Effect of ghrelin on proliferation, apoptosis and secretion of progesterone and hCG in the placental JEG-3 cell line

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SUMMARY

To determine the effect of ghrelin on placental cell proliferation, apoptosis and hormone secretion we cultured human JEG-3 cells with 100, 250, 500 or 1000 pg/ml of ghrelin for 48 hours. Ghrelin stimulated cell proliferation and decreased caspase-3 activity. All of the investigated ghrelin concentrations decreased progesterone (P₄) but had no effect on human chorionic gonadotrophin (hCG) secretion. Stimulatory effects on cell proliferation paralleled inhibitory effects on cell apoptosis suggesting a possible role for ghrelin in placental formation or remodeling. Reproductive Biology 2010 10 2: 159-165.

Key words: ghrelin, JEG-3, proliferation, apoptosis, progesterone, hCG

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INTRODUCTION

In recent years, compelling evidence has implicated ghrelin in reproductive processes, such as the regulation of embryo development and implantation. The presence of ghrelin mRNA and ghrelin receptors in the placenta clearly suggests a role for this hormone in placental physiology. In humans, high placental ghrelin mRNA and peptide levels are seen in the first trimester of pregnancy (primarily in cytotrophoblasts) but not in the third trimester [4]. Ghrelin is involved in the decidualization of human endometrial stromal cells. During pregnancy, the ghrelin level is the highest at mid-pregnancy and lowest in the third trimester (at the time of increased body weight; [3]). This suggests that ghrelin is an important autocrine/paracrine factor for the growth and maintenance of the placenta during pregnancy.

Due to the limited availability of first trimester placental tissue for trophoblast isolation, the human choriocarcinoma cell line JEG-3 has been used in this study as an in vitro model to examine the direct effects of ghrelin on placental hormone secretion, cell proliferation and apoptosis. These cells maintain many characteristics of human trophoblast cells and have been widely used to study feto-placental physiology. Additionally, JEG-3 cells produce many peptides and steroid hormones found in normal trophoblast cells, such as hCG, GnRH and progesterone (P4).

MATERIAL AND METHODS

The JEG-3 choriocarcinoma cell line was obtained from the American Type Cell Culture (Rockville, MD, USA). The cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) without phenol red and supplemented with 10% charcoal-stripped, fetal bovine serum (FBS), 100 UI/ml penicillin and 100 µg/ml streptomycin (Sigma Chemical Co. St. Louis, MO, USA). The viability of the cells (91%) was determined before seeding by the Trypan blue exclusion test. The cells were plated in 96-well plates (Nunc, Denmark) at the density of $3 \times 10^5$ cells per well and initially cultured for 24 h under a humidified 5% CO$_2$/95% air atmosphere at 37°C. Then, the medium was
replaced and cells were cultured for 48 h with increasing doses (100, 250, 500 and 1000 pg/ml; [9]) of ghrelin (Sigma Chemical Co. St. Louis, MO, USA). Medium P₄ and hCG concentrations were determined by EIA (DiaMetra kit, Italy) according to the manufacturer’s instructions. DNA synthesis in proliferating cells was determined by measuring bromodeoxyuridine (BrdU) incorporation with the commercial Cell Proliferation ELISA System (Roche, Germany). Caspase-3 activity was used as a marker for cell apoptosis and was measured with the use of caspase-3 colorimetric substrate – Ac DEVD-pNA-(7-amino-4methyl coumarin, Sigma Chemical Co. St. Louis, MO, USA). Data are presented as means±SEM of three independent experiments. Proliferation and caspase-3 activity data are expressed as a percentage of control cultures (100%) but statistical analysis was performed on raw data. Each treatment was repeated three times (n=3) in quadruplicate. All data were analyzed by one-way analysis of variance followed by Tukey test.

RESULTS AND DISCUSSION

The data presented here are the first to show that ghrelin acts in a dose dependent manner on human placental JEG-3 cell proliferation, apoptosis and hormone secretion. Stimulatory action on cell proliferation with parallel inhibitory action on cell apoptosis was observed under the influence of 250 and 500 pg/ml of ghrelin (p<0.05; fig. 1). Both processes maintain placental tissue homeostasis. Placental growth is complex and influenced by many factors, depending on a delicate balance among cell proliferation, differentiation and death. There are data showing the stimulatory action of ghrelin on cell proliferation as well as the inhibitory action on cell apoptosis in adipocytes [5], cardiomyocytes [2] and ovarian granulosa cells [11]. Our previous published data showed an increase in proliferation with simultaneous inhibition of cell apoptosis in ovarian follicular cells of pigs [8].

Another interesting finding is the inhibitory action of ghrelin on P₄ secretion. Ghrelin concentrations found in plasma of obese (100 and 250 pg/ml) and anorectic (1000 pg/ml) women [9] significantly decreased P₄ secretion in the current study (fig. 2A; p<0.05). Only 500 pg/ml of ghrelin,
Ghrelin and JEG-3 cells function

A concentration observed in the blood of normal, non-obese women, did not affect P₄ secretion by JEG-3 cells. There are data showing the inhibitory effect of ghrelin at doses of 10⁻⁷ to 10⁻¹² M on P₄ secretion by human luteal cells [12] and the lack of an effect of ghrelin at doses of 10⁻⁸ to 10⁻¹⁰ M on P₄ secretion by the rat adrenal cortex [1]. This suggests a tissue-dependent effect of ghrelin. The increase in cell proliferation and inhibition of P₄ secretion demonstrated in the current study could be explained by the results of Leavitt and Takeda [6] who found that P₄ is not required for the proliferation of artificially induced decidualoma cells in vitro.

A number of studies showed an important role for hCG in the regulation of cell proliferation, differentiation and apoptosis during human placental
Key points:

- Results of the presented data showed that only 100 pg/ml of ghrelin, a concentration found in obese women [9], significantly decreased hCG secretion by JEG-3 cells (fig. 2B).
- Some studies in which placental cells were exposed to various chemical agents demonstrated changes in cell proliferation with alternating changes in hCG level; all these agents decreased DNA synthesis or cell proliferation.
- Prast et al [7] showed that hCG did not significantly affect the proliferation of trophoblast SGHPL-5 cells but was rather promoting trophoblast invasion and migration.

In conclusion, we observed the regulatory effect of ghrelin on cell proliferation and apoptosis, which suggests that ghrelin may promote placental formation and development. The decrease of P₄ and the lack of an effect on hCG production in response to ghrelin was not linked to cell proliferation, but it was rather a specific response. Only 100 pg/ml of ghrelin, the concentration observed in the blood of obese women, decreased JEG-3 cell secretion of hCG, a main marker for trophoblast differentiation. Probably, the low hCG level reflects an interruption of trophoblast function culminating in placental insufficiency and fetal growth restriction.
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REFERENCE
