Effects of steroid blockers on LH-induced ovulation in the domestic fowl, *Gallus domesticus*

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Summary. The spontaneous ovulation of hens was suppressed by daily injection of PMSG. The LH injected to overcome the block was accompanied by one of 4 compounds known to inhibit steroidogenesis at different sites in the biosynthetic pathway. A dose of 300 mg aminoglutethimide phosphate, which inhibits the conversion of cholesterol to 20 α -hydroxycholesterol, blocked the LH-induced ovulation and prevented the normal rise in plasma progesterone. Metyrapone, an inhibitor of 11 β -hydroxylase, and SC 12937 and AY 9944, inhibitors of cholesterol synthesis, did not prevent ovulation or the progesterone rise induced by exogenous LH. Administration of progesterone overcame the inhibitory effect of aminoglutethimide, and it is suggested that progesterone is involved in the ovulatory process of the fowl.

Introduction

It has been suggested that in the domestic fowl progesterone may induce the release of LH required for ovulation by stimulating the hypothalamus (Fraps & Dury, 1943; Ralph & Fraps, 1959, 1960; Wilson & Sharp, 1976). Measurements of plasma LH and progesterone concentrations during the ovulatory cycle seem to support this suggestion (Peterson & Common, 1971; Kappauf & Van Tienhoven, 1972; Furr, Bonney, England & Cunningham, 1973; Haynes, Cooper & Kay, 1973; Wilson & Sharp, 1973; Laguë, Van Tienhoven & Cunningham, 1975; Shahabi, Norton & Nalbandov, 1975; Shodono, Nakamura, Tanabe & Wakabayashi, 1975). Furr *et al.* (1973) reported that the rise in progesterone always preceded or occurred simultaneously with the LH increase. By contrast, Williams & Sharp (1978) found that the preovulatory release of LH was accompanied by a large increase in the secretion of androgen and progesterone. It has also been reported that corticosteroids are involved in the ovulation of the hen (Van Tienhoven, 1961; Soliman & Huston, 1974; Etches & Cunningham, 1976; Etches, 1977; Wilson & Lacassagne, 1978). However, the role of steroid hormones in the ovulatory mechanism is not well clarified. We therefore studied the possible significance of ovarian steroid hormones in the ovulatory process of domestic hens by using compounds that are known to block steroid synthesis.

Materials and Methods

Experiments

The compounds used to block steroid synthesis at different sites were aminoglutethimide phosphate, metyrapone (provided from CIBA Pharmaceutical Company), AY 9944 (trans-

1-4-bis-[2-chlorobenzylaminomethyl]-cyclohexane dihydrochloride, provided by Dr D. Dvornik, Ayerst Research Laboratories) and SC 12937 (20,25-diazacholesterol dihydrochloride, provided by Dr R. N. Saunders, Searle Laboratories).

White Leghorn laying hens with 4 or 5 eggs in a clutch were used. The hens were kept in individual cages and exposed to 14 h light/day (06:00–20:00 h). Food and water were provided *ad libitum*. The regimen of PMSG and LH injections was as reported by Imai (1973): 80 i.u. PMSG (Ferring AB, Malmo, Sweden) were injected subcutaneously daily to suppress the spontaneous ovulation. At 22:00 h on the third day after cessation of egg laying and 24 h after the final injection of PMSG, LH (NIH-LH-B10) was injected intravenously alone or with one of the inhibitory compounds. The compounds were given as 2 subcutaneous injections at 0 and 45 min after LH injection or as 3 injections at 0, 30 and 60 min after LH injection. Hens which had been given aminoglutethimide were also given a subcutaneous injection of 15 mg oestradiol or progesterone immediately after the LH injection. Control birds were injected with saline solution (9 g NaCl/l) instead of LH. The total dose of each compound (150 mg SC 12937; 300 mg for each of the others) was based on preliminary tests with up to 400 mg of each drug administered to 2 hens; both hens died after a dose of 250 mg SC 12937, 400 mg metyrapone and 400 mg AY 9944. AY 9944 and the hormones were suspended in corn oil, the other 3 drugs were dissolved in distilled water. Blood was collected from the wing vein immediately before LH or control injection and 30, 60 and 90 min later. The plasma was separated by centrifugation at 4°C and stored at –20°C until used. The hens were killed 10 h after the LH injection and examined for ovulated ova. The ovaries were removed and ruptured follicles and large yellow follicles >3 cm in diameter were counted. Hens which had extensive atresia of the follicles were excluded from the results.

