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Comparison of the effects of human and chicken ghrelin on chicken ovarian hormone release

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ABSTRACT

The aim of the present experiments was to examine the species-specific and cell-specific effects of ghrelin on chicken ovarian hormone release. For this purpose, we compared the effects of chicken and human ghrelin on the release of estradiol (E), testosterone (T), progesterone (P) and arginine-vasotocin (AVT) by cultured fragments of chicken ovarian follicles and on the release of T and AVT by cultured ovarian granulosa cells.

In cultured chicken ovarian fragments, both human and chicken ghrelin promoted E release. T output was stimulated by chicken ghrelin but not by human ghrelin. No effect of either human or chicken ghrelin on P release was observed. Human ghrelin promoted but chicken ghrelin suppressed AVT release by chicken ovarian fragments. In cultured ovarian granulosa cells, human ghrelin inhibited while chicken ghrelin stimulated T release. Both human and chicken ghrelin suppressed AVT output by chicken granulosa cells.

These data confirm the involvement of ghrelin in the control of ovarian secretory activity and demonstrate that the effect of ghrelin is species-specific. The similarity of avian ghrelin on avian ovarian granulosa cells and ovarian fragments (containing both granulosa and theca cells) suggests that ghrelin can influence chicken ovarian hormones primarily by acting on granulosa cells.

1. Introduction

The study of differences in hormone action between species is important both for understanding the principles and evolution of the endocrine system and for the correct application of hormones in various species. Ghrelin is a good example of a hormone with large species-specific differences in structure, receptors, action and physiological role. This hormone was discovered in 1999 and is produced by the stomach and other tissues. Ghrelin is involved in the control of a wide array of biological functions, ranging from the control of gastrointestinal secretion and motility, behavior, biological rhythms, and metabolism to the regulation of cell proliferation, food intake, adiposity, and pituitary, ovarian and adrenocortical hormone secretion (Kaiya et al., 2013; Rak-Mardyla, 2013; Müller et al., 2015).

Avian (chicken) ghrelin has only 54% homology to the amino acid sequence of mammalian (human and rat) ghrelin (Kaiya et al., 2002). In mammals, ghrelin acts through the GHSR1a receptor, but in chickens and quails, other GHSR receptors for ghrelin have been found (Kaiya et al., 2013). The differences between mammalian and avian ghrelin and their receptors result in differences in the effects of mammalian and avian ghrelin on avian target tissues. For example, chicken ghrelin but not human or rat ghrelin is able to promote chicken gastrointestinal tract contraction (Kitazawa et al., 2007). Chicken ghrelin promotes the release of chicken adrenal corticosterone more effectively than human ghrelin (Kaiya et al., 2002). In chickens but not in rats, the anti-lipolytic activity of ghrelin has been described (Kaiya et al., 2013). In mammals, ghrelin promotes food and water intake and respiration; however, ghrelin reduces these processes in birds (Shousha et al., 2005; Geelissen et al., 2006; Kaiya et al., 2013).

Therefore, substantial differences between the effects of mammalian and avian ghrelin on avian metabolism and muscle contraction have been identified. It remains unknown whether such differences also affect the reproductive system and reproductive hormones.

Ghrelin is a potent regulator of both mammalian and avian reproduction. This hormone can affect (predominantly suppress) mammalian reproduction at the level of the hypothalamus, the pituitary and the ovaries (Rak-Mardyla, 2013; Tena-Sempere, 2013). In chickens, human ghrelin administration is able to reduce plasma progesterone (P) levels (Sirotkin and Grossmann, 2015) and to directly alter proliferation,

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apoptosis, steroidogenesis and protein kinases in cultured ovarian cells (Sirotkin et al., 2006, 2013; Sirotkin and Grossmann, 2007, 2008). These effects of human ghrelin on chicken ovarian cells are probably mediated by GHSR1a (Sirotkin and Grossmann, 2008; Sirotkin et al., 2013). The specific effects of mammalian and avian ghrelin on avian ovarian functions have not yet been examined. Nevertheless, both previous (Kaiya et al., 2002, 2013; Kitazawa et al., 2007) and present comparisons of chicken and mammalian ghrelin effect on chicken tissues enable one (1) to find the species-specific effects of this hormone, (2) to understand the interrelationships between hormone structure and function, (3) to verify the previous published data concerning the role of ghrelin in birds which were obtained mainly on mammalian ghrelin and (4) to find hormonal preparations which, irrespective of its origin and structure, could be applicable for regulation of chicken reproduction.

The aim of the present experiments was to examine this specificity by comparing the effects of mammalian and avian ghrelins on the secretory activity of cultured chicken ovarian tissue. For this purpose, we compared the effects of chicken and human ghrelin on the release of estradiol (E), testosterone (T), P and arginine-vasotocin (AVT) by cultured fragments of chicken ovarian follicles. In addition, to verify the ovarian fragment data and to identify the site of hormone release and ghrelin action within the ovarian follicles, we compared the effects of chicken and human ghrelin on T and AVT releases by cultured ovarian granulosa cells. These hormones are considered as the important regulators (Jurkevich and Grossmann, 2003; Rangel and Gutierrez, 2014; Sirotkin, 2014) and markers (Sirotkin and Grossmann, 2003, 2006, 2007, 2008, 2015; Sirotkin et al., 2006) of chicken ovarian functions.

