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Effect of intermittent administration of neurokinin 3 receptor agonist on luteinizing hormone secretion, estrus, and ovulation in feed-restricted goats

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ABSTRACT

The aim of this study was to determine the short-term effects of feed restriction on follicular dynamics and hormone secretion patterns in goats, and to examine the effect of senktide, a neurokinin 3 receptor agonist, under the feed-restricted condition. Goats were administered 2 mg of dinoprost in the luteal phase of the estrous cycle, and an intravaginal progesterone device (CIDR) was inserted for 10 days. Feed was provided at the level of 50% (feed-restricted, $n = 12$) or 100% maintenance (control, $n = 5$) for 15 days, beginning on the day of CIDR insertion. At 12 h after CIDR withdrawal, feed-restricted goats were intravenously administered with 200 nmol of senktide ($n = 6$) or saline ($n = 6$) at 4-h intervals for 24 h. Fifteen days of feed restriction caused $12.4 \pm 5.5\%$ body weight loss. On the day of CIDR withdrawal, feed-restricted goats had fewer follicles with smaller diameter than control goats. Feed restriction caused anestrus and anovulation in one case, and all the other goats in the control and feed-restricted saline-treated groups showed estrus and ovulated within normal ranges. Therefore, there was no significant influence of feed restriction on the timing of these events and related hormone profiles. In senktide-treated goats, there was a rapid increase in luteinizing hormone (LH) after each injection, which was followed by an increase in plasma estradiol concentration. In the senktide-treated group, 5/6 goats did not show estrus, and 3/6 goats ovulated 2 days after treatment, while most goats ovulated 3 or 4 days after treatment. These results suggest that feed restriction negatively affects follicular development and that senktide can enhance pulsatile LH secretion and thereby accelerate the ovulatory process.

1. Introduction

Neurokinin B (NKB), a member of the tachykinin family of peptides, is comprised of 10 amino acid residues in length. NKB and its receptor, neurokinin 3 (NK3R) are found to be distributed in both the central and peripheral nervous systems. The role of NKB and NK3R signaling in the central control of reproductive function was firstly noted by a human genetic study showing that loss-of function mutations of *Tac3* and *Tacr3*, which encode NKB and NK3R, respectively, have been identified in patients with hypogonadotropic hypogonadism (Topaloglu et al., 2009). Recent *in vivo* studies for NKB in rodents (Navarro et al., 2011), sheep (Billings et al., 2010; Sakamoto et al., 2012), and goats (Wakabayashi et al., 2010a) have indicated a stimulatory action of NKB on gonadotropin-releasing hormone

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(GnRH) and luteinizing hormone (LH) release. Senktide is a highly selective and potent agonist of NK3R (Wormser et al., 1986) and has been used for experimental purposes. Although the precise mechanism underlying the action of senktide is not fully understood, the stimulatory effect on pulsatile LH/GnRH secretion, presumably via the activation of kisspeptin neurons in the arcuate nuclei (ARC) (Okamura et al., 2013), is an attractive feature for the clinical application of this drug in several types of reproductive disorders associated with insufficient GnRH/LH secretion. In fact, our pilot study showed a therapeutic potential for anestrus in goats, in which intravenous treatment with senktide could induce LH secretion and thereby promoted follicular development and ovulation in 5/6 cases (Endo and Tanaka, 2014). However, because of the limitations of clinical trials, further investigations are needed to determine the effect of senktide on reproductive function under various physiological and pathological conditions.

Suppression of both pulse and surge mode of LH release has been observed by fasting or feed restriction in experimental models, including sheep (Foster et al., 1989; McShane et al., 1993) and goats (Mani et al., 1996; Tanaka et al., 2002, 2004), which can alter ovarian activity as in the clinical cases of anestrus and ovarian quiescence. In a goat study, we reported that feed restriction (30% of requirement for one month) caused a reduction in the frequency of LH pulses followed by anovulation after treatment for estrous synchronization with a progesterone-releasing controlled internal drug releasing device (Tanaka et al., 2004). A low response to estrous synchronization was also found in another study, where feed restriction (50% of requirement for two month) of female goats decreased the expression of estrus and conception rates (Kusina et al., 2001). The purpose of the present study was to test the hypothesis that intermittent administration of senktide can restore or enhance pulsatile LH secretion and thereby stimulate follicular development and ovulation, even when gonadal activity is being suppressed owing to the metabolic changes caused by feed restriction. The specific aims were, firstly, to examine the short-term effects of feed restriction (50% of the maintenance level for 15 days) on ovarian dynamics, hormonal profiles of LH and estradiol, expression of estrus, and timing of ovulation in cycling Shiba goats, and secondly, to examine the effect of senktide on these parameters.