Steroid assays

The plasma oestradiol and progesterone concentrations were determined by the radioimmunoassay previously validated (Tojo & Huston, 1980).

[2,4,6,7-³H(N)]Oestradiol (sp. act. 115.0 Ci/mmol) and [1,2,6,7-³H(N)]progesterone (sp. act. 114.0 Ci/mmol) were obtained from New England Nuclear Co. and used without further purification. An antiserum produced in a sheep against 1,3,5(10)-oestratrien-3,17-diol-6-one-6-CMO-BSA was used at a final dilution of 1:100 000. The antiserum produced in a rabbit against 4-pregnen-3,20-dione-3-CMO-BSA was used at a final dilution of 1:15 000. The buffer used was 0.1 M phosphate-buffered saline (PBS, pH 7.0) containing 0.1% gelatin. Bound steroids were separated by the dextran-coated charcoal method. The specificity of the antisera was tested by cross-reactivity with 12 different steroids. The antiserum to oestradiol cross-reacted 100% against oestradiol-17β, 17.8% against oestriol, 1.5% with oestrone and <0.1% with progesterone, pregnenolone, testosterone, 17α-hydroxyprogesterone, cortisone, corticosterone, hydroxycortisone, 5α-androsten-17β-ol-3-one, Δ⁴-androsten-3-one, and cholesterol. Cross-reactions of the progesterone antiserum were 100% with progesterone, 1.5% with 17α-hydroxyprogesterone, 0.5% with pregnenolone and <0.1% with the other steroids mentioned above. The least amount distinguished significantly from zero amount by *t* test was 31 pg oestradiol-17β/ml and 39 pg progesterone/ml. Recovery of the added oestradiol-17β from plasma was 84.2 ± 4.7% (*n* = 5, 250 pg/ml) and 83.3 ± 3.8% (*n* = 5, 500 pg/ml). Progesterone recovery was 87.2 ± 5.2% (*n* = 5, 250 pg/ml) and 83.3 ± 5.2% (*n* = 5, 500 pg/ml). The between-assay coefficient of variation was <15% for both assays. The hormone concentrations of the samples were calculated by comparison to standard curves composed of 8 points ranging from 31 to 2000 pg/ml for oestradiol-17β and from 39 to 5000 pg/ml for progesterone.

Results

The ability of the 4 compounds to prevent the ovulation induced by LH injection is shown in Table 1. The numbers of ovulated ova and of large yellow follicles remaining in the ovary indicated that the rate of follicular growth in the PMSG-treated hens was similar in all the groups. A dose of 200 µg LH induced ovulation in all the hens but <200 µg was not always

Table 1. Effects of aminoglutethimide phosphate (AGP), metyrapone, SC 12937 and AY 9944 on ovulation induced by exogenous LH in hens in which spontaneous ovulation was suppressed by PMSG treatment

