Partial Purification of Gonadotropins from Chicken Anterior Pituitary and their Biological Activities

Hideaki Tojo, Tetsuo Tsukamoto*
and Kiyohiko Ogawa
(*Laboratory of Animal Reproduction*)
(Received for Publication August 30, 1975)

Introduction

There have been fewer attempts of separating avian gonadotropins in comparison with those of mammalian gonadotropins.

Follett *et al.*[4] had succeeded in purificating chicken pituitary LH and FSH, by using the methods which were effective in the purification of gonadotrotropins from human[10] and horse[11] pituitaries. However, it was reported by Furr and Cunningham[5] that the purified chicken gonadotropins were more effective on follicular growth in hens than mammalian gonadotropins. Moreover, Hartree and Cunningham[12] showed that the biological potencies of chicken LH and FSH preparations, when assayed in mammals, were far lower than those of the corresponding mammalian preparations on a weight-basis of the target organs.

Those findings indicate that there may be a species-specificity between hormone preparations and their target animals used for the bioassay.

The present study was carried out to partially separate chicken anterior pituitary FSH and LH, according to the method of Hartree and Cunningham[12], and to estimate their biological potencies in the same species as the test animals.

Materials and Methods

Chicken anterior pituitaries were collected from broilers of both sexes, and stored in acetone, for 2 weeks (replaced by fresh acetone every 2 days). They were then dried in air, crushed and weighed.

Extraction of glycoprotein

All operations were made at 4°C.

Acetone-dried pituitary powder was mixed with the extraction medium (6% w/v ammonium acetate in 40% v/v ethanol, pH 5.1).

The pituitary residue was re-extracted with fresh medium for another 48 hrs. After combination of supernatants, the glycoprotein fraction was precipitated by adding two volumes of cold 96% ethanol. The mixture was left for 2 days after stirring for 30 min.

* Nihon I. B. M. Co., Ltd., Tokyo

Glycoprotein was separated by centrifugation, washed once with cold 96% ethanol, twice cold ether, and dried *in vacuo*.

Column chromatography on CM-cellulose

The glycoprotein precipitate was extracted overnight with 4 mM ammonium acetate (pH 5.5) and the extract was applied to a column of CM-cellulose (Whatman CM-32) pre-equilibrated with the same buffer.

The unadsorbed fraction (designated to CM-1) was eluted with the starting buffer. When elution was complete, judged by the monitoring of the optical density at 280 mμ, the adsorbed fraction (CM-2) was eluted by elution of 1 M ammonium acetate. CM-1 fraction was recovered by freeze-drying, and CM-2 fraction, by ethanol precipitation.

Estimation of biological activities of fractions

The activity of CM-2 fraction was firstly estimated on the basis of induction of premature ovulation for the second egg of a clutch in hens. White Leghorn hens laying 3 to 4 eggs in a clutch were used.

After check of the first egg in the uterus by digital palpation, preparations were intravenously injected at 2 to 4 p. m. Induced ovulation was determined by autopsy 9 hrs after the injection.

Secondly, the ovulation-inducing effect of CM-2 fraction on hens pre-treated with PMSG was compared with that of CM-1 and ovine LH.

Laying-hens were consecutively treated subcutaneously with injections of PMSG at daily dose of 100 IU until the second day after the cessation of egg-laying. The preparations were given intravenously to those hens at 5 p. m. The hens were sacrificed 18 to 20 hrs after the injection.

The activity of CM-1 fraction was estimated, using a chick testes assay described originally by Byerly and Burrows [2].

Chicks were kept without food and water for the test period. Preparations were injected subcutaneously into the dosal neck region 24, 48, 72 and 96 hrs after hatching. Chicks were killed 24 hrs after the final injection and the testes were removed and weighed.

Results and Discussion

The chromatogram of the crude glycoprotein-extract on CM-cellulose is shown at Fig. 1. Two protein fractions were obtained by stepwise elution. The first fraction was eluted by 4 mM ammonium acetate (pH 5. 5) and the second fraction was obtained by the additional elution of 1 M ammonium acetate. They were designated to CM-1 and CM-2 respectively.

The weight-yield of each prepartion and its percentage to the acetone-dried powder were shown at Table 1, along with the yield obtained by Hartree and Cunningham [12].