2. Materials and methods

2.1. Animals

Young (approximately 8 months of age) White Leghorn hens (LSL), which weighed 1.1–1.2 kg and had an egg laying rate of >95%, were held in individual cages under standard conditions at the Experimental Station of the Institute of Animal Science with a photoperiod 12 L:12 D (illumination 8.00–20.00). The conditions of animal care, manipulation and use corresponded to the instruction no. 178/2002 and related European Commission documents and were approved by the local ethical commission. After two months of adaptation to the conditions of the experimental farm, the animals were killed by decapitation.

2.2. Collection and culture of ovarian follicular fragments and granulosa cells

The largest (F1) follicles were isolated from the ovary. The stage of folliculogenesis was determined by recording the time of the last oviposition and by determining the size and the position of the next ovarian follicle. Fragments of the follicular wall (5 mm in diameter, weight 24 ± 8 mg, 6 fragments from each follicle) were isolated as described previously (Sirotkin and Grossmann, 2003, 2006). After washing three times in sterile culture medium (1:1 DMEM/F-12 mixture supplemented with 10% bovine fetal serum and 1% antibiotic-antimycotic solution (all from Sigma, St. Louis, MO, USA), the fragments were cultured for 2 days in 2 ml of culture medium in Falcon 24-well plates (Becton Dickinson, Lincoln Park, NY, USA) at 38.5 °C under 5% CO_2 in humidified air. Either human or chicken recombinant research grade ghrelin (Peptides International Inc., Louisville, KY, USA, cat. no. PGH-3625-PI and PGH/3656-PI respectively) was added to the culture medium of different groups of follicles at doses of 0, 1, 10 or 100 ng/ml of medium.

To obtain ovarian granulosa cells, ovarian follicles were washed 3 times in a sterile solution of 0.7% NaCl. Granulosa cells were gently scraped from the inner surface of the follicular wall with a lancet and washed 3 times by centrifugation and resuspension in sterile culture medium (1:1 DMEM/F-12 mixture supplemented with 10% bovine fetal serum and 1% antibiotic-antimycotic solution. Thereafter, the cells were cultured in 2 ml of medium in Falcon 24-well plates at a density of 10^6 cells/ml (determined by hemocytometer) at 38.5 °C under 5% CO_2 in humidified air. After 4 days of pre-culture (when the cells achieved a 50–60% confluent monolayer), the medium was replaced with fresh medium with or without human or chicken ghrelin at doses of 0, 0.1, 1, 10, 100 or 1000 ng/ml of medium, and the cells were cultured in fresh medium for 2 days. Immediately after culture, the medium conditioned by the granulosa cells was gently collected from the culture wells and frozen at −18 °C until the time of RIA or EIA. This protocol provides the maximal accumulation of ovarian hormones in the culture medium and the most reliable ovarian secretory activity (Sirotkin and Grossmann, 2003, 2008).

2.3. Immunoassay

The concentrations of estradiol (E), testosterone (T), progesterone (P), and arginine-vasotocin (AVT) were determined in 25 μl aliquots of incubation medium by EIA and RIA, which were previously validated for use in culture medium. These hormones are considered to be indexes of ovarian secretory activity and the response to hormonal stimuli and key regulators of both mammalian (Sirotkin, 2014) and chicken (Sirotkin and Grossmann, 2006, 2007, 2008; Sirotkin, 2014) ovarian functions.

E concentrations were evaluated according to the protocol described by Münster (1989) using antisera against steroids (produced in the Institute of Animal Science, Neustadt, Germany), with an assay sensitivity of 5 pg/ml. The cross-reactivity of the E_2 antiserum was <2% to estrone, ≤0.3% to estriol, ≤0.004% to T and ≤0.0001% to P_4 and cortisol. The inter- and intraassay coefficients of variation did not exceed 16.6% and 11.7%, respectively.

T was assayed according to the protocol described by Münster (1989) using antisera against steroids (produced in the Institute of Animal Science, Neustadt, Germany). The sensitivity of the assay was 10 pg/ml. The cross-reactivity of the T antiserum was ≤96% to dihydrotestosterone, ≤3% to androstenedione, ≤0.01% to P_4 and E, ≤0.02% to cortisol and ≤0.001% to corticosterone. The inter- and intraassay coefficients of variation were 12.3% and 6.8%, respectively.

P concentrations were measured as described previously (Prakash et al., 1987). Rabbit antiserum against P was obtained from the Research Institute for Animal Production in Schoonoord, Netherlands. The anti-serum cross-reacted ≤0.1% with E, dihydrotestosterone, T and 17 beta hydroxyprogesterone. The sensitivity of the EIA was 12.5 pg/ml. The inter- and intraassay coefficients of variation did not exceed 3.3% and 3.0%, respectively.

AVT was measured using RIA, according to the protocol described by Gray and Simon (1983). The anti-AVT antiserum was kindly provided by Dr. D. A. Gray (Max-Plank Institute for Physiological and Clinical Research, Bad Nauheim, Germany), which cross-reacted ≤1.0% with mesotocin and angiotensin II. The sensitivity of the RIA was 0.3 pg/ml. The inter- and intraassay coefficients of variation did not exceed 8.8% and 7.2%, respectively.

2.4. Statistics

The data shown are the means of the values obtained in three separate experiments performed on separate days using separate animals (8 animals per group) and their ovaries. In each in-vitro experiment, each experimental group was composed of six culture wells with ovarian fragments or 4 wells with granulosa cells. Assays of hormone levels in the incubation medium were performed in duplicate. The values of the blank control were subtracted from the value determined by RIA/EIA in cell-conditioned medium to exclude any non-specific background (<15% of the total values). The rates of substance secretion were calculated per mg of tissue/day (for ovarian fragments) or per 10^6 cells/day (for granulosa cells). Significant differences between the experiments were evaluated using two-way ANOVA. When effects of the treatments