OBSTETRICS

The potential role of prolactin as a modulator of the secretion of proinflammatory mediators in chorioamniotic membranes in term human gestation

Veronica Zaga-Clavellina, MSc, PhD; Adalberto Parra-Covarrubias, MD; Jorge Ramirez-Peredo, MD; Rodrigo Vega-Sanchez, PhD; Felipe Vadillo-Ortega, MD, PhD

OBJECTIVE: To test the effect of prolactin (PRL) on expression of proinflammatory cytokines and matrix metallopeptidase 9 (MMP-9) in vitro.

STUDY DESIGN: Tissue explants were incubated from 4 to 48 hours alone or in the presence of 500 ng/mL PRL, and mRNA expression in tissues and secretion of interleukin (IL)-1β, tumor necrosis factor alpha (TNF-α), MMP-2, and MMP-9 was quantified.

RESULTS: Fetal membranes secreted IL-1β, TNF-α, and MMP-9 in culture with consistent low concentration during the first 24 hours and then increased progressively. The presence of PRL during explant incubation significantly decreased the patterns of IL-1β, TNF-α and MMP-9 secretion along culture ($P < .001$). MMP-2 secretion was unaffected by PRL. The relative basal expression of IL-1β mRNA (1.2 ± 0.87) was reduced by 80% in the presence of PRL after 32 hours of incubation of the membranes ($P = .001$). The expression of the TNF-α mRNA was not modified by the presence of PRL (0.06 ± 0.01) compared with the basal expression levels (0.05 ± 0.01). MMP-9 mRNA basal expression (0.018 ± 0.008) was significantly reduced ($P = .001$) in the presence of PRL after 32 hours (0.002 ± 0.0005).

CONCLUSION: PRL may be a potential candidate as a key signal controlling the expression of signals related to the proinflammatory reaction associated with human labor.

Key words: amniochorion, human labor, inflammation, prolactin

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The network of signals that elicits progressive and coordinated responses in the myometrium, the cervix, and the fetal membranes and regulates normal human labor is incompletely characterized.[1] The primary signal for the initiation of human labor is unknown, but evidence suggests that the activation of the choriodecidual microenvironment is central for the progress of labor.[2,3] Choriodecidua activation is associated with the secretion of a set of proinflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor alpha (TNF-α), and IL-6, among others.[4,5] These paracrine and autocrine signals induce the expression of a second wave of effectors that include uterotonic compounds, such as prostaglandins[6] and oxytocin, and extracellular matrix degrading enzymes (matrix metalloproteinases).[7]

The initiation of labor in some animal species is related to progesterone-withdrawal[8] or activation of the adrenal axis,[9] but no such primary mechanisms have been identified in humans, and the equivalent triggering signal remains uncovered. Among the candidate signals, prolactin (PRL), a pleiotropic neuroendocrine hormone synthesized by the decidua, has been proposed as a mediator of the initiation of labor.[10] Values of this peptide increase 10 to 20-fold above their normal concentrations during human pregnancy, with peak values at 20-24 weeks of gestation, followed by a decline.[11,12] The maximum decline in serum, amniotic fluid, or decidual tissue occurs during the first stage of labor, which is followed by a second rise that persists during second phase of labor and even postpartum.[13] PRL is an inhibitor of prostaglandins synthesis,[14] and a sudden decrease in PRL promotes the local synthesis of prostaglandins, which will exert their uterotonic effects in the next phase of labor. Increasing concentrations of PRL in the second wave also directly affect the induction of myometrial contractions.[13]

Recent information on the composition and function of the choriodecidua has pointed to the selective activation of this microenvironment during human labor.[3] This information opens new targets for local signaling between tissues and migrating cells, resulting in the induction of some responses contributing

From the Departments of Cell Biology (Dr Zaga-Clavellina), Endocrinology (Dr Parra-Covarrubias), Rheumatology (Dr Ramirez-Peredo), and Nutrition (Dr Vega-Sanchez), Instituto Nacional de Perinatología Isidro Espinosa de los Reyes, and Unidad de Vinculación Faculty of Medicine, UNAM en el INMEGEN (Dr Vadillo-Ortega), Mexico City, Mexico.

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to labor initiation or promotion. In this study, we evaluate the effects of PRL on the secretion of mediators involved in the rupture of the fetal membranes, including signals such as IL-1β, TNF-α, and MMP-9, an enzyme involved in the degradation of the fetal membrane's extracellular matrix.

