Effect of the pretreatment with prolactin on the distribution of immunoreactive beta-endorphin through different ovarian compartments in immature, superovulated rats

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Summary

Beta-endorphin and prolactin (PRL) are natural inhibitors of ovulation via central and peripheral mechanisms, but their possible interactions within the ovary are still unknown. The aims of the present study were to determine the gene expression and the topographic distribution of beta-endorphin, and the possible changes evoked by the pretreatment with PRL on the ovarian beta-endorphin localization in immature, superovulated rats. Prepuberal female Wistar rats weighing 60–70 g were superovulated with 20 IU equine gonadotrophins and, 48 h later, 20 IU human chorionic gonadotropin (hCG). Four hours after the hCG injection, the rats received either 200 μg rat PRL i.p. (n = 12) or saline vehicle (n = 10). In the following morning the rats were killed and their ovaries were quickly removed. Beta-endorphin localization was assessed by immunohistochemistry and proopiomelanocortin (POMC) mRNA was detected by reverse transcription polymerase chain reaction (RT-PCR). Beta-endorphin was expressed mostly in the corpora lutea and perivascular stroma, but a weak to moderate immunostaining was also present in the theca cells and some granulosa cells of tertiary/antral and preovulatory follicles. The main differences observed in the distribution of ovarian beta-endorphin between the two groups were a more intense immunostaining in the granulosa cells of antral follicles, corpus luteum and stroma of PRL-treated rats. POMC gene transcripts were detected in 2/5 samples from the control group and in 3/7 samples from the PRL-treated group. Thus, the expression of beta-endorphin in tertiary/antral follicles is enhanced by PRL treatment in immature, superovulated rats, providing a putative mechanism by which PRL could inhibit the ovarian response to induced ovulation.

Introduction

Beta-endorphin, an endogenous opioid peptide derived from proopiomelanocortin (POMC), is present in both rodent and human ovaries with variable concentrations and distribution across the different phases of the reproductive cycle (Lolait et al. 1985, Aleem et al. 1986, Petraglia et al. 1987). In the human follicular fluid, the concentrations of beta-endorphin increase during the preovulatory period whereas other POMC-related peptides remain unchanged, suggesting a specific up-regulation of ovarian beta-endorphin production by the time of ovulation (Petraglia et al. 1987).

Despite the periovulatory increase in ovarian beta-endorphin concentration, its physiological role remains uncertain due to some divergence between the temporal and spatial distribution and some pharmacological effects of the peptide. In the rat ovary, during spontaneuous estrous cycles, beta-endorphin appears to be predominantly localized in the corpus luteum and, to a lesser extent, in developing follicles and interstitial cells (Lolait et al. 1985). The maximal intra-ovarian beta-endorphin concentration has been found at proestrus, when ovulation occurs (Lolait et al. 1985). However, the microinjection of beta-endorphin into the ovarian capsule is able to inhibit pharmacologically induced ovulation in immature rats (Faletti et al. 1995), suggesting that the periovulatory increase of ovarian beta-endorphin concentration favors an inhibitory rather than stimulatory effect of the peptide on the ovulation process. Indeed, beta-endorphin inhibits intra-ovarian molecular pathways involved in the triggering of ovulation (Faletti et al. 2003).

Excess circulating levels of the pituitary hormone prolactin (PRL) leads to ovulation arrest and interruption of the reproductive cycles. PRL inhibits ovulation by...
several mechanisms, including the stimulation of endogenous opioid peptide release in the hypothalamus (Quigley et al. 1980, Grossman et al. 1982), which in turn blunts the pulsatile release of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) (Cohen-Becker et al. 1986, Matsuzaki et al. 1994). However, direct ovarian effects of PRL have also been suggested, the mechanisms of which remain obscure.

In the present study we sought to determine the gene expression and the topographic distribution of beta-endorphin in the ovaries of immature rats submitted to superovulation with exogenous gonadotropins. In addition, we have investigated the possible effects of PRL on the ovarian beta-endorphin localization in this model of immature, superovulated rats.

Materials and methods

Twenty-two prepuberal female Wistar rats weighing 60–70 g were housed in a light and temperature-controlled environment (lights on from 5.00 a.m. to 7.00 p.m., 23 ± 2 °C) and were fed with commercial chow and tap water ad libitum.

