Maturational changes in the LH response of domestic fowl to synthetic chicken LHRH-I and -II

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ABSTRACT

Treatment of chickens at different stages of sexual development with a single i.v. injection of synthetic chicken LHRH (cLHRH)-I or -II stimulated a rise in the plasma concentration of LH within 1 min. The activity of cLHRH-II was 1.3- to 2.7-fold greater than that of cLHRH-I in sexually immature cockerels and hens as determined by the changes in the plasma concentration of LH during the 5 or 10 min after injection. This could be attributed to both a greater effectiveness of cLHRH-II to stimulate LH release and to a more prolonged action. Thus, LH concentrations in plasma were maximal within 1–2 min of injection of all doses of cLHRH-I but within 2–5 min of injection at the higher doses of cLHRH-II. The responsiveness of the pituitary gland to cLHRH-I and -II was substantially greater in the sexually immature cockerel than in the hen and diminished during sexual development of the hen. Coincident with the onset of egg laying, the characteristics of the LH response to cLHRH-II changed to consist of an initial rise during the first 2 min, followed by a more sustained increase with LH concentrations still rising 10 min after injection. In contrast, after injection with cLHRH-I, plasma concentrations of LH rose to a peak at 2 min and thereafter declined gradually. Treatment of the sexually immature hen with oestradiol, progesterone or a combination of both steroids did not enable the expression of a laying hen-type response to the injection of cLHRH-II. It would appear, therefore, that unidentified events associated with the final stages of sexual maturation bring about changes in the mechanism of action of cLHRH-II which differ from those of cLHRH-I.

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INTRODUCTION

Two forms of luteinizing hormone-releasing hormone (LHRH) have been isolated from chicken hypothalami. These have been sequenced and described as LHRH-I (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Gln-Pro-GlyNH₂; King & Millar, 1982) and LHRH-II (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-GlyNH₂; Miyamoto, Hasegawa, Nomura et al. 1984).

Comparisons of the potencies of the two peptides with respect to their releasing activity in vivo indicate that chicken LHRH-II (cLHRH-II) is more potent than cLHRH-I in laying hens (Sharp, Dunn & Talbot, 1987), whilst both peptides are equipotent in cockerels (Chou, Johnson & Williams, 1985; Sharp et al. 1987). These findings were interpreted as reflecting a sex difference in the responsiveness of the anterior pituitary gland to stimulation by the two peptides (Sharp et al. 1987) and may imply that gonadal steroids differentially modify the pituitary response to each of the peptides. If this were true, the response to each of the peptides might be expected to vary in birds in different physiological states.

This possibility was therefore investigated by comparing the effectiveness of synthetic cLHRH-I and -II to release luteinizing hormone (LH) in vivo in sexually immature cockerels and hens, in sexually immature hens pretreated with oestradiol and progesterone, in 16-week-old hens during the period of rapid follicular growth (Wilson & Sharp, 1975) and in laying hens.

The response, in terms of LH release, to a bolus injection of mammalian LHRH is very rapid, particularly in sexually immature birds in which a maximal incremental change is observed between 2 and 6 min after injection (Wilson & Sharp, 1975). Thus, differences in potency of the two peptides could be masked by an insufficient frequency of sampling. To minimize this possibility blood was taken at frequent intervals throughout the first 5–10 min after injection.
MATERIALS AND METHODS

Chickens, of a strain derived from a broiler × layer cross, were maintained on a lighting schedule of 8 h light and 16 h darkness and had free access to food and water.

Doses of between 0·06 and 5·55 nmol cLHRH-I/kg body wt (Sigma Chemical Company Ltd, Poole, Dorset, U.K.) and cLHRH-II (Peninsula Laboratories Europe Ltd, St Helens, Merseyside, U.K.) were injected into a wing vein in a volume of 0·25 ml NaCl (0-9%, w/v). Purity of the LHRH preparations was confirmed by gradient high-pressure liquid chromatography using a C18 column. Blood (1 ml) was taken from the contralateral wing vein immediately before injection and at 1, 2, 5 and 10 min thereafter. The blood was centrifuged and the plasma separated and stored at −20 °C until assayed for LH. Injections of cLHRH-I or -II were made during the second half of the photophase which, in laying hens, avoided stimulating with LHRH in the period between 12 h before and 2 h after the occurrence of an ovulation when cyclical changes in the responsiveness of the pituitary gland to synthetic mammalian LHRH are known to occur (Bonney, Cunningham & Furr, 1974).

