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Evidence for a potential role of neuropeptide Y in ovine corpus luteum function

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Abstract

Neuropeptide Y (NPY) is a neurohormone that is typically associated with food intake, but it has also been reported to affect the production of progesterone from luteal tissue in vitro. However, NPY has not been previously immunolocalized in the ovine ovary or in the corpus luteum (CL) of any species, and the effects of this neurohormone on luteal function in vivo are not known. Thus, we performed fluorescent immunohistochemistry (IHC) to localize NPY in the ovine ovary and used avidin-biotin immunocytochemistry (ICC) to further define the intracellular localization within follicles and the CL. We then infused NPY directly into the arterial supply of the autotransplanted ovaries of sheep to determine the in vivo effect of exogenous NPY on ovarian blood flow and on the luteal secretion rate of progesterone and oxytocin. Immunohistochemistry revealed that the NPY antigen was localized to cells within the follicles and CL, in the nerve fibers of the ovarian stroma, and in the vessels of the ovarian hilus. In the follicle, the NPY antigen was localized to nerves and vessels within the theca interna layer, and strong staining was observed in the granulosal cells of antral follicles. In the CL, NPY was localized in large luteal cells and in the vascular pericytes and/or endothelial cells of blood vessels, found dispersed throughout the gland and within the luteal capsule. In vivo incremental infusions of NPY at 1, 10, 100, and 1,000 ng/min, each for a 30-min period, into the arterial supply of the transplanted ovary of sheep bearing a CL 11 d of age increased ($P \leq 0.05$) ovarian blood flow. The intra-arterial infusions of NPY also increased ($P \leq 0.05$) in a dose-dependent manner the secretion rate of oxytocin, which was positively correlated ($P \leq 0.05$) with the observed increase in ovarian blood flow. The infusions of NPY had a minimal effect on the secretion rate of progesterone, and similar intra-arterial infusions of NPY into sheep with ovarian transplants bearing a CL over 30 d of age had no significant effect on ovarian blood flow or on the secretion rate of progesterone. These results suggest that NPY acts on the luteal vascular system and the large luteal cells to rapidly stimulate blood flow and the secretion of oxytocin, respectively, which collectively implies a putative role for NPY during the process of luteolysis when increasing amounts of oxytocin are secreted from the ovine CL in response to uterine pulses of prostaglandin F2α.

Keywords: Corpus luteum; Neuropeptide Y; Ovary; Oxytocin; Progesterone; Sheep

1. Introduction

Neuropeptide Y (NPY) is a highly conserved, 36-amino-acid peptide that was originally isolated from the porcine brain [1]; it is also the most widely distributed

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neurohormone in the body [2]. The greatest concentration of NPY is found in the hypothalamus [3], where NPY stimulates food intake [4–6] and facilitates the release of gonadotrophins by the hypothalamic–pituitary axis [7–10]. As might be expected, the greatest concentration of NPY immunoreactive (ir) nerve fibers are also located within the hypothalamus [5], but large quantities of NPYir fibers have also been localized in tissues of the reproductive tract in several species, including the cow [11,12] and the human [13,14]. However, the distribution of NPYir fibers and cells in the reproductive tract of sheep has not been reported previously, and NPY has not been localized to the corpus luteum (CL) in any species.

The lack of information regarding the localization of NPY in the CL is notable, because results from several in vitro studies have demonstrated that NPY can affect the production of progesterone by dispersed luteal cells in several species [15–19]. However, the effect of NPY on steroidogenesis was not consistent among species, because NPY inhibited progesterone production by porcine luteal cells [19] but stimulated the production of progesterone by bovine luteal cells [17]. The infusion of NPY via a microdialysis system inserted into the bovine CL in vitro caused a dose-dependent increase in the release of progesterone and oxytocin [17], suggesting that NPY might influence hormone secretion from the ruminant CL in vivo.

The putative intraluteal relationship between oxytocin and NPY is also of considerable interest. In addition to the in vitro data generated by Miyamoto et al [17], NPY has also been shown to stimulate the release of oxytocin from the posterior pituitary gland in lactating rats in vivo [20]. In the ovine CL, oxytocin mRNA concentrations are highest after approximately 2 d of luteal development, and they decline rapidly thereafter [21]. Oxytocin is translated almost immediately [22], and the mature peptide is subsequently stored in secretory granules in large luteal cells [23]. The luteal concentration of oxytocin slowly declines until the peptide is completely depleted from the CL after about day 18 of the ovine estrous cycle [24]. The functional role of luteal oxytocin is still unknown [25], but the peptide is released in large amounts from the ruminant CL in response to pulses of uterine prostaglandin F2α (PGF2α), which may also suggest a mediating role for NPY during luteolysis.

Therefore, to further explore the role of NPY in luteal function, we first defined the pattern(s) of localization for NPY in the ovine ovary and CL. To investigate the effect(s) of NPY on luteal function, we used the well-established ovarian autotransplant model in sheep, which permits the direct intra-arterial infusion of substances of interest into the ovary bearing a CL in conscious, unstressed animals [26,27]. We report that in the ovine ovary, specific staining for NPY was observed in nerves and vessels, in the granulosal cells of follicles, and in the large luteal cells in all stages of the CLs examined. The sequential infusions of NPY in vivo into the ovarian transplant sheep bearing a CL on day 11 of the cycle caused a dose-dependent increase in the secretion rate of oxytocin and stimulated ovarian blood flow. Collectively, these results suggest a role for NPY during luteolysis, when it is reported that blood flow in the periphery of the ruminant CL increases [28] and large amounts of oxytocin are released from granules stored within the large luteal cells of the CL [29].

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Connecticut. Intact Merino ewes (*Ovis aries*) were group-housed during the fall and winter breeding seasons, and then maintained on pasture during the spring and summer anestrous periods. Merino sheep with transplanted ovaries were group-housed in pens year round, fed twice daily, and given water ad libitum. During intra-arterial infusions, sheep with ovarian autotransplants were housed in metabolism cages, subjected to a 24-h experimental period, and then returned to their group pens.

2.2. NPY localization

2.2.1. Tissue collection and preparation

To determine NPY localization in the CL during the breeding season, intact ovaries were collected from mature, cycling ewes (n = 4) by midventral laparotomy, performed under 1.5% isoflurane/oxygen anesthesia. Ovaries were divided into halves then immersed in 4% paraformaldehyde for 24 h, rinsed in phosphate buffered saline (PBS; 0.01 M, pH = 7.4) and then stored in 30% sucrose at 4 °C. Tissues were then repeatedly rinsed for 72 h through a series of PBS and embedded in paraffin. Porcine ovaries were collected from a local abattoir and processed in a similar manner to serve as NPY positive control tissue [30].

2.2.2. Fluorescent immunohistochemistry

Paraffin-embedded tissues were cut into 5-μm serial tissue sections and mounted on silane-coated slides. Sections were subsequently deparaffinized and then air-dried. Slides were then washed twice in PBS and incubated with 200 μL of a blocking solution (10% NGS,