Effect of leptin on prolactin and insulin-like growth factor-I secretion by cultured rat endometrial stromal cells

Kamani H. Tennekoon,Ph.D.[a,b] *Thampoe Eswaramohan,B.Sc.*[a,b] *and Eric H. Karunanayake, Ph.D.*[b]

[a] Department of Physiology, [b] Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Colombo, Sri Lanka

Objective: To study the possible effect of leptin on PRL and insulin-like growth factor (IGF)-I secretion from rat endometrial stromal cells.
Design: The effect of recombinant murine leptin on the secretion of PRL and IGF-I by cultured rat endometrial cells was investigated.
Setting: Academic institutions.
Animal(s): Laboratory bred virgin female rats aged 3–4 months.
Intervention(s): Endometrial stromal cell (ESC) cultures in the fourth passage stimulated with 1–1,000 ng/mL of leptin for 24 hours and with 1 ng/mL leptin for 24–72 hours.
Main Outcome Measure(s): Measurement of PRL and IGF-I levels in the conditioned media by enzyme immunoassay.
Result(s): Endometrial stromal cells grown in vitro secreted both PRL and IGF-I into the medium and the concentrations significantly increased with passage of time even in the absence of leptin. The increase in PRL was seen mainly at 72 hours and in IGF-I at 24 and 72 hours. Presence of leptin in the culture medium (1–1,000 ng/mL) further enhanced PRL secretion in a dose-dependent manner and this effect was seen with all leptin doses used. Leptin also increased PRL secretion in a time-dependent manner and the increase was seen at 24, 48, and 72 hours. Leptin did not significantly affect IGF-I secretion either in a dose- or a time-dependent manner.
Conclusion(s): Biological effects of leptin on the rat endometrium include dose- and time-dependent stimulatory effects on stromal cell PRL secretion. (Fertil Steril® 2007;88:193–9. ©2007 by American Society for Reproductive Medicine.)

Key Words: Leptin, prolactin, endometrial stromal cells

Leptin, the first adipocyte hormone to be characterized, is now well known to exert diverse effects on reproduction (1–4). Genetically leptin-deficient and leptin receptor-deficient humans and rodents have reproductive impairment (5–7). Exogenous leptin reverses sterility in genetically leptin-deficient mice (5), advances sexual maturation in normal mice (8), and restores menstrual cycles in women with hypothalamic amenorrhea (9).

Leptin acts through its cognate receptor Ob-R and several isoforms of the receptor exist (10). The long form, Ob-Rb, is expressed mostly in the hypothalamus and is thought to mediate the effects on food intake through the JAK-STAT pathway. Most of the peripheral tissues express the short isoforms as well. These are thought to act through other pathways such as the MAPkinase pathway. Leptin receptors have been identified at different sites of the reproductive system, including the anterior pituitary, gonads, endometrium, and placenta.

Endometrial leptin receptors have been identified in women and in several animal species (11–14). Both endometrial epithelial and stromal cells express leptin receptors, thus becoming targets for leptin action. In mice, leptin receptor expression is greater in the stromal cells than in the epithelial cells (14).

In women, endometrial leptin receptor expression varies with the stage of the menstrual cycle (15, 16). Highest leptin receptor expression was observed in the early luteal phase by some investigators (15) and in the late luteal phase by others (11, 16). One study reported a cyclic variation in leptin receptors in luminal and glandular epithelium with highest expression in the luminal epithelium (16). They further observed a slight expression in the stromal cells throughout the menstrual cycle. Furthermore, endometrial leptin receptor deficiency is associated with infertility in women (11).

Leptin has been shown to stimulate secretion of interleukin-6 and several chemokines by cultured human endometrial stromal cells and by the human endometrial epithelial

Received April 27, 2006; revised and accepted November 27, 2006.
Supported by the National Research Council, Sri Lanka (grant no.: 00-07), WHO Rockefeller Career Development Fellowship, and Swedish Agency for Research Corporation with Developing Countries Grant for Molecular Biology and Biotechnology. Parts of this work was presented at the Fertility 2005, United Kingdom and at Sri Lanka Association for the Advancement of Science, Annual Academic Sessions, 2005.
Reprint requests: Kamani H. Tennekoon, Ph.D., Department of Physiology, Faculty of Medicine, University of Colombo, Kynsey Road, Colombo 8, Sri Lanka (FAX: 94-11-2552529; E-mail: khtennekoon@med.cmb.ac.lk).

cell line HHUA (17). Some investigators have observed a stimulatory effect of leptin on the secretion of leukemia inhibitory factor by human endometrial cells (18), but other researchers have failed to observe a similar effect (17). Leptin up-regulates expression of β3-integrin, a marker of endometrial receptivity, in human endometrial epithelial cell cultures (19).