To examine the effect of gonadal steroids on the ability of cLHRH-I and -II to release LH, sexually immature (11 weeks of age) hens were given a single i.m. injection of between 23·5 and 376·5 nmol oestradiol benzoate/kg body wt (6·25–100 µg/bird; Sigma Chemical Company Ltd) or 1·6 µmol progesterone/kg body wt (500 µg/bird; Sigma Chemical Company Ltd) in 0·25 ml oil or oil alone at 24 h before the cLHRH stimulation test. A further group of hens (11 weeks of age) was given a series of priming injections consisting of 376·5 nmol oestradiol benzoate/kg body wt on alternate days for 7 days together with 1·6 µmol progesterone/kg body wt on days 5, 6 and 7 followed by a cLHRH stimulation test on day 8.

The concentration of LH in the plasma was determined using the radioimmunoassay method described by Follett, Scanes & Cunningham (1972). The standard and 125I-labelled tracer were a preparation of chicken LH (fraction IRC2) and the antiserum M201 was raised against the same material. In ten assays the minimum detectable level of LH ranged from 0·06–0·08 µg/l and the potency of a pooled sample of plasma included in each assay ranged between 2·35 and 4·32 µg/l (mean ± s.e.m. 3·78 ± 0·10 µg/l; n = 10).

For statistical analysis the LH responses were measured as the area of a plain figure under a curve (Simpson's rule; Crowe & Crowe, 1969) with the value for plasma concentration of LH at 0 min taken as the baseline. In some cases Student’s t-test was used for comparison of means as indicated in the text.

RESULTS

Treatment of chickens with a single i.v. injection of either cLHRH-I or -II at different stages of sexual development stimulated a rise in the plasma concentration of LH within 1 min. The response to cLHRH-II, in terms of LH released during the 5 or 10 min after injection was consistently greater than the response to cLHRH-I in both cockerels and hens. Thus, in 5-week-old cockerels (Fig. 1) cLHRH-II at doses of 0·18–2·78 nmol/kg body wt (0·1–1·6 µg/bird) stimulated a 40–60% greater release of LH than did cLHRH-I, as determined by measurement of the area of a plain figure under a curve (Simpson’s rule; Crowe & Crowe, 1969). Similarly, at 9 weeks, doses of 0·35–5·55 nmol cLHRH-II/kg body wt (0·4–6·4 µg/bird) stimulated responses which, in the hen, were 33–52% greater and, in the cockerel, were 30–172% greater than the responses to the same doses of cLHRH-I (Fig. 2).

The greater effectiveness of cLHRH-II compared with cLHRH-I to release LH was also evident when the lowest dose of 0·09 nmol cLHRH-II/kg body wt (0·1 µg/bird) stimulated a significant (P < 0·05; Student’s paired t-test) increase in the mean concentration of LH in plasma within 1–2 min in both cockerels and hens at 9 weeks of age, whereas the same dose of cLHRH-I was ineffective (Fig. 2).

The larger LH response to an injection of cLHRH-II as compared with cLHRH-I was also partially due to a more sustained action of cLHRH-II. This was illustrated in 5-week-old cockerels (Fig. 1) in which the maximal incremental change in LH was found at 1 min after injection of cLHRH-I, whilst LH concentrations continued to increase to a peak at 2 min after injection of cLHRH-II. In 9-week-old cockerels and 9- to 16-week-old hens, the maximal incremental change was at 2 min after injection of all doses of cLHRH-I whereas the two highest doses of cLHRH-II provoked a more sustained increase in LH to a peak at 5 min after injection (Fig. 2).

The response to the injection of both forms of cLHRH was greater in immature cockerels than in immature hens (Fig. 2) and decreased during sexual development of the hen (Figs 2, 3 and 4).

A marked difference in the profiles of the LH responses to cLHRH-I and -II was evident in laying hens. Although the responses to both peptides were of similar magnitude during the first 2 min after injection, plasma LH concentrations in hens given cLHRH-I then declined gradually. In contrast, in hens given cLHRH-II there occurred a further, more sustained increase in LH, to the extent that at 10 min after injection plasma concentrations of LH were still increasing (Fig. 4). Another feature of the response in the laying hen which contrasted with that of the...
sexually immature hen was the lack of a clear relationship between the dose of cLHRH-I or -II and the resulting plasma LH concentrations.

