Relationships between Ovarian Proteolytic Enzyme Activity and Multiple Ovulation in the Hypophysectomized Hen : Gallus domesticus

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Relationships between Ovarian Proteolytic Enzyme Activity and Multiple Ovulation in the Hypophysectomized Hen (*Gallus domesticus*)

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Introduction

It is generally conceded that mature follicles rupture in response to the action of lutenizing hormone (LH). Since the domestic hen has a large ovary and ovulates almost daily, it is a convenient animal for the study of the mechanism of ovulation. In the intact hen, even if LH is administered in a broad range of dosages, it is impossible to induce more than one ovulation, which always occurs from the largest mature follicle.

A multiple ovulation could be induced in hypophysectomized laying hens by a single intravenous injection of LH at 3–15 h after the operation[10,12]. From these findings, Nalbandov[7] proposed that the ability of LH to induce a follicle to ovulate is facilitated by prior withdrawal of follicle stimulating hormone (FSH) stimulation. This was later confirmed by Ogawa *et al*[10].

There are so many reports suggesting that the digestion of stigma tissue with proteolytic enzyme may be essential in the process of rupture[1,2,5,6,8,13,18,20].

The present study was carried out to examine the role of ovarian proteolytic enzymes in follicle rupture of hens which had been forced to induce multiple ovulation.

Materials and Methods

Materials: Human chorionic hormone (HCG) and pregnant mare serum hormone (PMSG) were purchased from Teikoku-zoki Co. (Tokyo, Japan). Casein (No. 2242) was obtained from Merck (Dermstadt, West Germany) and hemoglobin (substrate powder) was from Worthington Biochemicals Corp. (Freehold, New Jersey, USA).

Methods: White Leghorn hens having regular clutches of at least 5 consecutive eggs were used. The birds were kept in individual cages, being exposed to 14 h light/24 h (06:00–20:00) and provided food and water *ad libitum*. The anterior pituitary was removed by using a stereotaxic instrument[17] 18 h before the expected time of ovulation.

The first group of hens was injected intravenously with 0.9% NaCl (1 ml) 2 h after hypophysectomy, followed by the injection of HCG (1000 iu/ml) 8 h after. The second group of hens was injected intravenously with PMSG (100 iu/ml) 2 h after hypophysectomy, followed by the injection of HCG (1000 iu/ml) 8 h after. All hens were killed 5 h after the injection of HCG, because Ogawa *et al.*[11] reported that the primary ovum was enforced to ovulate at the range of 5 3/4 to 7 5/6 h after ovine LH injection.

In this experiment, HCG and PMSG were used as the substitutes for LH and FSH,

respectively, since HCG exhibits properties like those of LH, and PMSG has follicle-stimulating activity.

Sample preparation and enzyme assay: The methods for sample preparation and enzyme assay were described elsewhere[8].

Statistical analysis: Student's unpaired t test was used to assess the statistical significance of difference between the means of groups.

Results

There were no significant differences in the weights of follicle walls between HCG and PMSG–HCG groups for each follicle type (Table 1).

Acid protease (AP) activity in the four largest follicles (F_1, F_2, F_3, F_4) walls of HCG group tended to be greater than those of PMSG–HCG group but they were not statistically significant except F_3 (Table 2).

Table 1. Weights of follicle walls of the four largest follicles.

Treatment	Type of follicle	Weight (mg±S.D.)
HCG	F1	581±131
PMSG–HCG	F1	503±120
HCG	F2	495±110
PMSG–HCG	F2	450±100
HCG	F3	388± 63
PMSG–HCG	F3	334±107
HCG	F4	312± 60
PMSG–HCG	F4	217±108

Values are mean±S.D. of 5 hens. No significant differences between means within each follicle type ($P<0.05$).

Table 2. Activities of acid and neutral proteases of the four largest follicles.

Treatment	Type of follicle	Acid protease	Neutral protease
		($\times 10^3$ units/follicle)	
HCG	F1	3.7±0.6	3.7±0.6
PMSG–HCG	F1	3.1±0.4	2.9±0.3*
HCG	F2	3.2±0.7	3.1±0.4
PMSG–HCG	F2	2.6±0.4	2.5±0.3*
HCG	F3	2.6±0.4	2.3±0.3
PMSG–HCG	F3	1.7±0.6*	1.7±0.3*
HCG	F4	2.1±0.8	1.9±0.4
PMSG–HCG	F4	1.5±0.6	1.4±0.3*

Values are mean±S.D. of 5 hens. Asterisk indicates statistically significant differences between means within each follicle type ($P<0.05$).

Neutral protease (NP) activity in the F_1, F_2, F_3 and F_4 follicle walls of the HCG group was significantly higher than those of the PMSG–HCG group (Table 2).

Discussion

Significant increase in NP activity and non-significant increase in AP activity of follicle walls of hens in HCG group, when compared with PMSG–HCG group, appear to suggest the involvement of proteolytic enzymes in the mechanism of multiple ovulation of hypophysectomized hens. The present results may support the proposal made by Nalbandov[7] and Ogawa *et al.*[11], FSH had an inhibitory effect on the multiple ovulations induced by exogeneous ovulation-inducing hormone in the hypophysectomized laying hens.

Progesterone levels in plasma and follicle were high several hours before ovulation[3,14,16]. Exogeneous LH significantly increases only the progesterone values in the plasma and follicle of hen[4,15,19]. LH and progesterone are related with the enzymic mechanism controlling the ovulation in the hen[8]. Furthermore, Ogawa *et al.*[9] reported that ovine LH and FSH stimulated progesterone production in granulosa cells isolated from the follicles of hypophysectomized and control (sham operation) hens when they were collected 6 h after operation, but the steroidogenic response to LH was greater for granulosa cells from hypophysectomized hens.

Therefore, a further study would be needed to clear the interrelationships between humoral and enzymic factors in order to understand the mechanism of multiple ovulation in the hypophysectomized hen.

Summary

This study was conducted to examine the role of proteolytic enzymes in the multiple ovulation of hypophysectomized laying hens. The hypophysectomy was performed 18 h before the expected time of ovulation.

The first group of hens was injected intravenously with 0.9% NaCl 2 h after hypophysectomy, followed by the injection of HCG (1000 iu/ml) 8 h after. The second group of hens was injected intravenously with PMSG (100 iu/ml) 2 h after hypophysectomy, followed by the injection of HCG (1000 iu/ml) at 8 h after. All hens were killed 5 h after the injection of HCG and the four largest follicles (F_1, F_2, F_3, F_4) were collected from each hen.

Neutral protease (NP) activity in the F_1, F_2, F_3 and F_4 follicle walls of the first group was significantly higher than those of the second group. Acid protease (AP) activity in the F_1, F_2, F_3 and F_4 follicle walls of the group 1 tended to be higher than those of the group 2 but they were not significant except F_3.

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