2. Materials and methods

2.1. Animals

Thirteen female Shiba goats (3.5 ± 1.8 [mean ± standard deviation (SD)] years of age) maintained at the Tokyo University of Agriculture and Technology were used in this study. Ten of the goats were nullipara, and the remaining three were primipara (1.8–2.5 years from the last delivery). Shiba goats are non-seasonal breeders under natural daylight conditions in Japan, and have been used as an experimental model for ruminants. The goats were housed in outside paddocks with sheltered areas, under natural photoperiod. They were normally fed a diet based on alfalfa hay cubes (approximately 700 g of dry matter/day) and additional concentrate supplements (0–50 g of dry matter/day) according to their body weights. All goats were clinically healthy and in good condition, and had normal estrous cycles before the study. All procedures were approved by the University Committee for the Use and Care of Animals of Tokyo University of Agriculture and Technology (#22–67).

2.2. Feeding regimen and senktide treatment

The study was conducted between November 2013 and February 2014. Prior to the experiment, goats were checked for estrus with bucks, and ovarian ultrasonography was performed using a B-mode scanner (HLS-375M, Honda Electronics, Co., Ltd., Aichi, Japan) every other day or daily to determine the time of ovulation. They were administered 2 mg of dinoprost ($PGF_{2\alpha}$ analog, Pfizer, Tokyo, Japan) on the day of the luteal phase (7–14 days after ovulation), and then a controlled internal drug-releasing device containing 0.3 g of progesterone (CIDR-G®, Pfizer New Zealand, Auckland, New Zealand) was inserted for 10 days. Feed (crushed alfalfa hay cubes only) was provided once daily at the level of 50% ($n = 12$) or 100% maintenance ($n = 5$) for 15 days, beginning on the day of CIDR insertion and continued until 5 days after CIDR removal. The amounts of feed were formulated according to the Nutrient Requirements of Small Ruminants (2007). Body weights were measured at the beginning and end of the feed restriction period.

At 12 h after CIDR withdrawal, feed-restricted goats were intravenously administered with 200 nmol of senktide in 5 ml of saline containing 0.25% dimethyl sulfoxide (50%-fed Senktide; $n = 6$) or saline (50%-fed Saline; $n = 6$) at 4-h intervals for 24 h (a total of 7 injections). The dose and procedure of senktide administration were determined based on previous studies in Shiba goats (Wakabayashi et al., 2010b; Endo and Tanaka, 2014). It was shown that the pulsatile LH secretion induced by 4-h intervals of senktide treatment could effectively promote follicular development and ovulation in anestrous goats (Endo and Tanaka, 2014). Goats receiving 100% feed were administered 5 ml of saline (100%-fed Control; $n = 5$) in the same manner.

Four goats in the 100%-fed Control group were used again in the 50%-fed Saline or Senktide group. At least one estrous cycle was allowed to elapse between two trials.

2.3. Blood sampling, estrus detection, and ovarian ultrasonography

Blood samples (1.5–2 ml) were collected by jugular venipuncture every other day for 10 days of the CIDR insertion period. They were also collected every 15 min from −2 to 6 h after the first administration for the analyses of LH pulsatile secretion and estradiol levels, and then collected every 4 h from 12 to 24 h just before the injection of senktide or saline for the analyses of LH and estradiol peaks associated with the time of ovulation. A catheter (18 gauge, 30-cm length; MEDICUT Catheter Kit, Sharwood Co., Tokyo, Japan) was inserted into the jugular vein for the collection of these samples. All goats were placed in a stanchion during the 24 h of the treatment period. Thereafter, the catheter was removed, and goats were returned to the outside paddock. Observation of estrus and blood sampling were commenced at 6-h intervals from 24 to 96 h after the start of treatment. Estrus and estrous signs were checked just before the blood sampling, according to a method described previously (Nagai et al., 2013). Estrus was defined as when a female goat stood to be mounted by a male goat. The time of onset of estrus was defined as the time when estrus was observed for the first time, and the duration of estrus was defined as the time interval from the first observed estrus to the first absence of estrus. Blood samples were centrifuged at 1750 × *g* for 20 min at 4 °C, and the plasma was stored at −20 °C until assay.

Ovarian ultrasonography was performed every other day during the period of CIDR insertion, and then daily until 5 days after senktide treatment. All follicles and CL that grew to greater than 2 mm in diameter were recorded by two or three cross-sectional images with maximal areas. Ovulation was comfirmed by the dissapearance of large follicles that were detected on the previous day of examination and by the development of corpus luteum (Orita et al., 2000).

2.4. Assays

Plasma concentrations of glucose and non-esterified fatty acid (NEFA) were measured by commercial assay kits according to the manufacturer's instructions (Wako Pure Chemical Industries, Osaka, Japan). LH concentrations in plasma were measured for all samples by radio-immunoassay as described previously (Suganuma et al., 2007). Intra- and inter-assay coefficients of variation were 6.1% and 11.9%, respectively, and sensitivity was 0.09 ng/ml. Estradiol-17β concentrations were measured for