Effect of progesterone on LH-releasing activity of the hypothalamus in non-laying hens primed with oestradiol

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Summary. LH-releasing activity of the hypothalamus in non-laying hens following the injection of progesterone was increased in hens that had been treated with oestradiol 4 h earlier. Short-term priming with oestradiol may be effective in increasing the responsiveness of the hen hypothalamus to progesterone.

Introduction

In laying hens (*Gallus domesticus*), the concentrations of plasma progesterone and LH change during the ovulatory cycle and each shows a peak some several hours before ovulation (Laguë, van Tienhoven & Cunningham, 1975; Shodono, Nakamura, Tanabe & Wakabayashi, 1975). Since progesterone is known to stimulate the release of LH from the pituitary (Wilson & Sharp, 1975a, b; Etches & Cunningham, 1976) by acting on the central nervous system including the hypothalamus (Fraser & Sharp, 1978), a positive feedback action of progesterone for the release of LH during the ovulatory cycle has been proposed (Sharp, 1980). Although the injection of progesterone causes an increase in the hypothalamic gonadotrophin-releasing activity in the laying hen, the effect was not apparent in the non-laying hen (Tanaka, Kamiyoshi & Sakaida, 1974). The cytoplasmic progesterone receptor concentration in the hypothalamus is less in non-laying than in laying hens (Kawashima, Kamiyoshi & Tanaka, 1979a). A single intramuscular injection of oestradiol into non-laying hens causes an increase in the cytoplasmic progesterone receptor concentration in the hypothalamus within 4 h of injection (Kawashima *et al.*, 1979b). It is assumed therefore that the effect of progesterone on the hypothalamus may be enhanced by the single injection of oestradiol 4 h before the injection of progesterone. The present experiments were performed to examine this assumption.

Materials and Methods

Birds and treatments

The White Leghorn hens used (20 months of age; 1.9–2.2 kg body wt) had not laid an egg for 1 week after a 3-day deprivation of food and water. The 80 birds were divided into 4 groups, and each received an injection of oestradiol or olive oil and a subsequent injection, 4 h later, of progesterone or oil. The steroids were dissolved in olive oil, and the injections were made intramuscularly at a dose of 0.5 mg/bird. The volume of injections was 0.5 ml. The birds were killed 1 or 4 h after the second injection, and the hypothalamic tissue was obtained by dissecting the brain just posteriorly to the optic chiasma, anteriorly to the oculomotor nerve, to 1 mm each

side of the midline, and to a depth of approximately 1 mm (20–28 mg/hen). The part of the hypothalamus obtained was the same as in our earlier investigations on progesterone receptors (see Kawashima *et al.*, 1980).

Preparation of hypothalamic extracts

The hypothalamic tissues were pooled and homogenized in 10 volumes of ice-cold 0.1 N-HCl. The homogenates were centrifuged (1000 g, 15 min, 4°C), and the supernatant fluid was neutralized with 1 N-NaOH and again centrifuged. The supernatant fluid was adjusted to pH 7.3 with 0.1 N-NaOH, and filtered (0.5 µm millipore filter). The filtrates were diluted with Eagle's minimal essential medium (MEM) containing Romanoff's avian Ringer salt (RRA) (Romanoff, 1943), and used immediately for LH-RH assay.

LH-RH assay

LH-releasing activity of the hypothalamic extracts was assayed on isolated hen pituitary cells *in vitro* by a method similar to that reported by Bicknell & Follett (1975) and Bonney & Cunningham (1977) with modifications. Anterior pituitary lobes were excised from laying hens. In each assay, 40 hens were used as donors of the pituitary. Trypsinization was carried out with 0.25% (w/v) trypsin in Ca²⁺- and Mg²⁺-free RRA solution to which bovine serum albumin (BSA; Fraction V, Armour, Illinois, U.S.A.) was added. The addition of BSA has been reported to be helpful in maintaining the function of the cellular membrane of rat pituitary cells (Hymer *et al.*, 1973). The pituitary cells were suspended in Eagle's MEM-RRA at a concentration of 1×10^6 /ml, and 0.5 ml of the suspension was placed in tubes. The hypothalamic extracts (0.5 ml) or standard LH-RH solution (0.5 ml) was added to the tubes, and also to another series of tubes into which 0.5 ml Eagle's MEM-RRA not containing the pituitary cells was dispensed. Four tubes containing the pituitary cells and 3 tubes not containing the pituitary cells were used for each dose of the hypothalamic extracts and of the standard LH-RH solution, and 4 tubes containing the pituitary cell suspension (0.5 ml) and Eagle's MEM-RRA (0.5 ml) served as controls. All tubes were incubated for 2 h at 41°C under an air atmosphere in a shaking (120 r.p.m.) water bath. After the incubation, the tubes were immediately cooled in an ice-bath, and gently centrifuged (180 g, 5 min, 4°C). The supernatant fluid was decanted and stored at -20°C. The amount of LH was measured by a radioimmunoassay for avian LH (Hattori & Wakabayashi, 1979). A highly purified chicken LH (Fraction IRC-2, Gunma) was used as a standard. The amount of LH measured in the tubes containing the pituitary cells minus the amount of LH in the tubes not containing the pituitary cells is equivalent to the amount of LH released both spontaneously and by the test material. The increase in the amount of LH released by the test material was obtained by further subtracting the amount of LH in the control tubes. The LH-RH concentration of the hypothalamic extracts was determined by a 4-point design (2 + 2), and expressed as ng equivalents of NIH-LH-RH/FSH-RH (NICH0-72-2722 CPR) per mg hypothalamic tissue. Statistical significance of the difference of the LH-RH concentration between groups was analysed by *t* test (Snedecor, 1956) on the values of distance (*M*) from the standard line in the 4-point assays (Finney, 1964).