As shown, the yields of all preparations obtained in this experiment were nearly same as their results.

Table 2 shows the ovulation-inducing effect of CM-2 fraction on the normal laying-hens. Its potency was compared with ovine LH.

The potency was investigated on the basis of ovulation-inducing for the second

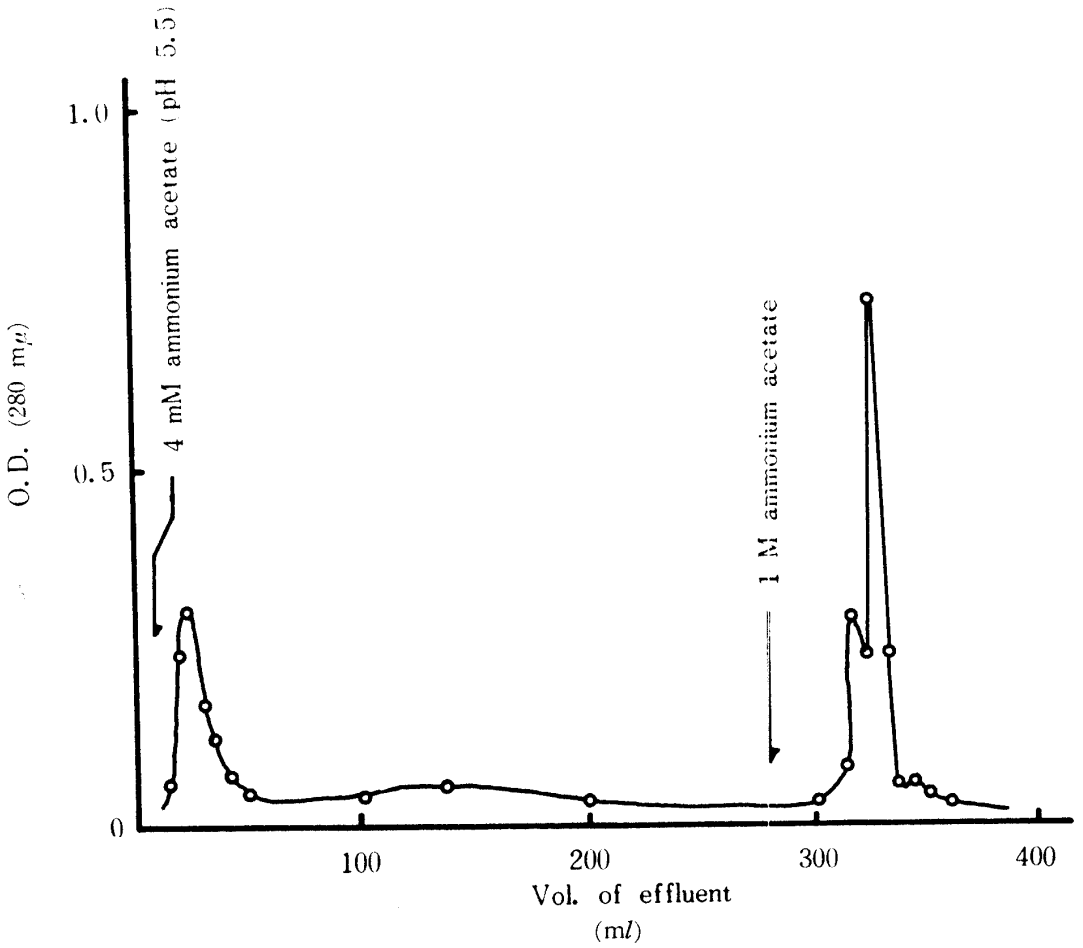

Fig. 1. Chromatography of the crude chicken glycoprotein extract on CM-cellulose.
The column was equilibrated with 4 mM ammonium acetate (pH 5.5). Column size : 1.0×30 cm. Flow rate : 4 ml/hr.

Table 1. Weight yields of preparations produced from chicken anterior pituitary powder.

