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Mu opioid receptor in the human endometrium: dynamics of its expression and localization during the menstrual cycle

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Objective: To study the dynamics of the expression and localization of the mu opioid receptor (MOR) in human endometrium throughout the menstrual cycle.
Design: Analysis of human endometrial samples from different menstrual cycle phases (menstrual, early/midproliferative, late proliferative/early secretory, midsecretory, and late secretory) by reverse transcription–polymerase chain reaction, Western blot, and immunohistochemistry.
Setting: Academic research laboratory.
Patient(s): Women from the Human Reproduction Unit of the Cruces University Hospital, fulfilling the following criteria: normal uterine vaginal ultrasound; absence of endometriosis, polycystic ovary syndrome, implantation failure, or recurrent miscarriage; and no history of opioid drug use.
Intervention(s): Endometrial samples of 86 women categorized into groups for the menstrual cycle phases: 12 menstrual, 21 early/midproliferative, 16 late proliferative/early secretory, 17 midsecretory, and 20 late secretory.
Main Outcome Measure(s): MOR gene and protein expression and localization in the different compartments of the human endometrium at different stages of the menstrual cycle.
Result(s): The expression of MOR mRNA and protein changed throughout the cycle in human endometrium. MOR expression increased during the proliferative phase and decreased during the secretory one. Lower values were found at menstruation, and maximum values around the time of ovulation. Small variations for each endometrial compartment were found.
Conclusion(s): The presence of MOR in human endometrium and the dynamic changes during the menstrual cycle suggest a possible role for opioids in reproduction events related to the human endometrium or endometriosis. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Mu opioid receptor, MOR, endometrium, menstrual cycle

The endogenous opioid peptides (EOPs) are derived from proopiomelanocortin (POMC), proenkephalin (PENK), and prodynorphin (PDYN) precursors and exert their effects by binding to the G-protein-coupled receptors δ-opioid receptor (DOR), κ-opioid receptor (KOR), and μ-opioid receptor (MOR) (1). EOPs are known to participate in the regulation of reproductive physiology at multiple sites (2) since opioid peptides and their precursors and receptors have been described in many of the male and female reproductive tissues. One of the best-known effects of opioid peptides on the reproductive system is their inhibitory role in the secretion of GnRH at the hypothalamic level and the tonic inhibition of the release of LH (3–5). But there is a growing body of evidence that indicates a participation of opioid peptides in the regulation of reproductive function through a direct local action within reproductive tissues (6).

One of the tissues where it is believed that the opioids are acting is the endometrium. Human endometrium is a complex tissue that regenerates and regresses with each menstrual

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cycle under hormonal control but also due to regulation by other factors like opioid peptides (7). In fact, the three opioid peptide precursors' mRNA has been described in endometrium: POMC in rat endometrial cells (6) and Ishikawa human endometrial adenocarcinoma cell line (8, 9); PENK in endometrial cells of human (10), cow (11), mouse (12), rat (6), and primate (13); and PDYN in human endometrial cells (14) and Ishikawa human endometrial cells (9). Moreover, opioid peptides such as β-endorphin and met-enkephalin have been found in the uterine fluid of the human and cow (15), and dynorphyns have been described in human and Ishikawa human endometrial cells (14), where β-endorphin has also been detected (8). Finally, DOR and KOR have been described in Ishikawa human endometrial cells, while MOR is absent (14, 16); in fact, MOR has been localized only in the uterine luminal epithelium cells of the pregnant mouse (17) and in endometriosis stromal cells (18–20). It has been reported that specific agonists for KOR bind to epithelial and stromal primary endometrial cell cultures (14, 21).