Treatment (total dose)	No. of hens	No. of ovulating hens (%)	Mean $\pm$ s.d. no. of ovulated ova/ovulating hen
LH (50 µg)	6	2 (33.3)	1.0 $\pm$ 0
LH (100 µg)	5	2 (40.0)	1.0 $\pm$ 0
LH (200 µg)	13	13 (100)	1.4 $\pm$ 0.7
LH (200 µg) + AGP (100 mg)	5	4 (80.0)	1.8 $\pm$ 0.5
LH (200 µg) + AGP (150 mg)	8	2 (25.0)*	1.0 $\pm$ 0
LH (200 µg) + AGP (300 mg)	9	2 (12.5)**	1.0 $\pm$ 0
LH (200 µg) + AGP (300 mg) + progesterone (15 mg)	6	4 (66.7)	1.5 $\pm$ 0.6
LH (200 µg) + AGP (300 mg) + oestradiol (15 mg)	5	0 (0)***	—
LH (200 µg) + metyrapone (150 mg)	6	5 (83.3)	1.2 $\pm$ 0.4
LH (200 µg) + metyrapone (300 mg)	7	4 (57.1)	1.5 $\pm$ 0.6
LH (200 µg) + SC 12937 (150 mg)	8	7 (87.5)	1.5 $\pm$ 0.8
LH (200 µg) + AY 9944 (300 mg)	5	5 (100)	2.1 $\pm$ 1.1
Saline	6	0 (0)***	—

Values significantly different from that of 200 µg LH alone, * P < 0.05, ** P < 0.01, *** P < 0.001.

Table 2. Effects of aminoglutethimide phosphate (AGP), metyrapone, SC 12937 and AY 9944 on plasma oestradiol (E_2) and progesterone (P) concentrations (mean $\pm$ s.e.m.) after LH injection (200 µg) in hens in which spontaneous ovulation was suppressed by PMSG treatment

Treatment	No. of hens	Hormone	Plasma hormone conc. (pg/ml)			
			0 min	30 min	60 min	90 min
LH	9	E_2	708 $\pm$ 81	721 $\pm$ 81	715 $\pm$ 45	670 $\pm$ 48
		P	1130 $\pm$ 110	2062 $\pm$ 170***	2130 $\pm$ 185***	1685 $\pm$ 160*
LH + AGP (300 mg)	7	E_2	583 $\pm$ 24	362 $\pm$ 32	345 $\pm$ 28	342 $\pm$ 25
		P	1095 $\pm$ 85	1135 $\pm$ 78	1093 $\pm$ 95	1114 $\pm$ 140
LH + metyrapone (300 mg)	5	E_2	781 $\pm$ 65	754 $\pm$ 40	692 $\pm$ 43	723 $\pm$ 25
		P	1220 $\pm$ 65	2115 $\pm$ 140***	2184 $\pm$ 225***	1790 $\pm$ 170*
LH + SC 12937 (150 mg)	6	E_2	683 $\pm$ 72	667 $\pm$ 42	773 $\pm$ 63	756 $\pm$ 52
		P	1050 $\pm$ 85	1867 $\pm$ 180**	2013 $\pm$ 162**	1862 $\pm$ 180**
LH + AY 9944 (300 mg)	5	E_2	752 $\pm$ 57	702 $\pm$ 50	594 $\pm$ 46	832 $\pm$ 32
		P	1153 $\pm$ 75	1952 $\pm$ 102***	1894 $\pm$ 162**	1904 $\pm$ 170**
Saline	6	E_2	550 $\pm$ 49	620 $\pm$ 38	650 $\pm$ 42	902 $\pm$ 26
		P	1035 $\pm$ 65	1197 $\pm$ 72	1145 $\pm$ 45	1150 $\pm$ 81

Values significantly different from those at 0 min in each treatment; * P < 0.05, ** P < 0.01, *** P < 0.001 (t test).

effective. Aminoglutethimide at a dose of 300 mg was completely effective in preventing the ovulation, but ovulation occurred if progesterone (but not oestradiol) was also injected immediately after the LH. Metyrapone prevented ovulation in only a few of the hens and SC 12937 and AY 9944 were also ineffective. The concentrations of plasma oestradiol and progesterone in the treated hens are shown in Table 2. A significant rise in progesterone concentration was obtained after the injection of LH alone, metyrapone, SC 12937 and AY 9944. The peak value was at 30 or 60 min after the LH injection. Aminoglutethimide injection prevented the plasma progesterone rise but oestradiol concentrations were lower after treatment than in the other groups of hens.

