Estradiol-induced GnRH Surge in Ovariectomized Goats

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Abstract. Neurosecretion of hypothalamic GnRH was monitored during the LH surge in ovariectomized goats given estradiol using microdialysis sampling. The microdialysis probe with a polycarbonate dialysis membrane was stereotaxically implanted in the median eminence, and was perfused with the artificial cerebrospinal fluid at a flow rate of 5 µl/min under conscious and unrestrained conditions. The LH surge was induced by means of an intravenous infusion of estradiol (3 µg/h, i.v.) for 16 h and hourly fractions of microdialysis perfusate were collected for GnRH measurement. The perfusate GnRH showed a marked increase to peak levels of 25.3 ± 6.9 pg/ml over basal levels of 2.6 ± 1.1 pg/ml at the onset of the LH surge, and then gradually decreased so that the GnRH surge lasted longer than the LH surge. These results suggest that the LH surge is induced by a surge release of GnRH from the hypothalamus but ends despite a continued supply of GnRH.

Key words: GnRH, Hypothalamus, Microdialysis, LH surge, Goat.

It has been well established that exogenously administered estradiol can induce a preovulatory-like LH surge in ovariectomized females in a wide range of species including the goat [1]. However, the exact mechanism of how estradiol elicits the release of a massive amount of LH from the pituitary gland remains unclear. Direct effects of estradiol on the pituitary to increase its sensitivity to GnRH were implicated in a wide range of species such as rats [2, 3], sheep [4-6] and monkeys [7, 8], whereas the importance of hypothalamic input was also demonstrated in ewes subjected to hypothalamic-pituitary disconnection [9, 10] or immunization against GnRH [11]. Recently an abrupt increase in the GnRH concentration in the pituitary portal circulation was shown at the onset of the LH surge in ewes given estradiol [12, 13] providing further evidence for the involvement of the hypothalamus.

The present study was therefore conducted to examine whether there is any increase in the neurosecretion of GnRH at the onset of the LH surge induced by estradiol in ovariectomized goats. The pattern of GnRH release was investigated by measuring the extracellular concentration of GnRH in the median eminence of conscious and unrestrained goats by means of a microdialysis technique that has been proved applicable for monitoring the neurosecretory activity of GnRH neurons in this species [14].

Materials and Methods

Adult female Shiba goats were from a closed colony at the University of Tokyo [15, 16]. They had been ovariectomized for at least one month before the experiment, and kept under controlled
temperature (23°C), photoperiod (lights on 7:00–19:00) and relative humidity (40%).

The procedures for surgical implantation and for perfusion of a microdialysis probe at the median eminence were described in detail previously [14]. Briefly, the animal was implanted with a guide tube under halothane anesthesia at a position such that the tip of the microdialysis probe would be located in the median eminence when it was inserted into the guide tube according to the stereotaxic method described elsewhere [17]. After a 1–3 week recovery period the following experiments were undertaken. The microdialysis probe (CMA/10, Carnegie Medicine, Sweden) with a 4 mm polycarbonate membrane of 20 KDa cut-off [18] was inserted into the guide tube and the perfusion with an artificial cerebrospinal fluid (CSF) at a flow rate of 5 µl/min was commenced on the day before the experiment. The relative recovery rate for GnRH in vitro at this flow rate was 2.8% [14]. A preovulatory-like LH surge was induced by estradiol infusion at the rate of 3 µg/h for 16 h via one of the catheters (18G, 95 cm in length, Japan Sherwood) fitted to the jugular vein bilaterally as previously described [19]. A fraction of the perfusate was collected at 1 h intervals for 30 h starting from 2 h prior to the estradiol infusion and stored at -20°C until assayed for GnRH concentrations. Matched blood samples were withdrawn at the time of perfusate collection via the catheter on the opposite side, and plasma was separated by immediate centrifugation and stored at -20°C until assayed for LH concentrations. The goat was loosely tied to a stanchion, and fed pelleted diet twice daily and allowed free access to water throughout the experimental period.