In preliminary experiments, (1) viability of the pituitary cells during the incubation, (2) time course of LH release by the hypothalamic extracts and by the standard LH-RH during the incubation, (3) dose-response relationship for the hypothalamic extracts and the standard LH-RH, and (4) specificity of the response for the hypothalamic extracts among the extracts of various tissues were examined. It was found that (1) the viability was 99% or more up to 16 h of incubation, (2) the increase in the amount of LH released showed a maximum (hypothalamic extracts) or a plateau (standard LH-RH) at 2 h of incubation, (3) a linear relationship between the log dose and the amount of LH released occurred for the hypothalamic extracts (2.5–10 mg

equiv.) and the standard LH-RH (80–320 ng), and the lines were parallel, and (4) there was no increase in LH release when extracts from cerebellum (5, 10 and 20 mg), posterior lobe of pituitary (0.5, 1 and 2 mg) and superficial pectoral muscle (5, 10 and 20 mg) were incubated for 2 h.

Results

As shown in Table 1, the concentration of LH-RH in the hypothalamus of non-laying hens was greater in the hens receiving the injection of oestradiol before the injection of progesterone (Group 1) than in the hens in Groups 2, 3 and 4 at 1 h and Groups 3 and 4 at 4 h. Although Group-2 hens showed a greater LH-RH concentration at 1 h, there was no appreciable difference between values for hens in Groups 3 and 4.

Table 1. LH-RH concentration (and 95% fiducial limits) in the hypothalamus 1 and 4 h after the injection of progesterone or oil into non-laying hens (20/group) pretreated with oestradiol or oil

Group	Treatment*		LH-RH concentration (ng/mg)†	
			1 h	4 h
1	Oestradiol	Progesterone	^a 41.21 (27.16–62.37)	^c 32.43 (25.24–41.69)
2	Oil	Progesterone	^b 24.60 (19.32–31.62)	24.27 (19.54–30.06)
3	Oestradiol	Oil	15.89 (11.43–22.13)	18.29 (14.62–24.49)
4	Oil	Oil	16.79 (13.74–20.51)	19.59 (15.78–24.32)

* Intramuscular injections 4 h apart.

† Equivalents of NIH-LH-RH/FSH-RH determined by 4-point assays. The doses used were 5 and 10 mg equiv. of the extracts, and 160 and 320 ng standard LH-RH. The indices of precision (λ) of assay ranged from 0.074 to 0.100.

^a Significantly different from value for Group 2 ($P < 0.05$), Group 3 ($P < 0.01$) and Group 4 ($P < 0.001$).

^b Significantly different from value for Group 4 ($P < 0.02$).

^c Significantly different from value for Group 3 ($P < 0.001$) and Group 4 ($P < 0.001$).

Discussion

The results indicate that, in respect of the LH-releasing activity of the hypothalamus of non-laying hens, a single intramuscular injection of oestradiol induces a greater response to progesterone given 4 h after the injection of oestradiol. Since the cytoplasmic progesterone receptor concentration in the hypothalamus of the non-laying hen increases within 4 h of the injection of oestradiol (Kawashima *et al.*, 1979b), the greater response to progesterone may be due to the increase in the amount of receptors in the tissue. Although priming the hypothalamus by repeated injections of oestradiol benzoate on alternate days for 7 days before the induction of LH release by progesterone has been reported for ovariectomized pullets (Wilson & Sharp, 1976), the present data suggest that only one priming injection of oestrogen is effective for the induction of LH release in non-laying hens.

The data also indicate that the injection of progesterone alone causes the increase in the hypothalamic LH-releasing activity in non-laying hens (Group 1 versus Group 3, and Group 2 versus Group 4). This is not in agreement with previous results which did not show any appreciable increase in the gonadotrophin-releasing activity of the hypothalamus in non-laying moulting hens (Tanaka *et al.*, 1974). The discrepancy may be due to a difference in the method of assay and/or the birds used, because in the previous study the assay method was less sensitive

(a bioassay using ^{32}P uptake by chick testes which measures LH and FSH activities) and the hens had not laid for a longer period. Nevertheless, the results in the present study support the concept that oestrogen primes the hypothalamus of the hen for the release of LH-releasing hormone and that progesterone exerts its action on the hypothalamus as an inducing agent for the release of LH for ovulation.

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