Preparation	Yield (mg)	
	Present exp.	Hartree and Cunningham*
Pituitary powder	7520 (100)	100000 (100)
Glycoprotein-extract	358.9 (1.77)	4300 (1.30)
CM-1 fraction	37.7 (1.80)	1500 (1.50)
CM-2 fraction	11.8 (0.56)	620 (0.62)

* It was cited from data published in *J. Endocr.* (1969)
The figures in parentheses indicate the percent of each praparation to the pituitary powder

Table 2. Inducing-effects of CM-2 fraction to graded doses on the ovulation of C_2 in hens.

Preparation	Dose of injection (μg)	No. of hens	Ovulating hens
Saline	—	5	0
CM-2	2000	3	3
	1000	2	2
	500	2	2
	100	2	2
	40	2	2
	10	5	2
	1	2	0
Ovine LH*	50	5	5
	40	5	3
	20	10	2
	10	5	0

* NIH-LH-S18

follicle of a clutch (C_2).

Intravenous injection of CM-2, to 10 μg of dose level, induce prematurely ovulation of C_2, but at the dose of 1 μg the ovulation was not induced any more. Whereas, ovine LH, to 20 μg of dose level, indicated ovulation-inducing potency, but at the dose of 10 μg, it was not indicated.

This result shows that, as to ovulation-inducing effect, CM-2 fraction is approximately ten times as much potent as ovine LH.

Hitherto, it was reported that CM-2 fraction contained much LH activities. As above, it was indicated that CM-2 fraction obtained in this experiment contained much LH activities.

Secondly, it was investigated whether the multiple ovulations would be induced or not, when the fractions were injected to the hens having many large follicles developed by pre-treatment of PMSG. The results are shown at Table 3.

Intravenous injection of CM-2 fraction induced ovulation of one or two ova (mean : 1.2±0.3 ova) in almost all the treated hens (5/6). Whereas, CM-1 and ovine LH did not wholly induce the ovulation at the dose of 40 μg.

As it was previously reported that CM-1 fraction contained mainly FSH and TSH activities, it was assumed that CM-1 fraction obtained in this experiment scarcely

Table 3. Ovulation-inducing effects of CM-1 and CM-2 fractions on the hens pretreated with PMSG.

Preparation*	No. of hens	Ovulating hens
Ovine LH	6	0
CM-1	4	0
CM-2	6	5

* 40 μg dose per hen

contained LH activities too.

Therefore, from the above findings, it is postulated that LH component in the anterior pituitary is almost contained in the adsorbed fraction (CM-2) of CM-cellulose, but not in the unadsorbed fraction (CM-1).

On the contrary, 40 μg dose ovine LH did not induce the ovulation in the hens pre-treated with PMSG. It is well-known that the ovulation-suppressed hens produced by treatment with PMSG, are apt to become hard to ovulate and that ovulations in those hens couldn't be induced by exogenous ovulation-inducing hormone if the dose or timing of the injection was not appropriate.

Therefore, 40 μg dose of ovine LH is assumed to be inadequate for ovulation-inducing in those hens. By contrast, CM-2 fraction induced ovulation at the same dose. This finding indicates that also in the hens pre-treated with PMSG ovulation-inducing potency of CM-2 fraction was higher than that of ovine LH.

Hitherto, it was reported that avian pituitary preparation is more effective than mammalian gonadotropins in stimulating ovarian function in immature pullets [3, 13], starving pullets [6] and hypophysectomized hens [9]. Imai [7], comparing the fowl anterior pituitary preparation with mammalian hormone (FSH), showed that the mammalian pituitary preparations were less effective than avian preparation in inducing follicle growth in hens with regressed ovaries.

This superiority of the avian preparation may be due to a qualitative difference between avian and mammalian gonadotropins. Our results were well in accord with previous works.

The effects of preparations on the chick testes-weights are shown at Table 4. The residue produced after the extraction of glycoprotein did not indicate any effects on the chick testes, in spite of high dose (1000 and 1500 μg). This shows that the residue did not nearly or wholly contain gonadotropic activities.

Chicken anterior pituitary powder (CAP) and CM-1 fraction significantly increased

Table 4. Effects of preparations on the weight of chick testes.

