鶏の排卵に対する Cycloheximide の影響(畜産学科)

The Effect of Cycloheximide on Ovulation in the Domestic Fowl

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Summary

This paper investigates the effect of intraveneous injection of cycloheximide on spontaneous and LH—induced ovulation and on the proteolytic enzyme activities of follicles blocked by cycloheximide.

Ovulation was completely inhibited when cycloheximide was injected at the expected time of the endogeneous LH surge leading to ovulation. Such an inhibiting effect of the drug occured immediately before the injection of exogeneous LH.

The proteolytic activities of follicles blocked by cycloheximide are statistically lower than those of follicles near spontaneous ovulation.

From these results, it appears that the inhibitory effect of cycloheximide on ovulation in the domestic fowl was induced as a result of diminishing the production of proteolytic enzyme by LH.

Introduction

It has been established that the luteinizing hormone (LH) is involved in ovulation in the domestic fowl. But it remains unknown how LH plays a role in the rupture of matured follicles which lead to ovulation.

Nakajo *et al*[10]. and Dukelow and Maatman[1] report that the direct injection of several proteolytic enzymes into the follicular wall or application of them to a small piece of filter-paper placed on the follicular surface was capable of induction of ovulation in the domestic fowl. This has led to the suggestion that tearing of the follicular wall might result from the action of proteolytic enzymes. Yoshimura and Fujii[16] also suggest that the proteolytic enzymes participate in the process of follicular rupture on the basis of their observation that the morphological changes in spontaneous ovulation were similar to the proteolytic enzyme-induced rupture of matured follicles.

In order to investigate the role of proteolytic enzyme in the rupture of follicles which resulted in ovulation in the domestic fowl, the present experiment was made by injecting an inhibitor of protein synthesis, cycloheximide into the fowl.

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Materials and Methods

Birds

White Leghorn hens (15 months of age) laying 4－6 eggs in a clutch with one-day pause between clutches were used. They were kept in individual cages under 14 hrs (0500～1900) during day light hours and supplied with feed and water *ad libitum*. Times of oviposition were recorded for at least two weeks prior to the experiment in order to grasp the character of the clutch for each individual hen. When times of oviposition could be predicted with some accuracy, it was possible to determine the approximate time of ovulation, which normally follows oviposition, by adding 30 minutes to the observed time of oviposition[11]. In these hens, ovulation of the second ovum of the clutch (C_2) was expected to occur at about 0800 hrs.

Cycloheximide and LH injection

Cycloheximide (Wako Pure Chemical Industries, LTD) was dissolved in 0.9% saline and injected intraveneously at a dose of 1 mg/hen at scheduled times described below. Times of injection of the drug were 8 to 9, 10 to 11 and 15 to 16 hrs before the expected C_2 ovulation. These injection times were determined by the periods near the LH release, 2 to 3 hours before and 7 to 8 hours before the release of endogeneous LH for inducing ovulation, on the basis of the result that the time interval from the release of LH to the occurrence of ovulation is about 8 hrs[5]. The drug was also injected intraveneously three times: previous to, 2 hrs before, and 8 hrs before the injection of LH (NIH-LH-S18, ovine. 0.2mg/ml/hen). This hormone was dissolved in 0.9% saline and injected intraveneously at 15:00 to 16:00 on the day when the hen laid the terminal egg in a clutch (C_t). All of the injected hens were butchered after the time of expected ovulation and the occurrence or non-occurrence of ovulation was ascertained on autopsy.

Determination of proteolytic enzyme activity

The proteolytic enzymes on the follicle membrane of the stigma and in the non-stigma region were measured: the follicles near C_2 ovulation and of the ones blocked by cycloheximide. A 20% follicle membrane homogenate was prepared in cold 0.15 M NaCl with a Potter-Elvehjem homogenizer. The individual homogenates were then centrifuged (7,500, r. p. m.) for 10 min at 2°C. The resulting supernatant was used for the determination of the proteolytic enzyme activity was measured in the reaction mixture containing M/20 Lactic acid buffer (pH 3.8), casein of 0.5 mg/ml and supernatant corresponding to 20% homogenate in final volume of 1 ml. The tubes containing the reaction mixture were immersed in a 45°C bath. The reaction was started by the addition of casein dissolved in Lactic acid buffer. After incubation for two hours, the reaction was stopped by adding 1 ml of 20% TCA. And then

hydrolysates in the filtrate were determined by the method of Lowrey *et al.*[9] with bovine serum albumin (Armour Pharm. Co., Chicago) as a standard. The data were analyzed statistically with t−test.

Results

The effect of cycloheximide injected at different times before C_2 ovulation are shown in Table 1.

The cycloheximide injected at 8 to 9 hrs before C_2 ovulation was unable to block ovulation. However, cycloheximide injection at 10 to 11 hrs before C_2 ovulation resulted in failure of ovulation of 25%. The injection of the drug at 15 to 16 hrs before C_2 ovulation resulted in complete inhibition of ovulation.

Table 1 Effect of cycloheximide on spontaneous ovulation

Times of injection	Hens used	No. of hens		
		ovulated	blocked	blocked %
8 − 9 hrs before ovulation	13	13	0	0
11 − 12 hrs before ovulation	8	6	2	25
15 − 16 hrs before ovulation	9	0	9	100

Table 2 Effect of cycloheximide on induced−ovulation

Times of injection	Hens used	No. of hens		
		ovulated	blocked	blocked %
Immediately before LH injection	6	6	0	0
2 hrs before LH injection	7	3	4	57
8 hrs before LH injection	10	0	10	100

The effect of cycloheximide injected at different times before LH injection on ovulation are shown in Table 2. In all the hens receiving the drug immediately before LH injection, their ovulations were not blocked. However, when we increased the intervals between the injection of the drug and LH, the percentage of the inhibition of ovulation tended to increase. Furthermore, the drug injected at 8 hrs before LH injection inhibited ovulation completely.