Discussion

Aminoglutethimide was the only compound able to suppress the LH-induced ovulation and the increase in plasma progesterone levels caused by LH injection. Aminoglutethimide is reported to prevent the conversion of cholesterol to 20 α -hydroxycholesterol (Kahnt & Neher, 1966) and it is possible that this was the site of action in the treated hens, in which plasma oestradiol concentrations were also decreased. However, the ovulation blocked by aminoglutethimide could be overcome by a single injection of progesterone, indicating that progesterone may be involved in the subsequent ovulatory process after sensitization of the mature follicle by LH. Oestradiol appeared not to be an essential steroid in relation to follicular rupture. The results support those of Lipner & Greep (1971) who found that a high dose of aminoglutethimide was effective in blocking ovulation induced by exogenous LH in immature rats primed with PMSG and that an agent which inhibited progesterone synthesis significantly blocked the induced ovulation. As in the domestic fowl (Shahabi, Bahr & Nalbandov, 1975; Imai & Nalbandov, 1978), plasma progesterone values are also significantly increased in rats and rabbits shortly after a rise in endogenous LH or LH injection (Labhsetwar, Joshi & Watson, 1973; Shaikh & Harper, 1972), indicating a similar ovulatory mechanism. However, in the domestic fowl, it has been suggested that progesterone induces ovulation through stimulating the hypothalamus (Fraps & Dury, 1943; Ralph & Fraps, 1959, 1960; Wilson & Sharp, 1976). Williams & Sharp (1978) reported that there was first an increase in LH level which caused a small rise in plasma androgen and progesterone while the preovulatory release of LH was accompanied by a much larger increase in the secretion of these steroids. This suggests that a large increase in progesterone after LH release is associated with the follicular rupture.

Metyrapone inhibits the hydroxylase that converts 11 β -deoxycorticosterone to corticosterone (Liddle, Island, Lance & Harris, 1958). In this study, metyrapone had no significant effect on the block to ovulation although corticosteroids are able to induce ovulation in the hen (Van Tienhoven, 1961; Soliman & Huston, 1974; Etches & Cunningham, 1976; Etches, 1977; Wilson & Lacassagne, 1978). Therefore, the results of metyrapone injection did not indicate any involvement of the adrenal in the LH-induced ovulation and it is possible that the injected adrenal steroids will be converted to progesterone or other steroids. SC 12937 and AY 9944 are considered to prevent cholesterol synthesis (Counsell, Klimstra, Ranney & Cook, 1962; Dvornik, Kraml, Dubuc, Givner & Gaudry, 1963). Daily administration of AY 9944 also caused the cessation of egg laying accompanied by decrease in blood cholesterol (Martin & Lupien, 1969). In the present study, neither compound affected the ovulation or the rise in progesterone level induced by LH, in spite of the almost lethal dose used. Lipner & Greep (1971) reported that AY 9944 was not effective in preventing ovulations in rats. Prevention of cholesterol synthesis therefore does not affect the ovulatory process after sensitization of the mature follicle by LH. We conclude that in the domestic fowl progesterone may be an important steroid in the ovulatory process after sensitization of the mature follicle by LH, probably in the synthesis or activation of the enzyme required for follicular rupture.

This work was financed by the Experimental Station, College of Agriculture, University of Georgia. We thank Dr D. N. Marple, Auburn University, for the antioestradiol serum; Dr F. J. N. Thompson, University of Georgia, for the antiprogesterone serum; Dr R. R. Kraeling, Richard B. Russell Agricultural Research Center (U.S.D.A.), for helpful advice on the RIA; NIH for gifts of bovine LH; CIBA Pharmaceutical Company (New Jersey) for aminoglutethimide phosphate and metyrapone; Dr D. Dvornik, Ayerst Research Laboratories (Montreal, Canada), for AY 9944; and Dr R. N. Saunders, Searle Laboratories (Chicago) for SC 12937.

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Received 13 May 1980