EFFECT OF LEPTIN, INSULIN, AND IGF-I ON GnRH-INDUCED LH SECRETION FROM PORCINE PITUITARY CELLS IN VITRO

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Received for publication October 17, 2006

Abstract

The objective of this study was to analyse the response of porcine pituitary cells to leptin, insulin, and IGF-I in vitro. Pituitary cells were cultured in McCoy 5A medium without hormones (negative control), with GnRH (4x10^-9 M/L) (positive control), with GnRH and 10^-11-10^-6 M/L of leptin, with GnRH and 3.9-91.0 mIU/L of insulin, or with GnRH and 1.03-39.2x10^-9 M/L of IGF-I. After 6, 12, 18, 24, 30, and 36 h of cell incubation, the secretion of LH was determined. The obtained results showed that the effect of leptin and insulin on LH secretion from pituitary cells was dependent on the used dose of leptin. Leptin in concentrations 10^-9, 10^-8, and 10^-7 M/L were significantly (P≤0.05) enhanced, whereas in concentration 10^-6 M/L suppressed a LH secretion. Insulin at a dose from 3.9 to 13.0 mIU/L caused an increment, whereas in higher concentrations – a significant (P≤0.05) drop in LH secretion. Almost full positive correlation (r=0.98) between the level of IGF-I in the culture medium and LH secretion was found.

Key words: swine, pituitary cells, leptin, insulin, IGF-I, LH.

Leptin plays a significant role in the control of female reproduction. The rise in leptin level is one of the earliest signals initiating the puberty by the activation of the hypothalamic–pituitary–ovarian axis. The physiological increase in plasma leptin concentration in adult ewes, especially during the late luteal and follicular phase, contributes to the augmentation of the ovulation rate (4, 13). However, in many species, inordinately high plasma leptin concentration is related to fertility disorders, among other things, to the inhibition of ovulation followed by ovarian cysts (OC) development. OC is one of the essential reasons of the temporary or persistent infertility in sows (8). The main etiological factor of the OC is an inappropriate, usually too low, amplitude of preovulatory LH surge, whereas fatness and insulin resistance predispose to OC development. Fatness is connected with the augmented quantity of white fatty tissue - the main source of leptin. Insulin resistance, on the other hand, causes an increase in blood concentration of insulin (12) – the significant stimulator of leptin synthesis and secretion (6). Additionally, leptin is the most significant metabolic signal that can regulate growth hormone secretion from the anterior pituitary (9). In normal conditions GH stimulates the hepatic- or peripheral-derived IGF-I secretion and most of the actions of the growth hormone are mediated through IGF-I. In this respect, leptin may influence pituitary and ovarian activity also via IGF-I (11). Therefore, the objective of the present study was to examine the direct effect of leptin, insulin, and IGF-I on GnRH-induced LH secretion from porcine pituitary cells.

Material and Methods

The pituitary glands were obtained at slaughter from sows with active healthy ovaries being at the follicular phase of their oestrous cycle. The isolation of pituitary cells was carried out through the digestion of the pituitary glands with 0.25% trypsin solution. The suspension of the cells in the trypsin solution, combined with the preparatory medium (DMEM supplemented with 0.1% BSA, 0.08% glucose, 0.59% HEPES, and gentamicin in the final concentration 20 µg/mL) was centrifuged at 1 200 rpm for 10 min. The sedimented cells were twice washed and finally cultured in McCoy 5A medium containing 2.5% foetal calf serum, 10% horse serum, mixture of amino acids and vitamins, 0.59% HEPES, gentamicin (20 µg/mL), and adjusted to pH 7.4. One ml (in the case of LH secretion analysis) or 100 µl (in the case of proliferation index determination) of dispersed cell suspension (2.5 × 10^5/mL) was transferred to each culture dish of 24-well (or 96-well, respectively) culture plate and incubated for 84 h at 37°C under the atmosphere of 5% CO2. After attachment to the dishes, the cells were washed and finally incubated with McCoy 5A medium:
- without hormones (negative control);
- with GnRH (4x10^{-9} M/L) (positive control);
- with GnRH (4x10^{-8} M/L) and 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} or 10^{-6} M/L of leptin, respectively;
- with GnRH (4x10^{-8} M/L) and 3.9, 9.0, 13.0, 34.0 or 91.0 mIU/L of insulin, respectively;
- with GnRH (4x10^{-8} M/L) and 1.03x10^{-9}, 3.03x10^{-9}, 7.22x10^{-9}, 11.7x10^{-9}, or 39.2x10^{-9} M/L of IGF-I, respectively.

After 6, 12, 18, 24, 30, and 36 h of incubation, the samples of the media were withdrawn for analysis of LH secretion. Simultaneously proliferation index of cells treated with leptin, insulin, and IGF-I were determined.