Superovulation was induced with 20 IU equine gonadotrophins (PMSG, supplied by the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases [NIADDK]) in 0.2 ml saline given subcutaneously. Forty-eight hours later, the animals were injected i.p. with 20 IU human chorionic gonadotropin (hCG, Serono) dissolved in 0.2 ml saline to trigger ovulation. Four hours after the hCG injection (5.00 p.m., 23 ± 2 °C), the ovaries were fixed in 4% phosphate-buffered formaldehyde, pH 7.4 (% v/v) until 12 h at 4 °C. This is a specific polyclonal antibody that cross reacts with rat beta-endorphin but not with other related peptides. Sections were treated with biotinylated goat anti-rabbit IgG for 30 min at room temperature and incubated with the avidin–biotin–peroxidase complex (Vector, Burlingame, CA) for 60 min. Peroxidase activity was visualized by exposing the slides for 3 min to 1 mg/ml 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) in PBS containing 0.3% H2O2. The sections were then counterstained with hematoxylin. In the negative controls the primary antibody was preadsorbed with synthetic rat beta-endorphin (Peninsula Laboratories, San Carlos, CA, USA). Additional negative controls consisted in the replacement of the primary antibody by normal rabbit serum at equivalent dilution.

RNA extraction, complementary DNA synthesis and RT-PCR

Both ovaries of each animal were removed from the −80 °C freezer, pooled and homogenized in phenol–guanidine isothiocyanate (Trizol, Gibco BRL, Gaithersburg, MD, USA). Total RNA was extracted with chloroform and precipitated with isopropanol by 12,000 × g centrifugation at 4 °C. The RNA pellet was washed with 75% ethanol, resuspended in diethylylpyrocarbonate-treated water and quantified by light absorbance at 260 nm. First strand cDNA was synthesized from 2 μg total RNA using the Superscript first-strand synthesis system purchased from Invitrogen (Carlsbad, CA, USA). After denaturing the template RNA and primers at 70 °C for 10 min, 50 U reverse transcriptase was added in the presence of RT buffer (50 mM KCl, 20 mM Tris–HCl, pH 8.4), 2.5 mM MgCl2, 0.5 mM dNTP mix and 40 U RNAse inhibitor. The mixture (20 μl) was incubated at 42°C for 55 min, then heated at 70 °C to stop the reaction and stored at −20 °C.

RT-PCR was carried out in a final volume of 50 μl. Two microliters of the first strand synthesis reaction were incubated with PCR buffer (supplied with the enzyme), 1.5 mM MgCl2, 0.2 μM sense and anti-sense primers, 0.2 mM dNTP mix and 2 U Taq DNA polymerase (Life Technologies, São Paulo, Brazil). In order to improve sensitivity, a nested PCR strategy was applied to detect beta-endorphin precursor mRNA. The external primers (GenBank Accession No. AH002232) sense (5′ → 3′ AACTGCTGGCTTGATCC, bases 191–199 of exon 2 and 89–98 of exon 3, spanning intron B) and anti-sense (CGTCTGTGAGAGGGTACC, bases 610–629 of exon 3) generated a product of 550 base pairs (bp). The internal primers, sense (ATGGGTCACCTCGCTGGGA, bases 191–210) and anti-sense (CAGGACTTGCTCAACC, bases 470–490), both located in exon 3, generated a product of 300 bp. The housekeeping gene beta-actin

Immunohistochemistry

Formalin-fixed, paraffin-embedded ovarian specimens were cut into 4 μm slices, which were stained by immunohistochemistry using the avidin–biotin–peroxidase method as previously described (Reis et al. 2002). All samples and controls were processed together. After exposure to 1% H2O2 in methanol to block endogenous peroxidase, sections were treated with normal goat serum for 30 min to suppress non-specific binding. Rabbit anti-human beta-endorphin serum (kindly donated by Dr. A.F. Parlow, National Hormone & Peptide Program, NIDDK) was diluted 1:500 in phosphate-buffered saline (PBS) containing 1% bovine serum albumin and applied on the slides for

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