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A high level of male sexual activity is necessary for the activation of the medial preoptic area and the arcuate nucleus during the 'male effect' in anestrous goats

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HIGHLIGHTS

- We studied the activation of mPOA and ARC depending on the level of sexual activity.
- In both areas, sexually active males induced a higher activation than inactive ones.
- Sexually active males did not specifically activate kisspeptin cells.

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ABSTRACT

In small ungulates such as sheep or goats, the introduction of a male among a group of anovulatory females during the anestrus season leads to the reactivation of the gonadotrope axis and ovulation, a phenomenon known as the 'male effect'. In goats, our previous studies have demonstrated the importance of male sexual activity for an efficient reactivation of the gonadotrope axis assessed through ovulation and blood LH pulsatility. In the present experiment, we assessed whether the level of male sexual activity would also induce differential activation of two brain regions of key importance for the reactivation of GnRH activity, namely the medial preoptic area and the hypothalamic arcuate nucleus. In both structures, we observed a differential activation of Fos in females, depending on the level of buck sexual activity. Indeed, goats unexposed to males showed low levels of expression of Fos while those exposed to sexually inactive bucks showed an intermediate level of Fos expression. Finally, the highest level of Fos expression was found in females exposed to sexually active males. However, and contrary to our initial hypothesis, we were not able to find any specific activation of kisspeptin cells in the arcuate nucleus following the introduction of highly sexually active males. As a whole, these results demonstrate that the level of male sexual activity is a key factor to stimulate brain regions involved in the control of the gonadotrope axis in the context of the male effect in goats.

1. Introduction

Female sheep and goat show a seasonal pattern of reproductive activity. Indeed, females exhibit, during the year, an alternation between periods of ovarian activity during the breeding season, and a period of anestrus [1]. Interestingly, this state of anovulation can be overcome through socio-sexual interactions: when anestrous ewes or goats are exposed to sexually active rams or bucks, a high proportion of the females will ovulate and display estrus, and if they mated will become pregnant. This male-induced ovulation was first reported in sheep over 70 years ago and was termed the 'ram effect' [2]. In goats, this phenomenon was described later [3].

The peripheral response to the introduction of the male has been quite well described: females show a nearly immediate reactivation of

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LH pulsatility that is itself the consequence of a reactivation of pulsatile GnRH secretion [4,5]. This short-term response leads to the synthesis of estrogens by the ovaries and allows the establishment of a positive feedback inducing the GnRH preovulatory surge and therefore ovulation after a few hours (long-term response) [6]. However, it should be noticed that the occurrence of a GnRH/LH surge and ovulation observed in females is highly variable and is thought to be the result of a low integration of either male sensory cues or E2-positive feedback signal at the level of the hypothalamus [6].

In goats, we demonstrated the key role of male sexual activity to counteract the huge variability observed in the response among females. Indeed, during the anestrus period, the inhibitory influence of the photoperiod also induces a reduced level of sexual activity in bucks [7,8]. In particular, males show low levels of sexual behavior and interest towards females [8]. In this context, we have demonstrated that introducing a male that has previously been exposed to a photoperiodic treatment to stimulate its sexual activity, virtually reduces all the variability in the response, thus resulting in >90% of females showing ovulation [8–10]. The effect of the intense sexual behavior displayed by the males subjected to the photoperiodic treatment is so powerful that durations of contact shorter than 4 h per day between males and females are enough to efficiently stimulate the anestrous females [10,11].

More generally, the comparison of the responses induced by males remaining under normal photoperiod, thus expressing a low level of sexual activity during the anestrus season and photo-stimulated males appear to be a powerful model to understand the mechanisms controlling the response of the females. In this context, the central mechanisms and the precise neuroendocrine pathway linking male sensory cues to GnRH secretion is yet to be precisely determined.

Importantly, kisspeptin signaling appears as an essential step for GnRH secretion as it stimulates LH secretion in a GnRH dependent manner into the hypophysial portal blood [12]. In sheep and goat, kisspeptin neurons are mainly located in the medial preoptic area (mPOA) and the ARC [13–16] and expression of the Kiss1 gene as well as peptide production is downregulated in the ARC during the anestrous season in comparison to the breeding season [17]. In addition, the number of kisspeptin fibers in close apposition to GnRH neurons is higher in the breeding season [17]. Kisspeptin administration to anestrous females induces ovulation in seasonally acyclic females [18]. Interestingly, the kisspeptin response (stimulating GnRH) is greater during the non-breeding season and this may be due to higher kisspeptin receptor expression on GnRH neurons at this period of the year.

