Timing of Proceptive and Receptive Behavior of Female Goats in Relation to the Preovulatory LH Surge

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ABSTRACT. The temporal relation between the luteinizing hormone (LH) surge and the occurrence of two components of sexual behavior was investigated in female goats. Behavior observation and blood sampling were carried out every 2–6 hr from 26 hr before through 116 hr after prostaglandin (PG) administration which was given to induce luteolysis during the mid-luteal phase. The preovulatory LH surge appeared 30–100 hr after PG injection, and it continued for 6.5 ± 0.4 hr. Although the interval from PG injection to the onset of the LH surge differed considerably among individuals, the proceptivity (defined as an approach to a male with vigorous tail-swishing) and the receptivity (defined as an acceptance of male's mounting or copulation) occurred well synchronized with the LH surge in each female. The proceptivity and receptivity were observed from -7.4 ± 1.7 hr to 16.8 ± 1.5 hr and from -2.6 ± 1.6 hr to 13.0 ± 2.0 hr, respectively, with regard to the LH surge onset (0 hr). Thus the proceptivity appeared first and lasted longest, and the receptivity was always seen around the time of the LH surge suggesting an existence of causal relationship between these two preovulatory events. The present results support the view that the female goat has evolved an efficient behavioral strategy such that it actively attracts males before it becomes fully receptive of male's approach to mate, when the LH surge occurs simultaneously to ensure a high conception rate at the subsequent ovulation. — **KEY WORDS:** goat, LH surge, proceptivity, receptivity, sexual behavior.

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Females estrous behavior can be classified into three major components in many mammalian species, namely the attractivity or stimuli emanating from a female towards males, the proceptivity or active movements of females to solicit males, and the receptivity or passive acceptance of males sexual approach including mounting and copulation [1, 6, 7, 9, 23]. In the wild, prey animals such as herbivores must have evolved their behavior to meet the most efficient breeding strategy to minimize the chance of being hunted by predators and at the same time achieve their reproductive success. Therefore, it is supposed that the duration of mating period has been selected to be as short as possible yet with enabling sufficient communication between opposite sexes to attract each other. To materialize high conception rate with this short lasting estrus, the timing of copulation and the ovulation should have been arranged at a temporal order which ensures successful fertilization.

The hypothalamo-pituitary-gonadal axis is of central importance in the reproductive endocrine system, since it not only controls follicular growth and ovulation but also regulates the reproductive behavior. In the ruminant species, for example, an increasing estradiol level in the circulation induces the LH surge, an endocrine signal necessary for ovulation, within a couple of days of luteolysis, and this estradiol rise is essential for the manifestation of estrous behavior [5, 17]. Based on such a close correlation between the estrous behavior and endocrine changes, one can postulate a common neural mechanism responsible for both

of these preovulatory events. However, available information is limited on the temporal relationship between the estrous behavior and the endocrine changes. In the present study, we therefore examined the temporal sequence of proceptive and receptive sexual behaviors with reference to the timing of preovulatory LH surge in the female Shiba goat.

MATERIALS AND METHODS

Subjects

Eleven multiparous female and 4 sexually experienced male Shiba goats were from the closed colony at the experimental station of the University of Tokyo [8]. Females and males were divided into three groups for behavior observation. During the experiment, females and males were kept in different pens separated from each other except for the period of behavioral observations, when the males were brought into the females' pen.

Sexual behavior observation

Female goats were allowed to feed hay and concentrates and drink water in the experimental pen (2.6×2.6 m), where a removable cage (1×1 m) was placed at one corner. They received an intramuscular injection of 3 mg prostaglandin F_{2α} (PG, Panaseran F) during the mid-luteal phase. Blood sampling was performed via the jugular vein catheter (Argyle Medicut Catheter, 18G and 38 cm in length). Behavior observation for 10 min was carried out every 2–6 hr at the time of blood sampling as described below. The frequency of blood sampling and behavior observation was every 6 hr during the period from the PG injection to the appearance of first estrous signs (e.g. swollen and blushed vulvae with discharge of viscous fluid) and

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every 2–3 hr thereafter until the disappearance of estrous signs. In addition to manual recording on the check sheet all the behaviors of female goats during the 10 min were videotaped for further analysis.

Each session of behavior observation consisted of three parts as follows; at first the females were gently held and blood sample (1 ml) was collected via the catheter for measurement of LH concentrations, and then males were brought into a cage to test the proceptivity of females for 5 min, and finally the males were released from the cage to test the receptivity of females for another 5 min. Criteria for proceptivity and receptivity are described below. After the end of each session, the pen floor was cleaned to remove the olfactory effect of residual urine or feces on subsequent analysis of estrous behavior. At 12 hr intervals additional blood samples (6 ml) were collected for progesterone and estradiol assays in 5 female goats. Plasma was separated by immediate centrifugation and stored at -20°C until assayed for concentrations of LH and ovarian steroids.

In order to examine the sexual behavior under the natural mating condition, one additional female goat was kept with males in a large pen (4.6×2.7 m) and its behavior was recorded for 143 hr continuously on the videotape from the luteal phase through the follicular phase without any interference by observers.

