen, 1987. Release of luteinizing hormone releasing hormone (LHRH) and neuroactive substances in unanesthetized animals as estimated with push-pull cannulae (PPC). *Biol. Reprod.* **36:**59-76.

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

Shin, H. T., 1983. Cattle for beef production. In: Animal Agriculture in Korea (K. S. Im and Y. I. Parks eds), The Asian-Australian Association of Animal Production Societies, Sisa Corp. Ltd., Seoul, pp. 97-105.

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한우의 신경내분비학적 연구 : 시상하부의 체외배양 조직에서 맥동적 LHRH 분비 양상에 관하여

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본 연구는 한우에서 적출한 시상하부조직을 superfusion 하여 체외배양 하면서 in vitro LHRH의 내인성 분비양상을 조사하였으며, 한우의 in vitro LHRH 분비양상과 수입소인 홀스타인 젖소의 LHRH 분비양상을 비교하였다. 시상하부의 median eminence조직을 적출하여 조각을 내어서 superfusion chamber에 넣은 후 37°C 배양기에서 배양하였다. superfusion은 4시간 동안 계속되었으며, 10분간격으로 분획을 받아내어 LHRH분비능을 LHRH방사면역 측정법으로 측정하였다. LHRH 분비 양상의 특성을 PULSAR algorithm으로 분석하였다. 한우와 수입 젖소에 있어서 내인성 LHRH분비는 맥동적인 양상을 보였다. 한우에 있어서 LHRH 분비의 평균분비, 펄스의 진폭과 그 간격은 11.08 ± 1.50 pg/min/mg × 10^{-3}, 21.43 ± 7.28 pg/mg × 10^{-3}, 39.42 ± 3.08 min이었고 이 값은 홀스타인 젖소에서의 측정치와 거의 유사하였다. 이와같은 한우의 펄스 발진기의 기본적인 특징은 한우의 신경내분비적 연구에 중요하리라 사료된다.