The effect of cycloheximide injection on the proteolytic enzyme activities in both follicle membranes of the stigma and non−stigma region of follicles near ovulation and of the ones which kept on blocking ovulation by cycloheximide are shown in Fig. 1. In the stigma and non−stigma region near ovulation, the proteolytic activities were significantly higher than those of the same region of the drug−blocked follicles. In follicles near ovulation and follicles blocked by the drug, the proteolytic activities of the stigma and non−stigma region were not statistically different.

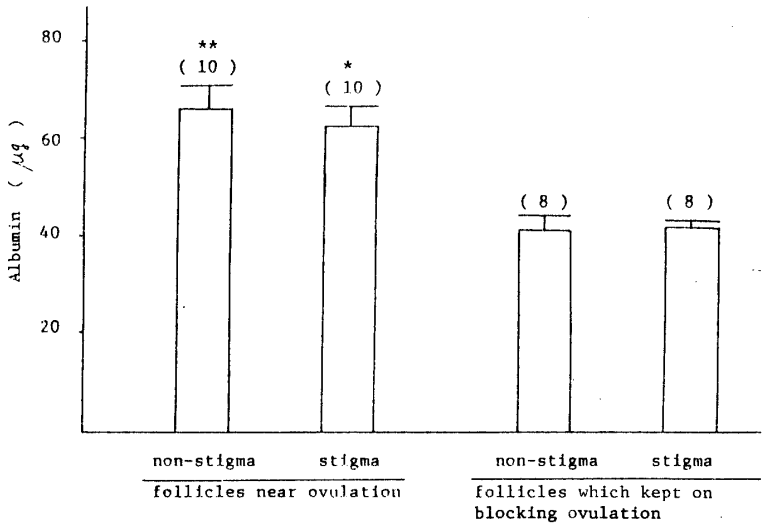

Fig. 1 Proteolytic enzyme activities in the stigma and non-stigma region of follicles near ovulation and ones which kept on being blocked by cycloheximide against ovulation

The values in parentheses are number of follicle membranes. ⊢⊣ Mean ± SD

*$P < 0.05$ (stigma × stigma in both follicles near ovulation and blocked)

**$P < 0.01$ (non-stigma × non-stigma in both follicles near ovulation and blocked)

Discussion

Although it has been well known that LH plays the role of trigger for ovulation in the domestic fowl[4, 5, 6, 7, 12, 15], the changes which are induced in the follicle and which result in ovulation remain unknown.

LH[14] and HCG[8, 14] have been proved to be able to increase the proteolytic enzyme activities in both rabbit and rat ovaries. The injection of collagenase, protease, and Nagase directly into follicles induced rupture of the follicles just as in the normal ovulation in the rabbit[2, 3].

In the study on ovulation of the hen, Nakajo *et al.*[10] performed similar experiments on follicles of the hen as reported in mammal described above. They postulated that rupture might be induced in the follicles in the same manner as in the mammalian follicles. Yoshimura and Fujii[16] reported that the same morphological changes appeared in the rupture induced by proteolytic enzymes and spontaneous rupture. These findings suggested that proteolytic enzymes may be involved in the rupture of hen's follicle.

Cycloheximide is believed to inhibit specific protein synthesis by preventing the transfer of amino acid from s-RNA to nascent polypeptide chains[13]. When cycloheximide was injected at the expected time of LH surge which is responsible for inducing ovulation in normal hen, ovulation was completely inhibited (Table 1).

It is suggested that the protein synthesis in the follicle which is necessary to induce ovulation and which resulted from the release of LH was inhibited. Such an inhibitory effect of cycloheximide on ovulation was also confirmed when the drug was injected immediately before the injection of LH on the day when C_t was laid (Table 2). Proteolytic activities in follicles where cycloheximide kept on ovulations were significantly lower than those of follicles near spontaneous ovulation (Fig. 1). It is, therefore, possible that LH participates in the rupture of follicle by stimulating the production of proteolytic enzyme in the follicle membrane. It is also suggested that the inhibitory effect of cycloheximide on ovulation could be ascribed to the suppression of protein synthesis (production of proteolytic enzymes) which is involved in the rupture of follicles. In the hen, the rupture of the follicle occurs in the limited region of the stigma at ovulation. However, the proteolytic activities in the stigma and non-stigma region of each follicle were not statistically different (Fig. 1). It appears that the tearing is easily induced in the stigma even though proteolytic activities are the same at the stigma and non-stigma region, because the follicular wall at the stigma is formed with less mechanical strength than that of the non-stigma region[16].

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鶏の排卵に対するCycloheximideの影響

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要　約

蛋白合成阻止剤であるCycloheximideの排卵に対する影響を調べるため，同薬剤を自然あるいは誘起排卵前の色々な時間に投与した。また同薬剤投与による蛋白分解酵素活性への影響も調べた。

Cycloheximideが，排卵をもたらす内因性ＬＨの放出時期に合わせて投与された場合排卵は完全に阻止された。このような排卵に対する阻止効果は，外因性ＬＨの注射直前における同薬剤の投与によっても認められた。

Cycloheximide投与によって排卵が阻止されている卵胞のスチグマ部とそれ以外の部における蛋白分解酵素の活性は，自然排卵直前の卵胞の同部位における蛋白分解酵素活性値に比較して有意に低い値を示した（$P < 0.05 \sim 0.01$）。

これらの結果から，CycloheximideはＬＨによる卵胞膜における蛋白分解酵素の合成を阻止することによって排卵に影響を及ぼしていると思われた。

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