Parameters for proceptivity

Approaching to males: The cumulative period of time when females' heads were located within the area of 30 cm around the cage, in which males were enclosed, was measured during the first half of 10 min observation period.

Tail swishing: The number of bouts of vigorous tail swishing was counted in each female during the 10 min observation period.

Parameters for receptivity

Standing heat: The number of occurrence of female's receptive behavior, in which it stood still in response to male's approach including mounting and copulation, was counted during the second half of the 10 min observation period.

Copulation: The number of copulation was counted during the second half of 10 min observation period.

Hormone assays

The plasma concentrations of LH, progesterone and estradiol were measured by specific double antibody radioimmunoassays using ^{125}I -oLH (NIADDK-oLH-I-3) as a tracer and anti-ovine LH rabbit serum (YM#18) for LH, progesterone-11 α -glucuronide-(^{125}I tyramine) and anti-progesterone rabbit serum (GDN#337) for progesterone, and oestradiol-6-(o-calboxymethyl)oximino-(2- ^{125}I histamine) and anti-estradiol sheep serum (GDN#244) for estradiol, according to the procedures described elsewhere [18]. The minimum detectable levels for LH, progesterone and estradiol were 0.4 ng/ml, 33.6 pg/ml and 4.1 pg/ml, respectively. Intra- and inter-assay coefficients of variation for LH were 7.3% and 11.0%. All the samples were assayed at the same time for progesterone and estradiol, and the intra-assay coefficients of variation for progesterone and estradiol were 10.5% and 7.0%, respectively.

RESULTS

Consecutive behavioral analysis in a female goat throughout the 143 hr period revealed that sexual behaviors appeared from 48 hr after PG administration as shown in Fig. 1. Before the onset of receptive period there was a time of proceptivity when the female showed interest in the male and approached to it, although the female yet not accepted the male and escaped from its trial to sniff or mount. Thereafter the female started to show vigorous tail swishing and frequent urination, and the male's approach dramatically increased. Finally the female began to stand still in response to the approach and mounting by males, which resulted in a series of copulations. Each copulation took only a few seconds, but repeated several times until the female started to escape or the male seemed to loose interest. This series of estrous behavior, which continued usually less than 30 min, was repeated with various intervals ranging from 10 min to 5 hr, and the interval tended to get prolonged toward the end of estrus.

In 8 out of 11 female goats, which received PG in the luteal phase, estrous behavior was observed. The profiles of plasma concentrations of LH, progesterone and estradiol in 5 female goats, from which serial blood sampling was conducted, are shown in Fig. 2. Following the abrupt decline of plasma progesterone at the luteolysis induced by PG, plasma estradiol showed gradual increase until the time of LH surge. These hormones were maintained at low levels following the LH surge until the end of experimental period.

Behavioral change was conspicuous in all the females in which the LH surge was induced. The timing of occurrence of proceptive and receptive sexual behaviors is shown with reference to the LH surge in Fig. 3 (left). Data are normalized to the onset of the LH surge. The timing of both the two aspects of estrous behavior, namely the proceptivity and receptivity, were found to be well synchronized with the LH surge. Particularly the receptivity, which was indicated in terms of copulation frequency, was observed exclusively around the time of the LH surge. On the other hand, the occurrence of the proceptivity was rather scattered throughout the experimental period, although the main part of it coincided well with that of the receptivity. By contrast, in 3 female goats, which showed no LH surge during the experimental period, neither the proceptivity nor the receptivity was apparently seen as shown in Fig. 3 (right).

The group-means for the timing of the LH surge and for the proceptive as well as receptive behaviors are shown in Fig. 4. Although the latency from the PG administration to the LH surge varied considerably from animal to animal with the range between 30 and 100 hr ($n=8$), these three parameters, namely the proceptivity, receptivity and the LH surge, were observed being well synchronized among animals; the proceptivity started first, which was followed by the onset of receptivity, and finally the LH surge occurred. When the durations of these parameters were compared, the proceptivity lasted longest, while that of the

Fig. 1. Sequential patterns of sexual behavior in a representative female goat following prostaglandin (PG) administration in the luteal phase (0 hr). Data obtained for 4 hr during the luteal phase (from -24 to -20 hr) and the follicular phase (from 48 to 52 hr) are presented in the upper and lower panels, respectively.

Fig. 2. Changes in plasma concentrations of LH (upper), progesterone (middle) and estradiol (bottom) during the preovulatory period in female goats. Mean $\pm$ SEM for 5 animals are normalized to the onset of LH surge, which was designated as the time when plasma LH first exceeded the level of 10 ng/ml.

Fig. 3. Occurrence of the proceptivity (expressed as accumulated seconds approaching the male per 5 min), and the receptivity (number of copulation per 5 min) in female goats. Data are means $\pm$ SEM for 8 animals in which the LH surge was induced (left), for 3 animals in which the LH surge was not observed during the observation period (right).