Pretreatment of 11-week-old sexually immature hens with a single i.m. injection of 23·5–376·5 nmol oestradiol benzoate/kg body wt (25 or 100 μg/bird) depressed the concentration of LH in plasma within 24 h to levels which, in hens given either 94 or 376·5 nmol oestradiol benzoate/kg body wt (25 or 100 μg/bird) were significantly ($P < 0.05$; Student's unpaired $t$-test) lower than those in vehicle-injected controls (Fig. 5a,b). Whilst oestradiol benzoate reduced the effectiveness of both cLHRH-I and -II to stimulate the release of LH, it did not appear to affect the differential between the relative activities of the two peptides. Thus, in hens pretreated with 23·5–376·5 nmol oestradiol benzoate/kg body wt (6·25–100 μg/bird), the response, in terms of LH released during 10 min after injection of the peptides, was 37–100% greater after injection with cLHRH-II than after injection with cLHRH-I. This difference in the effectiveness of the two peptides was of a similar order of magnitude to that of hens pretreated with vehicle alone (Fig. 5a,b). Pretreatment of 11-week-old hens with a single injection of 1·6 μmol progesterone/kg body wt (500 μg/bird) neither modified the basal concentration of LH in plasma nor the magnitude and duration of the LH response to injected cLHRH-I or -II (Fig. 5a,b). Treatment of 11-week-old hens with a combination of progesterone and oestradiol benzoate injections over 7 days depressed the basal concentration of LH in plasma and reduced the responsiveness of the pituitary gland to the extent that the LH response to cLHRH-I or -II during the first 2 min after injection was similar to that observed in the laying hen (Figs 4 and 5c,d). This combined treatment with oestradiol benzoate and progesterone did not, however, bring about the development of the extended response to cLHRH-II characteristic of the laying hen.

**DISCUSSION**

This study demonstrates that cLHRH-II is more potent than cLHRH-I in releasing LH in vivo in

**FIGURE 1.** Concentrations of LH in plasma of 5-week-old cockerels given a single i.v. injection of saline (●) or chicken (a) LHRH-I or (b) LHRH-II at 0·18 (▲), 0·7 (○) and 2·78 (▼) nmol/kg body wt. Values represent means ± S.E.M. for six birds.
chickens in different physiological states. This cannot be attributed to dissimilar half-lives of the two peptides (Sharp et al. 1987), and the extent of the difference in LH-releasing activity (1.3- to 2.7-fold) was of a similar order of magnitude in the sexually immature cockerel as in the immature hen, so discounting an influence of gender. However, marked changes in the profile of the LH response to cLHRH-II coincident with the onset of egg-laying, and not similarly observed in the response to cLHRH-I, would suggest some influence of events associated with sexual maturation on the mechanism of action of cLHRH-II on the anterior pituitary gland.

As found in studies using injected mammalian LHRH (Wilson & Sharp, 1975) the LH responses to cLHRH-I and -II in the female were maximal in sexually immature birds and diminished during the 3 weeks preceding the onset of lay. This period is associated with a fall in the basal concentration of LH in plasma (Wilson & Sharp, 1975) due, partially at least, to the suppressive action on the pituitary gland of raised blood levels of oestrogen (Peterson & Webster, 1981).
contradictions described in previous studies. Chickens LHRH-II, short duration of action, may not be equipotent to cLHRH-I, and the LH response to exogenous cLHRH-I and -II differed in sexually immature fowl in terms of the magnitude and duration of effect, a more pronounced difference in characteristics of the responses was apparent in the laying hen. Thus, in the immature hen the peaks of the LH responses to cLHRH-I and -II were attained within 1–2 min and 2–5 min respectively of injection and LH concentrations declined thereafter. In contrast, in the laying hen, the responses to cLHRH-I and -II were similar for the first 2 min after injection and then diverged, so that LH concentrations declined during the 2–10 min after injection of cLHRH-I, whilst in hens injected with cLHRH-II, LH concentrations underwent a further, more sustained increase during the same period.

In order to express the marked difference in characteristics of the responses to the two peptides in the laying hen and the less pronounced difference in duration of the responses in sexually immature chickens it was necessary to take blood samples at short intervals during the first few minutes after injection. This requirement may help to explain apparent contradictions between the findings of this and previous studies in vivo in which the two peptides were described as equipotent in releasing LH in the cockerel (Chou et al. 1985; Sharp et al. 1987).

