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In the ovine pituitary, CXCR4 is localized in gonadotropes and somatotropes and increases with elevated serum progesterone

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ABSTRACT

The pituitary is the central endocrine regulator of reproduction and in addition to various hormones regulating its actions, other molecules, such as chemokines, influence pituitary physiology as well. Despite reports over 2 decades ago that chemokines regulate the pituitary, much of the basic biology discerning chemokine action in the pituitary is unclear. A small number of chemokines and their receptors have been localized to the pituitary, yet chemokine ligand 12 (CXCL12) and its receptor, CXCR4, have received the most attention as both are increased in human pituitary adenomas. This chemokine duo was also reported in normal human and rat pituitary, suggestive of a functional role and that this chemokine axis might function in pituitaries from other mammalian species. To date, reports of CXCL12 and CXCR4 in pituitary from livestock are lacking, and research on pituitary during pregnancy in any mammalian species is limited. Moreover, progesterone regulates CXCR4 expression in a tissue-dependent manner, but whether differing concentrations of progesterone reaching the pituitary modulate CXCL12 or CXCR4 is not known. To address these gaps, our first objective was to determine if CXCL12 and CXCR4 expression and protein abundance differ in sheep pituitary during early gestation (days 20, 25, and 30 of gestation) compared to nonpregnant ewes. The second objective was to determine if CXCL12 or CXCR4 production was altered in the ovine pituitary when circulating progesterone concentrations are elevated. The expression of CXCL12 messenger RNA decreased on day 20 of gestation compared to nonpregnant ewes; CXCL12 protein was similar across all days tested. In nonpregnant and pregnant ewes, CXCR4 was localized to somatotropes and gonadotropes on all days tested. Abundance of CXCR4 increased in the pituitary tissue of pregnant ewes with elevated circulating progesterone compared with pregnant ewes with normal circulating progesterone concentrations (control). The present study details CXCL12 and CXCR4 in normal ovine pituitary and reveals that gonadotropes and somatotropes may be regulated by CXCL12/CXCR4, underscoring this signaling axis as a potential new class of modulator in endocrine functions.

1. Introduction

Functions of the pituitary gland are essential to reproductive biology and in addition to various hormones regulating pituitary actions, other molecules, such as chemokines, influence pituitary physiology as well [1]. Although reports of chemokines regulating the pituitary were proposed over 20 yr ago [2], an understanding of physiological roles and basic biology of chemokines and their receptors in the pituitary is lacking. More than 50 chemokines and approximately 20 chemokine receptors

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have been identified [3,4], although only a few have been observed in hypothalamus or pituitary. With respect to pituitary physiology, much of the research has focused on chemokine (C-X-C motif) receptor 4 (CXCR4) and its ligand CXCL12 as both proteins are increased in human pituitary tumors, suggesting this axis might promote adenoma development [5–7]. A paucity of information on potential roles for CXCL12 and CXCR4 in normal pituitary functions exists. Using immunohistochemistry in normal human pituitary, CXCR4 was localized to ~34% of anterior pituitary cells while CXCL12 was identified in ~12% of the cells [5]. The same study noted rat pituitaries expressed CXCR4, but less CXCL12. The fact that CXCL12 and CXCR4 are expressed in normal human and rat pituitary tissue would suggest a functional role and that this chemokine duo might function in pituitaries from other mammalian species.