In the context of the male effect in sheep, it was demonstrated that the introduction of the ram among a group of anovulatory females induces the activation of GnRH cells in the mPOA and also of kisspeptin cells in the ARC as evidenced by the higher number of cells that expressed the marker of cellular activation c-Fos in GnRH and kisspeptin cells respectively [15]. Moreover, the intracerebroventricular injection of the kisspeptin antagonist P-271 blocked the LH response induced by the ram [15]. In addition, it is well known that the male odor is the most important sensory cue for the induction of the male effect, despite the fact that there is some debate as to whether olfactory cues alone are sufficient to induce a complete response, i.e. ovulation [19,20]. A recent study in goats demonstrated that the male pheromone 4-ethyloctanal, when presented to females, increased the electrophysiological multiple-unit activity (MUA) [21] within the ARC thought to represent an important component of the GnRH pulse generator [14,22]. Indeed, neurons expressing kisspeptin, neurokinin B and dynoprhin (KNDys) are thought to be partly responsible for this MUA activity in sheep and goat [14,23].

On the basis of these results, we explored here the activation of the two key brain regions regarding the control of the gonadotrope axis in the context of the male effect in goats, namely, the mPOA and ARC. More specifically, our hypothesis was that the activation of these regions, measured by using c-Fos as a marker of cellular activation, would depend on the level of sexual behavior expressed by males. In addition, we also explored whether the intensity of male sexual activity would differentially impact the ARC kisspeptin cells.

2. Material & methods

2.1. General management conditions

The experiment was carried out during the non-breeding season using local goats (*Capra hircus*) from the Laguna region in the State of Coahuila, Mexico (latitude, 26°23′N and longitude, 104°47′W). In females isolated from males, non-breeding season lasts from March to August and from January to April in bucks isolated from females [24,25]. All females of the present study had given birth between August and September.

The animals were maintained under conditions that fulfilled the nutritional requirements of the animals and the procedures used in the experiments were in strict accordance with the Official Mexican Rule for the technical specifications for the production, care, and use of laboratory animals [26]. In the present experiments, animals were fed with 2 kg of alfalfa hay (18% CP) and 200 g/d of commercial concentrate (14% CP; 1.7 Mcal/kg) per animal, with free access to mineral blocks and water during the study.

2.2. Animals

2.2.1. Males

Males were 2-year-old at the beginning of the photoperiodic treatment. On November 1st, 4 males were randomly divided into 2 groups ($n = 2$/group) and remained into distinct shaded outdoor pens (5×5 m) until the study in April. One group was maintained under natural photoperiod during the whole experiment and therefore exhibited a low level of sexual activity at the time of the introduction to females [8,9]. These bucks were called sexually inactive males. The other group of males was subjected to a treatment of long days (16 h of light/8 h of darkness) from November 1st to January 15th. The light treatment consisted of providing natural light with additional artificial light from 6:00 h to 8:00 h and from 18:00 h to 22:00 h (in order to obtain a total of 16 h of light/d). The open pen had 15 daylight lamps of 68 W of energy each. Light-on and light-off were regulated by an electronic timer and light intensity was at least 300 lx at the level of eyes of the animals. Intensities similar to that of our photoperiodic treatment or lower were demonstrated to induce the effect of "long days" in goats and rams [27,28]. On January 16th, the light treatment was stopped and the bucks were exposed to natural variations of day-length. This treatment has previously been shown to take a further 45 to 60 days to produce a stimulatory effect on male reproductive activity [8,29]. Testosterone secretion is therefore stimulated from March to the end of April and, as a consequence, the intensity of odor and the sexual behavior of bucks are highly improved during these months that normally correspond to the sexual rest season [8,30]. Therefore, these bucks were labelled as sexually active, the condition corroborated by the results, see below.

2.2.2. Females

We used twenty-one multiparous anovulatory goats ranging from 4 to 5 years. All females were isolated from males from December 15th until April, when exposure to males was implemented (see below). On April 1st and 9th, each female was submitted to a transrectal ultrasonography using an Aloka SSD-500 machine connected to a transrectal 7.5 MHz linear probe to verify their anovulatory condition (i.e. absence of corpus luteum). This method was previously described [31] and proved to be reliable for the assessment of luteal activity in goats [32,33]. On April 10th, anovulatory females selected after ultrasound were scored for body condition score (BCS) and housed in shaded open pens (6×4 m). BCS was assessed by palpating the spinous and lateral processes and the musculature of the lumbar region of the spine, and