The underlying cause of the pronounced change in duration of the response to cLHRH-II in the laying hen is not clear. This difference was not evident in hens examined at 16 weeks of age, i.e. at less than 3 weeks before the onset of egg-laying when plasma concentrations of oestrogen are raised (Peterson & Webster, 1974; Senior, 1974), and thus is unlikely to be attributable to the effects of oestrogen alone. In support of this suggestion, pretreatment of sexually immature hens with oestradiol benzoate did not affect the time-course of the response to either peptide. Also, pretreatment of laying hens with the anti-oestrogen tamoxifen (ICI 46,474), which raises both the basal concentration of LH in plasma and the responsiveness of the pituitary gland to mammalian (Wilson & Cunningham, 1981) and chicken LHRH-I and -II, does not abolish the laying hen-type of response to cLHRH-II (S. C. Wilson, R. T. Gladwell & F. J. Cunningham, unpublished observations).

If a change in the response to cLHRH-II at the onset of egg-laying is brought about by the action of ovarian steroids, it is possible that progesterone is involved since secretion of this steroid comes principally from the largest ovarian follicle (Bahr & Johnson, 1984) and is only secreted in significant quantities during the final stages of follicular maturation. However, pretreatment of sexually immature hens with progesterone or with a combination of progesterone and oestradiol, previously shown to bring about the development of the positive feedback
system within the hypothalamus-pituitary system of the ovariectomized hen (Wilson & Sharp, 1976), did not enable the expression of the response to cLHRH-II characteristic of the laying hen. The possibility that a similar response to cLHRH-II was present also in the sexually immature hen but was masked by the comparatively large release of LH during the first 2–5 min after injection seems unlikely in view of the fact that oestradiol–progesterone treatment of the sexually immature hen substantially reduced both the basal concentration of LH in plasma and the LH response to injected cLHRH-I and -II without revealing a laying hen-type profile of response.

The difference in response to injected cLHRH-I and -II could suggest the existence of different receptors for the two peptides. Studies by King, Davidson & Millar (1988) to investigate the possibility of the existence of more than one type of LHRH receptor using LHRH antagonists and receptor desensitization techniques were unable to demonstrate more than a single receptor type. However, these authors used dispersed pituitary cells from immature cockerels which may not necessarily be representative of those of the laying hen. Indeed, the difference between sexually immature and mature hens in the profile of their LH response to injected cLHRH-II indicates a need for investigations of changes in LHRH receptor characteristics associated with sexual maturation. Direct studies of cLHRH receptor properties have not, as yet, proved viable due to difficulties in the preparation of receptor populations with high affinity for LHRH (Millar & King, 1983). Thus, although suggestions may be proposed as regards the presence of more than one receptor type, different affinities of cLHRH-I and -II for a single receptor site, or differences in action at the post-receptor level, such suggestions can, at present, be no more than speculative. Also, assessment of the distribution of cLHRH-I and -II in the

![Concentrations of LH in plasma of laying hens given a single i.v. injection of saline (●) or chicken LHRH-I (□) or LHRH-II (■) at (a) 0-18, (b) 0-70, (c) 2-77 and (d) 5-55 nmol/kg body wt. Values represent means ± s.e.m. for six hens.](image-url)
FIGURE 5. (a and b). Concentrations of LH in plasma of 11-week-old hens pretreated with a single i.m. injection of 94 (■) or 376-5 (▲) nmol oestradiol benzoate/kg body wt or 1·6 µmol progesterone/kg body wt (○) followed 24 h later by a single i.v. injection of (a) 0·35 nmol cLHRH-I/kg body wt or (b) 0·35 nmol cLHRH-II/kg body wt. Values represent means ± S.E.M. for eight hens. For clarity, LH values for hens pretreated with 23·5 nmol oestradiol benzoate/kg body wt are not shown, but did not differ significantly from those of controls pretreated with vehicle (●). (c and d). Concentrations of LH in plasma of 11-week-old hens (□) pretreated with 376·5 nmol oestradiol benzoate/kg body wt on days 1, 3, 5 and 7 together with 1·6 µmol progesterone/kg body wt on days 5, 6 and 7 followed by a single i.v. injection of (c) 1·39 nmol cLHRH-I/kg body wt or (d) 1·39 nmol cLHRH-II/kg body wt on day 8. Controls were pretreated with vehicle (●). Values represent means ± S.E.M. for eight hens.
brain using immunocytochemical (Mikami et al. 1988) or radioimmunoassay (R. T. Gladwell, S. C. Wilson & F. J. Cunningham, unpublished) techniques has revealed cLHRH-II to be low or absent in the median eminence, which must cast doubt on the significance of this peptide as a physiological LH-releasing hormone.

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REFERENCES