The presence of all the compounds of the opioid system in the endometrium denotes a role of this system in any of the processes holding the endometrium. On the one hand, it has been described that dynorphin, via KOR, may participate in the apoptotic processes related to endometrial tissue remodeling during early pregnancy or menstruation (21). On the other hand, it is known that estrogens and glucocorticoids suppress the secretion of endometrial β-endorphin, while the secretion of dynorphin is induced by GnRH, and that type-specific regulation of endometrial opioids suggests that each type of opioid peptide possesses a quite distinct physiological role within the uterine cavity (9). Regarding the possible role of the opioid system in implantation, it has been hypothesized that some peptides coming from PENK could locally modulate the immune response since the mRNA of PENK was dramatically increased in the vicinity of the implantation site of the pregnant mouse uterus (12). Finally, the absence of β-endorphin and met-enkephalin in the uterine fluid of postmenopausal women but the presence of both peptides during the menstrual cycle (with higher concentrations in the secretory phase than in the proliferative phase due perhaps to the stimulation by gonadal steroids) suggests a role for opioids during the menstrual cycle (15, 22). Moreover, the transient expression of KOR and MOR and the presence of PENK in the mouse myometrium could regulate the myometrial contractibility (17).

As can be seen, there is evidence to suggest that the opioid system is involved in some endometrial functions, but from our point of view there is a gap in the field, since to date there is no detailed study about MOR in the mammalian endometrium during the menstrual cycle. Therefore, the aim of the present study was to analyze the dynamics of the expression of the MOR gene and protein, as well as the localization of MOR, in human endometrium throughout the menstrual cycle.

MATERIALS AND METHODS

Ethics Statement

Written informed consent was obtained from all participants at the time of tissue collection, and ethical approval was provided by the Clinic Research Ethics Committee of the Basque Health System in the Cruces University Hospital (ethics approval no. CEIC EI4/36, 02/2015).

Human Endometrial Tissues

Endometrial tissues for this study were obtained by endometrial biopsy from 86 women ages 22–39 years with regular menstrual cycles (25–35 days) who had not undergone hormone treatment in the previous 3 months. Samples were collected from patients using a Cornier pipelle (Laboratoires CCD). All the participants were patients from the Human Reproduction Unit of the Cruces University Hospital, fulfilling the following criteria: normal uterine vaginal ultrasound; absence of endometriosis, polycystic ovary syndrome, implantation failure, or recurrent miscarriage; and no history of opioid drug use. Prophylactic antibiotics were not used. There was no case of infection or other side effects. Endometrial dating was determined histologically by an experienced pathologist (L.A.) according to the criteria of Noyes et al. (23).

These samples were categorized in groups for the different menstrual cycle phases: phase I, menstrual (days 1–5, n = 4); phase II, early to midproliferative (days 6–10, n = 7); phase III, late proliferative to early secretory (days 11–19, n = 9); phase IV, midsecretory (days 20–24, n = 7); and phase V, late secretory (days 25–28+, n = 8). Collected tissues were either snap-frozen in liquid nitrogen and stored at −80°C for protein and mRNA extraction or fixed in buffered formalin (pH 7.4) and processed to paraffin wax blocks for immunohistochemistry (IHC).

Reverse Transcription

RNA from endometrial tissue (obtained from some samples of each stage) and cerebral cortex (positive control) were isolated with the RNasy mRNA Purification Kit (Ambion). The procedure for obtaining the cDNA was performed with ImProm-II Reverse Transcription System (Promega) according to the manufacturer's instructions. Briefly, about 150 ng of RNA and random primers was heated at 65°C for 10 minutes and chilled on ice for 5 minutes. Then, after adding the reverse transcription mix, the mixture was annealed at 25°C for 5 minutes. A first-strand synthesis reaction was carried out at 55°C for 60 minutes, and the reverse transcriptase was inactivated at 70°C for 15 minutes.

Real-Time Quantitative Polymerase Chain Reaction (PCR) Analysis

Real-time quantitative reverse transcriptase (RT)-PCR was performed on 29 endometrial samples throughout the menstrual cycle; phase I (n = 5), phase II (n = 9), phase III (n = 3), phase IV (n = 6), and phase V (n = 6). Quantitative PCR was performed in three replicates with the StepOne thermocycler using a TaqMan assay (Applied Biosystems), specifically designed for recognizing MOR (Hs01053957_m1). The thermal profile for this PCR consisted of a "holding stage" of 20 seconds at 95°C and 40 cycles with two steps: 1 second at 95°C and 20 seconds at 60°C. We used GAPDH