LH surge being shortest (6.50 ± 0.35 hr, mean $\pm$ SEM, $n=8$). Proceptivity continued from -7.38 ± 1.69 hr to 16.75 ± 1.51 hr after the LH surge onset (0 hr), whereas the receptivity from -2.63 ± 1.55 hr to 13.0 ± 2.02 hr ($n=8$).

Fig. 4. Temporal relationship among the proceptivity, receptivity, and the LH surge. Data are normalized to the onset of the LH surge. The period of proceptivity is designated as the time when a female approached the fence restraining males more than 99.25 sec/5 min, which is the mean + 2SD of the control value obtained before the PG injection, whereas that of receptivity as the time when a female showed standing heat and allowed males to copulate.

DISCUSSION

The results of the present study showed that the timing of the preovulatory LH surge, and two components of estrous behavior, i.e. proceptivity and receptivity, were well coincided in female Shiba goats. Particularly, the temporal relationship between the onset of receptive period and that of the LH surge was very similar among individuals regardless of varying latency from the PG induced luteolysis. On the other hand, such apparent signs of proceptivity and receptivity were not observed in animals in which no LH surge was induced. The individual variation in the latency from PG administration to the onset of the LH surge is thought to be reflecting different patterns of folliculogenesis and estrogen secretion from them. However, in each animal the temporal relationship between the estrous behavior and the LH surge was virtually identical. This finding provides physiological evidence to support the view that there is an efficient breeding strategy, in which a female attracts as many males as possible with its proceptive behavior, in addition to pheromonal signals, before it enters the receptive phase. This is important to increase the chance of mating with the best male through the inter-male competition. The timing of actual mating, which occurs during the receptive period, is arranged at the time of the LH surge, so that the capacitated sperms can stay at the ampulla of the oviduct to fertilize the ovum shed on the next day of copulation [18]. Altogether, these behavioral sequence would guarantee high

conception rate with relatively short duration of estrus.

Both proceptivity and receptivity continued longer than the LH surge, with the former two covering the latter period. Particularly the onset of the LH surge was just preceded by that of the receptivity, and the receptivity continued for about 16 hr. Both the timing and the duration of the receptivity seems likely to be related to the timing of the surge release of gonadotropin releasing hormone (GnRH) in this species. The GnRH is neurosecreted from the hypothalamus into the pituitary portal circulation at the median eminence forming an endocrine signal responsible for inducing the LH surge. Manabe *et al.* [12] demonstrated by using a microdialysis technique that the start of the preovulatory GnRH surge was synchronized with the onset of the LH surge and the GnRH surge continued for about 16 hr in ovariectomized goats given estradiol. Similar results were reported in ewes [16] and estrogen-primed ovariectomized ewes [14, 15] by measuring GnRH profiles in the pituitary portal blood. Based on these observations we inclined to hypothesize that this prolonged nature of the GnRH surge, which continues far beyond the period of LH surge, may have some physiological role, besides its stimulatory effect on the pituitary gonadotropin secretion, probably for the control of estrous behavior, in particular the receptive behavior.

Estrogens are known to be indispensable for eliciting female sexual behavior in all mammalian species examined to date [6]. For example, the administration of estrogen to ovariectomized rats triggered lordosis response, a receptive behavior in rodents [2, 3]. GnRH has been speculated as a candidate substance mediating the estrogen action on the behavior. For instance, the GnRH injection was shown to potentiate lordosis in ovariectomized rats [10, 19–22, 25–27] and in hypogonadal mice [28] primed with estrogen in doses too low to initiate lordosis. Furthermore, administration of an anti-GnRH globulin [26, 27] or GnRH antagonists [19] disrupted lordosis response in estrogen-primed ovariectomized rats. The effect of GnRH could not be ascribed to LH or follicle-stimulating hormone (FSH) release, since lordosis is facilitated by the injection GnRH in estrogen-primed ovariectomized rats, which had been hypophysectomized [24]. All these observations favor the view that estradiol acts not only on the neuroendocrine system to induce the GnRH surge and consequent LH surge, but also stimulate the central nervous system regulating the receptivity via the molecule of GnRH, which may then be acting as a neuromodulator in the brain.

Besides, Moss and McCann [21] demonstrated that the administration of GnRH induced the receptivity but had little effect on the proceptivity (e.g. hop-darting, ear wiggling, and approaching to the male) in rats. Also in our results, the proceptivity preceded and continued much longer than the GnRH surge from Manabe *et al.* [12]. Therefore GnRH may be not indispensable for the proceptivity, and the two components of sexual behavior may therefore be under different regulatory systems involving different neurotransmitters or neuromodulators as suggested by

previous studies [4, 11, 13].

In conclusion, the present study revealed a close temporal correlation between the preovulatory LH surge and estrous behavior, especially the receptivity, in female goats. It was also speculated that the timing as well as the duration of GnRH surge would be tightly related to the period of receptivity, although this hypothesis should be examined in our future study, in which the neurosecretion of GnRH and behavior would be analyzed simultaneously in conscious and unrestricted animals by using neurophysiological technique such as brain microdialysis.

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