Preparation	Dose (μg)	No. of chicks	Testes weights (mg) (Means±S. D.)
Saline		10	5.8±1.5
Residue	1000	10	6.3±1.7
	1500	10	5.6±1.8
Pituitary powder	1000	9	11.8±3.6*
	1500	8	14.1±2.6*
CM-1	1000	10	12.2±3.5*
	1500	10	14.9±4.0*
Ovine LH[a]	200	11	13.1±3.0*
	2000	11	13.7±2.7*
Ovine FSH[b]	200	11	12.7±3.1*
	2000	10	21.7±4.8*

* Significant difference from saline at 1% level.

a NIH-LH-S18

b NIH-FSH-S10

the weights of chick testes at the dose of 1000 and 1500 μg respectively. However, the potency of CAP or CM-1, at the dose of 1000 μg, was almost equal to that ovine LH and FSH at the dose of 200 μg.

Thus, in this experiment, fractionation of FSH from CAP by chromatography on CM-cellulose, did not much increase its potency in weight-increasing of chick testes.

On the other hand, it was found that weight-increasing potency of ovine FSH was higher than LH at the dose level of 2000 μg.

Breneman *et al.*[1] reported that mammalian FSH was more potent than LH in chick testes assay. By contrast, Kamiyoshi *et al.*[8] indicated that LH is a little more effective than FSH on the basis of ^{32}P uptake by testes of 1-day-old chicks. Our findings, at the dose of 2000 μg, coincided with the report of by Breneman *et al.*[1]

As above, from the present study, it was indicated that fractionation of gonadotropins from chicken anterior pituitary powder by chromatography on CM-cellulose, increased its gonadotropic activities, especially LH activity. In addition, it was demonstrated that the biological potencies of chicken LH fraction, when assayed in the same species, were much higher than those of mammalian LH.

Therefore, it will be required to establish an original bioassay for avian hormones, based on the same species as the test animals.

Summary

Gonadotropins were partially fractionated from the acetone-dried powder of chicken anterior pituitaries and their biological activities were estimated.

The biological activities of fractions were estimated on the basis of inducing-potency, for the ovulation in the normal laying-hens or, of hens pre-treated with PMSG, and on the basis of weight-increasing of chick testes.

The adsorbed fraction of glycoprotein-extract on CM-cellulose was more effective than mammalian LH (ovine LH) on ovulation-inducing both in the normal laying-hens and in the hens pre-treated with PMSG.

On the contrary, the unadsorbed fraction was less potent in increasing chick testes than mammalian hormones (ovine FSH and LH), in spite of its potency increased by fractionation.

As above, the present study indicated that partial fractionation of chicken anterior pituitary powder increased its LH or FSH activity. Especially, when assayed in the same species, ovulation-inducing potency of adsorbed fraction was significantly higher than that of mammalian LH (ovine LH).

Acknowledgments

The authors wish to thank the National Institute of Health, U. S. A., for supplying NIH-FSH-S10 and NIH-LH-S18.

References

1) Breneman, W. R., Zeller, F. J. and B. E. Beekman.: *Poult. Sci.*, **38**, 152-158 (1959)
2) Byerly, T. C. and W. H. Burrows.: *Endocrinol.* **22**, 366-369 (1938)

3) Das, B. C. and A. V. Nalbandov.: *ibid.* **57**, 705-719 (1955)
4) Follett, B. K., Scanes, C. G. and F. J. Cunningham.: *J. Endocr.*, **52**, 359-378 (1972)
5) Furr, J. A. and F. J. Cunningham.: *Br. Poult. Sci.*, **11**, 7-13 (1970)
6) Imai, K.: *J. Reprod. Fert.*, **31**, 387-397 (1972)
7) : *ibid.* **33**, 91-98 (1973)
8) Kamiyoshi, M., Tanaka, K. and Y. Tanabe.: *Endocrinol.*, **91**, 385-388 (1972)
9) Opel, H. and A. V. Nalbandov.: *ibid.* **69**, 1016-1028 (1961)
10) Hartree, S. A.: *Biochem. J.*, **100**, 754-761 (1966)
11) Hartree, S. A., Mills, J. B., Welch, R. A. S. and M. Thomas.: *J. Reprod. Fert.*, **17**, 291-303 (1968)
12) and F. J. Cunningham.: *J. Endocr.*, **43**, 609-616 (1969)
13) Taber, E., Claytor, M., Knight, J., Gamberell, D., Floweres, J. and C. Ayers.: *Endocrinol.*, **62**, 84-89 (1958)