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Hypothalamic-pituitary GnRH/LH axis activity is affected by salsolinol in sheep during lactation: Effects of intracerebroventricular infusions of salsolinol and its antagonizing analogue

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ABSTRACT

The aim of the study was to test the hypothesis that salsolinol, a derivative of dopamine, is involved in the regulation of hypothalamic-pituitary gonadotropic (GnRH/LH) axis activity in lactating sheep. In the first experiment performed on sheep during the fifth week of lactation, a structural analogue of salsolinol (1-MeDIQ) was infused into the third brain ventricle (IIIv) to antagonize its action within the central nervous system (CNS). A push–pull perfusion of the infundibular nucleus/median eminence was performed simultaneously, and blood samples were collected from the jugular vein. In the second experiment, sheep received infusions of salsolinol into the IIIv, 48 hours after the weaning of their 8-week-old lambs. Blood samples were collected during the experimental periods, and the anterior pituitary (AP) tissue was dissected immediately after the end of the experiment. Perfusate GnRH concentration (experiment 1), plasma LH concentration (experiments 1 and 2), and relative LHβ mRNA levels in the AP tissue (experiment 2) were assayed. Blocking of salsolinol action in the CNS of lactating sheep caused a significant ($P < 0.001$) decrease in the perfusate GnRH concentrations in comparison with controls. Treatment with 1-MEDIQ also significantly decreased ($P < 0.001$) the LH concentration in the blood plasma. In turn, salsolinol infused 48 hours after lamb weaning significantly ($P < 0.001$) increased plasma LH concentration, reflected in the significant ($P < 0.05$) increase in the amplitude of LH pulses in the treated sheep as compared to the control animals. There was no significant difference in the relative levels of LHβ-subunit mRNA in the AP between control and salsolinol-infused sheep. The results lead to a conclusion that salsolinol affects the secretory activity of the GnRH/LH axis in sheep during lactation. Whether salsolinol infused into the IIIv evokes this stimulatory effect by itself or by modulation of other regulatory systems needs to be clarified.

1. Introduction

Lactation is characterized by a number of adaptive morphological and physiological changes in the female body. There is an increase in the activity of certain metabolic processes, leading to larger energy requirements than in nonlactating animals. Despite adrenal glucocorticoids, which play a significant role in this, the sensitivity of the maternal hypothalamic-pituitary-adrenal axis to stressors is reduced [1,2]. In addition, depending on the animal species, the weakening or even complete inhibition of the reproductive functions is observed [3–5]. These processes require close cooperation between the nervous

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and endocrine systems and have the effect of changing the physiology and behavior of the mother, allowing her to take care of the newborn.

The energy imbalance caused by high milk production is thought to be an important factor in the inhibition of gonadotropin secretion and in delaying the return of ovarian cyclic activity [6,7]. Suckling, which stimulates milk production, inhibits also the reproductive functions, especially during the intense suckling in the early period after parturition [8]. This is reflected in the reduction in the pulsatile GnRH and LH secretion in comparison with other phases of the reproductive cycle [9–11]. In pigs, the inhibitory effect of suckling on LH secretion already appears at 3 days postpartum, and the first postpartum estrus does not occur until suckling ceases [12]. In both milked and suckled cows, the circulating LH concentrations generally start to increase again between 10 and 20 days postpartum [13]. These changes in LH levels occur more rapidly in milked cows than in suckled ones, in which the onset of estrus can be considerably delayed, despite of the much higher milk production in dairy cows [11]. There is some evidence that feeding high protein levels to high producing cows and a high energy diet to suckled beef cows shorten considerably the time to the first ovulation [14]. It has also been shown that early weaning of piglets and calves causes a quick return to normal LH secretory activity. Consequently, a significant increase in the circulating LH, which resulted from the increase in LH pulse frequency, can lead to the reoccurrence of estrus and ovulation [11,15,16]. This is accompanied by an increase in the number of GnRH receptors in the pituitary gland and restoration of the positive feedback between LH and estradiol [17,18].