Our group and others have demonstrated key roles for CXCL12/CXCR4 signaling during early gestation, specifically during implantation and placentation in humans [8,9], baboons [10], sheep [11–14], and mice [15]. Interestingly, in separate studies investigating the role of CXCL12/CXCR4 in the corpus luteum (CL), we observed both proteins also increased in CL during early pregnancy compared to nonpregnant (NP) ewes (unpublished data). It was curious similar expression patterns for CXCL12 and CXCR4 existed in different reproductive tissues (ie, endometrium, fetal extraembryonic membranes, and CL) during the same period of early gestation. Whether CXCL12 or CXCR4 are present in ovine pituitary and which cell types may express these proteins have not been described in any livestock species; also, whether they fluctuate with pregnancy, as observed in other tissues, or if elevated serum progesterone (P4) in vivo influences CXCL12 or CXCR4 in ovine pituitaries are unknown. To address these gaps, our first objective was to determine if CXCL12 and CXCR4 synthesis differed in sheep pituitary during early gestation (days 20, 25, and 30 of gestation) compared with NP sheep (day 10 of the estrous cycle). As circulating P4 concentrations are similar during luteal phase and early gestation in sheep, we were able to evaluate CXCL12 and CXCR4 in the pituitary dependent on pregnancy and not P4. The second objective was to determine if CXCL12 or CXCR4 abundance in pituitary is altered when circulating P4 concentrations are elevated. In a previous study, we administered human chorionic gonadotropin (hCG) to sheep on day 4 of gestation, which resulted in sustained elevated serum P4 concentrations [16]. Intriguingly, we observed increased CXCR4 in CL and endometrium when endogenous P4 concentrations were elevated compared with pregnant ewes on the same day of gestation with normal physiological P4 concentrations. To determine if similar responses occurred in the pituitary, we elected to analyze CXCL12 and CXCR4 in pituitary tissue collected during this study. Because a major goal was to evaluate CXCL12 and CXCR4 in pituitary from pregnant sheep, this model not only permitted examination in pregnant ewes but also allowed us to evaluate the effects due to elevated circulating P4 in vivo as opposed to exogenous P4 administration. Our data provide novel information on CXCL12 and CXCR4 in normal sheep pituitary, underscoring this signaling axis as a potential new class of modulator in endocrine functions.

2. Materials and methods

2.1. Pituitary collection from ewes on days 20, 25, and 30 of gestation

The New Mexico State University Animal Care and Use Committee reviewed and approved all experimental procedures that used animals. Pituitary tissue used for the present study was collected from our previously published work [13]. To summarize, estrus was synchronized in 20 mixed-age western whiteface ewes during the mid-to-late luteal phase with 2 injections of dinoprost tromethamine (5 mg intramuscular [i.m.]; Lutalyse; Pfizer, New York, NY) administered 4 h apart. On detection of estrus (day 0) by a vasectomized ram, ewes were placed in experimental groups and mated to an intact ram of known fertility. Pregnant ewes (n = 5/d) were anesthetized with 20 mg/kg body weight of sodium pentobarbital (Vortech Pharmacy, Dearborn, MI) on day 20, 25, or 30 of gestation and on day 10 (n = 5/d) of the estrous cycle (nonpregnant [NP] control ewes). Ewes were considered pregnant based on the presence or absence of a conceptus. After exsanguination, the pituitary was removed and half snap frozen in liquid nitrogen and stored at −80°C for subsequent RNA and protein isolation. The other half was fixed in 4% paraformaldehyde.

2.2. Treatment with hCG to increase progesterone synthesis

Pituitary tissue collected from our previously published research [16] was used for the present study. Briefly, 19 mixed-aged western whiteface ewes were randomly assigned to 1 of 2 treatments: HiP4 (600 international units [i.u.] hCG i.m.; n = 9) or control (4.8 mL saline i.m.; n = 10). The ewes were treated 4 d after mating. Within each treatment, the ewes were randomly assigned to 1 of 2 groups where half the ewes were euthanized 13 d after mating (control n = 4; HiP4 n = 5), and the remaining ewes euthanized 25 d after mating (control n = 6; HiP4 n = 4). Days were selected to correspond to preattachment (d13) and postattachment (d25) of the conceptus to endometrium. Ewes were anesthetized with 20 mg/kg body weight of sodium pentobarbital via intravenous administration. Ewes were euthanized by exsanguination, and pituitary tissues were collected as previously described. Our previous publication details the statistically higher concentrations of serum progesterone observed in ewes exposed to hCG [16].

2.3. Ribonucleic acid isolation and complementary DNA (cDNA) synthesis

Total cellular RNA was extracted from 100 mg of pituitary tissue in 1 mL of Tri Reagent BD (Molecular Research Center Inc, Cincinnati, OH), according to manufacturer's directions, eluted in nuclease-free water, and subsequently treated with DNase using the TURBO DNA-free kit (Ambion, Foster City, CA) to ensure that samples were not contaminated with genomic DNA. The quantity and purity of RNA was determined using a NanoDrop-2000 spectrophotometer (Thermo Scientific, Waltham, MA) and stored at −80°C until further analysis. Complementary DNA was synthesized from 1-μg RNA using the iScript cDNA Synthesis Kit