Leptin and its receptors are expressed in the embryo, indicating a role for leptin in the embryo–maternal dialogue during implantation. Several investigators have shown that leptin is required for preimplantation embryo development (20, 21), whereas others have demonstrated a down-regulation of leptin receptors in the uterine implantation sites (14). Immunoreactivity to the long form of the leptin receptor has been observed in human implantation sites with positive signals in the cytotrophoblast and in the epithelial and stromal cells of the maternal decidua (16). In the mouse, trophoblast invasion was stimulated by leptin in vitro (22).

Prolactin is a secretory protein of endometrial stromal cells. PRL receptors are found in glandular epithelial cells, implicating a paracrine role for PRL in the endometrium (23). The chemical structure and immunogenecity of endometrial PRL is identical to that of anterior pituitary PRL (24). However, PRL of endometrial origin is transcribed from an alternative promoter resulting in a longer mRNA transcript (25). Factors that regulate endometrial PRL secretion differ from that regulating pituitary PRL secretion, and include substances such as cyclic AMP, relaxin, insulin-like growth factor (IGF)-I, and P (23).

The pattern of endometrial PRL secretion with higher levels in mid and late secretory phases, the concomitant increase in PRL receptor expression in the endometrial glands, and the known immunomodulatory and angiogenic actions of PRL suggests that PRL plays a significant role in implantation (26). Furthermore, PRL and PRL receptor-deficient mice are sterile (27, 28) and PRL expression is impaired in women with recurrent miscarriages or unexplained infertility (29).

Insulin-like growth factor-I is expressed in the mid and late proliferative, and early secretory endometrium. Insulin-like growth factor-I immunoreactivity is localized to endometrial epithelial and stromal cells, as well as to the myometrium (30). Type I IGF receptor, which mediates most of the IGF-I action, is localized to the same cells indicating an autocrine and a paracrine role for IGF-I in the uterus. Insulin-like growth factor-I is thought to mediate mitogenic effects of estrogen (E) and both E and P stimulate uterine IGF-I synthesis (31, 32). Furthermore, exogenous IGF-I stimulated PRL secretion and inhibited IGF-binding protein-1 (IGFBP-1) secretion from cultured endometrial stromal cells (33).

The IGFBP-1, in turn, has been shown to down-regulate mitogenic effects of IGF-I on endometrial stromal cells (34). Insulin-like growth factor-I has also been shown to stimulate proliferation and migration of mouse ectoplacental cone cells, suggesting a role in placental development (35). In view of the endometrium being a target tissue for leptin, and known and postulated effects of PRL and IGF-I in the endometrium, we investigated possible effect of leptin on PRL and IGF-I secretion by cultured rat endometrial stromal cells in the present study.

MATERIALS AND METHODS

Isolation and Culture of Rat Endometrial Stromal Cells

Primary endometrial stromal cell cultures were established using 3- to 4-month-old, virgin female Sprague Dawley rats from the Animal House at the Faculty of Medicine, University of Colombo. The study was approved by the Institutional Review Board (IRB). Rats were housed under standard conditions (12 hours light and 12 hours dark with ad libitum access to food and water). Stromal cells were prepared according to previously reported methods (36) with some modifications. Vaginal smears were examined microscopically to identify rats in the metestrus stage.

Rats in the metestrus stage were killed by injecting pentathiol sodium. Uteri collected, trimmed of fat, and washed in Hanks balanced salt solution (HBSS) to remove blood. Uteri were then minced into small fragments of approximately 1 mm^3 in HBSS and digested with collagenase type I (0.42% w/v) for 30 minutes at 37°C with intermittent agitation.

The supernatant was filtered through 100 μm filters to remove tissue debris and glandular parts and centrifuged at 800 x *g* for 5 minutes at 10°C. The cell pellet was suspended in Dulbecco's modified Eagle medium (D-MEM/F-12) supplemented with fetal calf serum (FCS) (10% v/v) and antibiotic antimycotic (1% v/v). An aliquot of the cell suspension was used for counting cells in a hemocytometer (Thoma, Hamburg, Germany) and for determination of viability by trypan blue exclusion.

Once the viability was ensured to be 90% or more, cells were seeded onto 24-well culture plates (Nunc, Rochester, NY) at a density of 300,000 cells per well. These were incubated in FCS and antibiotic antimycotic supplemented DMEM/F-12 at 37°C with 5% CO_2 and grown until confluence. Conditioned medium was aspirated and replaced with fresh medium 24 hours later and every 48 hours thereafter. Representative cells were stained for vimentin and cytokeratin to ensure that the monolayers comprised of 98% or more stromal cells.

Antibiotic antimycotic (15240-096), Collagenase Type I (17100-017), DMEM/F-12 (11039-021), FCS (10099-133), and HBSS (14170-088) were from Invitrogen Corporation, Grand Island, NY. Vimentin (N1521, clone V9) and cytokeratin (N1590, clone AE1/AE3) primary antibodies, secondary antibody (biotinylated link antibody and alkaline phosphatase labeled streptavidin; K 610, DAKO LSAP2 kit), substrate-chromogen (fast red substrate; K 0597), and 100