Materials and Methods

Collection of samples

The study was approved by the internal review board of the Instituto Nacional de Perinatología Isidro Espinosa de los Reyes in Mexico City (register 212250-02141). Patients provided written informed consent for the collection of samples and subsequent analysis. All patients included in this study lived in Mexico City, they were Hispanic and of medium socioeconomic status. Specific information from each patient is described in the Table. Patients with history of preterm delivery, hypertension, diabetes mellitus, thyroid, liver, or choric renal diseases were excluded.

Fetal membranes were collected from women who delivered by elective cesarean section, without evidence of active labor and with neither clinical nor microbiologic signs of chorioamnionitis or lower genital tract infection. General microbiologic cultures were conducted on the placenta and fetal membranes immediately after delivery, a sterile swab was rolled across randomly selected areas and only membranes with proven sterility were used for this study. The specimens were transported to the laboratory in sterile Dulbecco's modified Eagle medium (DMEM; Gibco BRL, Bethesda, MD), and rinsed in sterile phosphate-buffered saline solution to remove adherent blood clots.

Fetal membrane explants

The membranes (including amnion and choriodecidua) were cut into disks (12-mm diameter) using a biopsy punch. We did not capture areas near to the placenta. One piece of membrane was placed in each well of a 24-well tissue culture plate with 1.0 mL of DMEM supplemented with 0.1% lactoalbumin hydrolysate, one mM sodium pyruvate, and antibiotic-antimycotic solution (penicillin 100 U/mL; streptomycin 100 μg/mL; amphotericin B 0.25 μg /mL). The explants were cultured in triplicate at 37°C under an atmosphere of 5% CO_2/95% air and saturated water, separate cultures were performed for each time point. All reagents for cell culture were purchased from Gibco BRL. All explants were routinely followed for cell viability using the XTT Assay (R&D Systems, Minneapolis, MN).

PRL's effect on IL-1β, TNF-α, and MMP-9 secretion

To characterize the effects of PRL on the basal secretion and modulation of the different mediators, the explants were incubated alone or in the presence of 500 ng/mL human recombinant PRL (Sigma, St. Louis, MO), and in vitro secretion of IL-1β, TNF-α, and MMP-9 were plotted on a time-response curve at 4, 8, 24, 28, 32, and 48 hours.

In a different set of experiments, another set of explants from the same 15 women were incubated with 500 ng/mL PRL for the first 24 hours, and then conditioned media was eliminated and the explants were rinsed with phosphate-buffered saline solution Hanks solution and maintained for an additional incubation period of 24 in DMEM with 0.1% lactoalbumin hydrolysate (Gibco BRL) without PRL. Other explants were incubated during the first 24 hours in the absence of PRL and then, 500 ng/mL PRL was added, and the culture was maintained for an additional 24 hours.

A function-blocking anti-PRL antibody (MCA714 clone; Serotec, Oxford UK) was used as a control for the effect of PRL addition. The antibody was mixed (2.5 μg/mL) for 1 hour at 37°C with the media containing PRL and then added to selected independent experiments.

ELISA

IL-1β, TNF-α, MMP-2, and MMP-9 concentrations were measured in all conditioned media using specific DuoSet enzyme-linked immunosorbent sandwich assays (R & D Systems, Minneapolis, MN). Monoclonal antibodies against, IL-1β, TNF-α, MMP-2 and MMP-9 were used as the capture antibodies, and polyclonal biotinylated antibodies against both cytokines were used as the detection antibodies. For the IL-1β assay, a standard curve was developed from 4 to 260 pg/mL, and the sensitivity was 2 pg/mL. The TNF-α assay was linear from 15 to 960 pg/mL with a sensitivity of 5.0 pg/mL. The

TABLE
Clinical characteristics of patients

Patient	Age	Gestational age at birth	Parity	Marital status
1	34	38	1	Married
2	23	37	2	Single
3	38	38.1	3	Married
4	22	36.6	5	Married
5	25	38.1	4	Married
6	23	38.3	3	Married
7	31	39	4	Married
8	22	37.3	3	Married
9	37	38.4	2	Married
10	20	38.5	1	Single
11	34	39.5	3	Married
12	20	39.6	1	Single

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