Assessment of cell proliferation was based on the reduction of the tetrazolium salt (MTT) into a blue formazan. Both control and experimental cultures were pulsed with 15 µl of MTT (for 3 h at 37°C) and then solubilised with SDS overnight. The optical density (OD) of the formed blue formazan was measured by a ELISA microplate reader at the wavelength of 600 nm (16). The results were used to determine LH secretion levels. LH concentration in culture medium was determined using LH [^{125}I] IRMA KIT (Orion Diagnostica, Spectria, Finland). LH secretion was expressed as a concentration (IU/L) of the hormone, which was released to culture medium by about 2.5 x 10^4 gonadotrophs during 6, 12, 18, 24, 30, and 36 h, respectively.

Results

The effect of leptin on LH secretion from porcine pituitary cells in vitro. LH secretion by 2.5 x 10^4 cells averaged 0.745 ± 0.12 IU/L in the positive control culture. The addition of 10^{-8} M/L of leptin to culture medium resulted in a significant (P ≤ 0.05 or P ≤ 0.001, respectively, Fig. 1) increase in LH secretion during the whole period of experiment, with the maximum value 2.484 ± 0.27 IU/L after 36 h (Figs 1 and 2). In the presence of leptin in concentration 10^{-7} and 10^{-6} M/L, the marked (P ≤ 0.05) increase in LH was noted only after 24, 30, and 36 h. The treatment with 10^{-11} and 10^{-10} M/L of leptin did not affect LH secretion significantly. In contrast, pituitary cells incubated with the highest dose of leptin (10^{-6} M/L) decreased the amounts of secreted LH (Fig. 1).

![Fig. 1. The influence of leptin (10^{-11}-10^{-6} M/L) on GnRH-induced LH secretion from porcine pituitary cells in vitro. *, ** - statistical significance (*P ≤ 0.05, **P ≤ 0.001) of the difference between the respective values obtained after GnRH + leptin administration and positive control.](image-url)
Fig. 2. The picture of porcine pituitary cell culture – A. positive control; B. the culture after 36 h of incubation with leptin (10^{-8} M/L). Magnification: A: 400x, B: 800x. (SG – secretory granules).

Fig. 3. The changes in GnRH-induced LH secretion from porcine pituitary cells in vitro under the influence of insulin. *, ** - statistical significance (*P \leq 0.05, **P \leq 0.001) of the difference between respective value obtained after GnRH + insulin administration and positive control.  
Correlation coefficient r=0.98

Fig. 4. The relationship between IGF-I concentration in culture medium and LH secretion from porcine pituitary cells in vitro.
Influence of insulin and IGF-I on LH secretion. The effect of insulin on LH secretion from porcine pituitary cells in vitro was dose dependent. The dose of 3.9 to 13.0 mIU/L of insulin caused a significant or highly significant (P ≤ 0.05 or P ≤ 0.001) increment, whereas higher doses – a significant (P ≤ 0.05) drop in LH secretion (Fig. 3). IGF-I; on the other hand, independently of the used dose, acted as a stimulant on LH secretion. Almost full positive correlation (r = 0.98) between the quantity of secreted LH and the dose of IGF-I was found (Fig. 4).

Discussion

There is the evidence that changes in metabolic state and associated alterations in metabolic hormones and growth factors, such as leptin, insulin, and IGF-I, influence the reproductive axis in the sow (15) resulting in normal ovarian activity or ovarian disorders, like cyst development. In swine, leptin regulates hypothalamic gonadotropin-releasing hormone (GnRH) release and subsequent LH secretion (3). Our results show that leptin also directly affects LH secretion from the porcine pituitary cells, independently of hypothalamic input. Leptin in concentrations 10^{-9}, 10^{-8}, and 10^{-7} M/L significantly (P ≤ 0.05) enhanced, whereas at a dose 10^{-6} M/L suppressed LH secretion from porcine pituitary cells.

Although it is well established that the development of ovarian cyst and other ovarian disorders connected with inadequate LH secretion often is associated with hyperinsulinaemia (18), the effect of insulin and IGF-I on the pituitary gland are still relatively little known, especially in swine. There are the data showing that acute infusion of insulin in rodents and chronic insulin infusion in ruminants in vivo can influence the pituitary gland, through the hypothalamus (7, 10). Findings obtained on rats show that both insulin and IGF-I are able to increase LH secretion from cultured rat pituitary cells (1, 18, 19). However, there are not any data on the direct effect of insulin and IGF-I on LH secretion from pituitary cells of swine in vitro. The results of our study on isolated porcine pituitary cells showed that insulin at a dose from 3.9 to 13.0 mIU/L caused an increment, whereas in higher concentrations – a significant (P ≤ 0.05) drop in LH secretion. Additionally, almost full positive correlation (r = 0.98) between the quantity of released LH and the dose of IGF-I was found (Fig. 4).

In summary, our results show that leptin in concentrations 10^{-9}, 10^{-8}, and 10^{-7} M/L significantly (P ≤ 0.05) enhances, whereas in concentrations of 10^{-6} M/L suppressed LH secretion from porcine pituitary cells. Insulin, indeed, in a dose from 3.9 to 13.0 mIU/L causes an increment, whereas in higher concentrations – a significant (P ≤ 0.05) drop in LH secretion. Moreover, almost full positive correlation (r = 0.98) between the quantity of released LH and the dose of IGF-I (in the range from 1.03x10^{-9} to 39.2x10^{-6} M/L) was found.

References