Perfusate GnRH and plasma LH concentrations were measured by the radioimmunoassays described previously [14]. The minimum detectable levels were 0.048 pg/tube and 0.051 ng/tube for GnRH and LH, respectively. All the samples were assayed at once and the intra-assay coefficients of variation were 3.1% and 9.2% for GnRH and LH, respectively.

After the experiment was completed the position of the microdialysis probe was examined histologically in each animal according to the procedure described previously [14], and revealed that the probes were correctly placed in the median eminence (data not shown).

**Results**

There was a clear increase in the GnRH concentration in the microdialysis perfusate sequentially collected from the median eminence of ovariectomized goats given estradiol (Fig. 1). Although basal levels as well as peak levels of perfusate GnRH varied considerably among individual animals as indicated in Table 1, the relative pattern of perfusate GnRH in relation to the LH surge was similar in all the 3 goats examined. The onset of the GnRH surge, defined as when the ever-rising GnRH concentration exceeded twice the basal level, was 13.2±1.8 h after the start of estradiol infusion.

Sequential profiles of perfusate GnRH and plasma LH were normalized to the LH peak as shown in Fig. 2. The abrupt increase in perfusate GnRH was synchronized with the onset of the LH surge, and the perfusate GnRH then gradually declined in such a way that high GnRH levels were still maintained when plasma LH returned to the basal level.

**Discussion**

Results of the present study provided evidence for the presence of a surge release of GnRH from the hypothalamus during the LH surge induced by estradiol in ovariectomized goats. The results were consistent with recent findings in ovariectomized ewes given estradiol [12, 13] and preovula-

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Data are means±SEM. <sup>a</sup>: Average value (n=10–15) during the pre-surge period.
Patterns of GnRH neurosecretion during the estradiol-induced LH surge assessed by means of the microdialysis technique as shown in this study and those with the pituitary portal blood sampling [12, 13] were virtually identical, i.e. consisting of an initial abrupt increase at the onset of the LH surge, a longer acrophase exceeding the period of the LH surge, followed by a gradual decline. However, basal as well as peak concentrations of GnRH measured by these two methods were considerably different, and this is probably due to limited recovery rate of GnRH through the microdialysis membrane. Whereas these results suggest that estradiol acts primarily on the brain rather than on the pituitary gland to induce the GnRH surge that in turn elicits massive LH release, i.e. the LH surge, in ruminants such as ewes and goats, estradiol has also been shown to act directly on the pituitary to enhance its responsiveness to GnRH [5, 10, 21, 22], and this appears essential for the full expression of the LH surge.

The finding that the GnRH surge continued beyond the duration of the LH surge was in accordance with previous observations in ovariec томized ewes given estradiol [12, 13] and intact preovulatory ewes [20]. It is therefore considered to further support the current hypothesis that the
LH surge ends due to the loss of pituitary response to GnRH following either the down regulation of GnRH receptors [23] or the exhaustion of the releasable LH pool [5] rather than the elimination of GnRH input from the hypothalamus. Or alternatively, the LH surge may be terminated by some other unidentified mechanisms, since the presence of hypothalamic factors inhibiting GnRH-stimulated LH release has been suggested [24].

GnRH is secreted at the median eminence from the nerve terminal of GnRH neurons into the pituitary portal circulation. The majority of GnRH cell bodies reside in the preoptic/septal area in the ewe [25] and the Shiba goat [26]. GnRH cells themselves do not possess estrogen receptors [27], but neurons containing various neurotransmitters and/or neuromodulators are abundant in this region of the brain, and some of them such as neurons containing noradrenalin and inhibitory amino acid γ-aminobutyric acid (GABA) have been shown to accumulate estrogens [28]. Recently Robinson et al [29] reported changes in these neuroactive substances including a marked decline in the release of GABA in the preoptic/septal region prior to the LH surge in ewes using the microdialysis technique. Therefore, to further understand the mechanism of estrogen action on the neurosecretion of GnRH, it seems necessary to monitor changes in various neurotransmitters as well as neuromodulators concomitant with GnRH during the estradiol induced LH surge via microdialysis probes placed in specific brain regions such as preoptic/septal areas and the median eminence.

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References