In sheep, which are a species with marked seasonality of reproduction, an inhibition of estrus during lactation passes to seasonal anestrus, coinciding in the natural reproductive cycle with long photoperiod. The speed with which pituitary LH content is replenished depends on whether lactation occurs during seasonal anestrus or during the breeding season [19]. According to Mandiki et al. [19], the return of the ovaries' secretory activity occurred more rapidly in sheep that were in lactation in the autumn than in those that were in lactation in the spring, and weaning lambs in autumn hastened the resumption of estrous cycles. It was also demonstrated that the duration of infertility in sheep and cows was proportional to the number of offspring fed and the frequency of suckling [8,10,20].

Numerous compounds, including neurotransmitters, regulatory peptides, and hormones, are involved in modulation of the secretory activity of the hypothalamic-pituitary gonadotropic (GnRH/LH) axis in females [21]. They include catecholamines, opioid peptides, neuropeptide Y, kisspeptin, and oxytocin and prolactin, and some of them are released in response to suckling. In recent years, much attention has been focused on the dopamine derivative salsolinol, the concentration of which increases in the extracellular matrix of the sheep hypothalamus during suckling [22]. Evidence shows that salsolinol stimulates prolactin secretion [23] and inhibits stress-induced activity of the hypothalamic-pituitary-adrenal axis [2] in lactating sheep. Dopaminergic regulation also influences the GnRH/LH axis in both breeding and anestrous seasons [24–26]; thus, we can expect that salsolinol will modulate the release of GnRH and/or LH during lactation. To test this hypothesis, we performed a series of intracerebroventricular (ICV) infusions of salsolinol and its structural antagonistic analogue (1-MeDIQ) [27] in post-weaning and nursing sheep, respectively.

2. Material and methods

2.1. Animal management

All animal procedures were conducted in accordance with the Polish Guide for the Care and Use of Animals (1997) and were approved by the local ethics committee. Experiments were performed on Polish Longwool sheep (n = 24, from 3- to 4-year-old). They were maintained indoors in individual pens under natural lighting conditions (52°N, 21°E), mated naturally in September, and lambed in the following February. Sheep were fed twice a day with a diet formulated for pregnant and lactating animals according to the recommendations of the National Research Institute of Animal Production (Norms 1993); hay and water were available *ad libitum*. Animals were divided into two experiments, which were conducted during 2 consecutive years. The first experiment with the 1-MeDIQ treatment was performed during the fifth week of lactation (n = 8), whereas the second one, with the salsolinol treatment, was performed 48 hours after the weaning of 8-week-old lambs (n = 16). During the experimental procedures, sheep were kept in comfortable cages, where they could lie down. In the 1-MeDIQ experiment, ewes were kept together with their lambs, and lambs had a restrained access to the udder.

2.2. Brain surgery

During the second month of pregnancy, a stainless steel guide cannula (1.2-mm outer diameter) was implanted into the third ventricle (IIIv) of the brain of all ewes. In addition, eight ewes that would be infused with 1-MeDIQ were also implanted with an additional cannula (1.6-mm outer diameter) into the infundibular nucleus/median eminence (IN/ME). The implantation was performed under general anesthesia through a hole drilled in the skull, in accordance with the procedure described by Traczyk and Przekop [28]. The cannulae were positioned according to the stereotaxic coordinates of the sheep hypothalamus [29]: frontal 29.5 to 31 mm and sagittal 0.3 to 0.5 mm for the cannulation of the IIIv and frontal 31.0 to 32.5 mm and sagittal 1.0 mm for the cannulation of the IN/ME. Guide cannulae were fixed to the skull with stainless steel screws and dental cement. External opening of the canal was closed with a stainless steel cap. After the surgery, ewes were injected daily with antibiotics for 5 days and with analgesics for 4 days. Placement of the cannulae into the IIIv was confirmed by the outflow of cerebrospinal fluid during surgery, and placement into the IN/ME was confirmed with an injection of blue ink after slaughtering. The sheep used in the study were in good health conditions, showed normal behavior and food intake, and had the correctly localized cannulae.