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Effects of Chicken (Gln^8)- and Mammalian (Arg^8)-Luteinizing Hormone-Releasing Hormones on the Release of Gonadotrophins *in Vitro* and *in Vivo* from the Adenohypophysis of Japanese Quail

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Both chicken luteinizing hormone-releasing hormone (Gln^8-LH-RH) and mammalian luteinizing hormone-releasing hormone (Arg^8-LH-RH) increased the release of FSH and LH from adenohypophysial halves of adult male Japanese quail when the halves were incubated in a medium containing LH-RH. Elevation of the FSH release was less marked than that of the LH release irrespective of the LH-RH species. A pulse stimulation (10 min) of adenohypophysial halves with chicken as well as mammalian LH-RH *in vitro* resulted in a burst of both FSH and LH release. The increment of the FSH release was smaller than that of the LH release. A single intravenous injection of each LH-RH to adult male quail induced rapid increases of plasma FSH and LH levels within 5 min. The plasma levels of FSH and LH returned to their initial levels at varying times after the injection depending on the dose of LH-RH. Again, the increment of the FSH level was smaller than that of the LH level, showing a similar profile to the *in vitro* pulse stimulation experiment. No significant difference in ability to stimulate FSH and LH secretions from the quail adenohypophysis was detected between chicken and mammalian LH-RHs under either *in vivo* or *in vitro* conditions, whereas chicken LH-RH has been reported to be far less potent than mammalian LH-RH when tested on the rat adenohypophysis.

Gonadotrophin secretion from the hypophysis in birds is considered to be controlled by a hypothalamic neurosecretory hormone, LH-RH, as it is in mammals. Follett (1970) first demonstrated in birds that chicken and quail hypothalamic extracts stimulated LH release from adenohypophysial halves of the chicken. Similar results were obtained with quail adenohypophysial halves (Smith and Follett, 1972) and dispersed chicken adenohypophysial cells (Bicknell and Follett, 1975). Authentic mammalian LH-RH could increase the release of LH *in vivo* in the chicken (Furr *et al.*, 1973; Bonney *et al.*, 1974) and quail (Davies and Bicknell, 1976), and also *in vitro* in the chicken (Bicknell and Follett, 1975; Bonney and Cunningham, 1977b). FSH release was enhanced by authentic mammalian LH-RH in the Japanese quail *in vivo* (Davies and Collins, 1979) and *in vitro* (Gledhill, 1977), and in the dove *in vivo* (Balthazart *et al.*, 1981).

The isolation and structural determination of an LH-RH-like substance from chicken hypothalami were recently reported by Miyamoto *et al.* (1982) and King and Millar (1982). They isolated a substance which stimulated LH release from the adenohypophysis of mammals. The substance had a structure identical to mammalian LH-RH except that an arginine residue at position 8 was substituted by glutamine. More recently, Millar and King (1983) showed that chicken LH-RH stimulated the release of LH from chicken ade-

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nohypophysial cells *in vitro*, and Hasegawa *et al.* (1984) also reported similar results in an *in vitro* experiment. For *in vivo* effects of chicken LH-RH in birds, no study has been published except three brief notes (Hattori *et al.*, 1983; Ishii *et al.*, 1984, for LH and FSH releases; Chan *et al.*, 1984, for LH release).

However, the effect of chicken LH-RH on FSH release from the avian adenohypophysis has never been examined. The present paper reports the effects of authentic chicken LH-RH as well as mammalian LH-RH on the release of FSH and LH in the Japanese quail.

MATERIALS AND METHODS

Materials. Five-week-old male Japanese quail (*Coturnix coturnix japonica*) were purchased from a commercial source. For *in vitro* experiments, they were exposed to daily photoperiods of 16 hr light and 8 hr darkness (lights on from 0800 to 2400) for about 3 weeks before each experiment. For the *in vivo* experiment, they were exposed to daily photoperiods of 8 hr light and 16 hr darkness (lights on from 0800 to 1600) for about 4 weeks. Just before the start of the experiment, each bird was visually examined and only individuals with a regressed cloacal protrusion were used.

In vitro experiments. Quail with developed testes were sacrificed by decapitation and adenohypophyses were immediately removed. The glands were cut midsagittally into two halves. In the continuous-exposure experiment, three halves were placed in each of several incubation flasks containing 0.5 ml of Medium 199 (pH 7.4) and preincubated without hormone at 37° under an atmosphere of 95% O_2 and 5% CO_2 for 3 hr with shaking. Then, the medium was renewed and 50 μl of a saline solution containing varying concentrations of chicken LH-RH (Miyamoto *et al.*, 1982), mammalian LH-RH (NIAMDD), or vehicle (0.9% NaCl) was added to each flask. The final concentration of the hormones in each flask was 1, 5, 25, 125, and 625 ng/ml with four replicate flasks. The gland halves were incubated in the presence of LH-RH for 2 hr.

In the pulse stimulation experiment, three gland halves were placed in each of several test tubes containing 1 ml of medium. After preincubation for 3 hr, the medium was changed every 10 min for 90 min. Chicken LH-RH (30 ng/ml) or mammalian LH-RH (30 ng/ml) was added only into the medium of the second renewal. In other words, the glands were exposed to LH-RHs for only 10 min just after the initial 10-min period. The experiment was performed in triplicate and all the incubation media were recovered from the tubes and stored at −20° until they could be assayed for gonadotrophins.

In vivo experiment. Nine groups of five to eight birds were injected intravenously with 120, 600, 3000, and 15000 ng of chicken or mammalian LH-RH, or vehicle (0.05 ml saline). Blood collections were performed from a wing vein before the LH-RH injection and 5, 15, 30, 60, and 120 min after the injection. The volume of blood samples was 0.15 to 0.25 ml per bird for each time. Plasma was separated by centrifugation and stored at −20° until it could be assayed for FSH and LH.

Radioimmunoassay of gonadotrophins. Concentratins of FSH and LH in incubation media or plasma samples were determined by the radioimmunoassay methods of Sakai and Ishii (1983) for FSH, and Hattori and Wakabayashi (1979) for LH. In the latter, we used chicken LH prepared by T. Yoshida and S. Ishii (unpublished) for radioiodination. This preparation is practically FSH free, and immunologically and biologically as potent as one of the most highly purified chicken LH preparations, IRC-2 of Hattori and Wakabayashi (1979).

Statistical analysis. Results were analyzed for significance by Duncan's multiple range test or two-way layout analysis of variance. Dose–response relations were evaluated by the linear regression analysis.

RESULTS

Effects of Continuous Exposure of Adenohypophysial Halves to Chicken and Mammalian LH-RH on the Release of FSH and LH in Vitro

Chicken LH-RH had a significant enhancing effect on the release of FSH ($P < 0.05$) (Fig. 1a). The formula of the regression line of the dose–response relationship was $Y = 15.4X + 167.3$. Mammalian LH-RH could also increase the mean FSH release (its regression line being $Y = 9.47X + 150.1$) but the difference in the response among the treated and control groups was not statistically significant ($P > 0.05$), when tested as a whole (Fig. 1a). This could be accounted for by large variation in FSH values, especially in the highest dose group. The maximum increase of FSH release with chicken LH-RH was 1.6 times the control level, being lower than the rate of the LH release. No significant difference was detectable between responses to

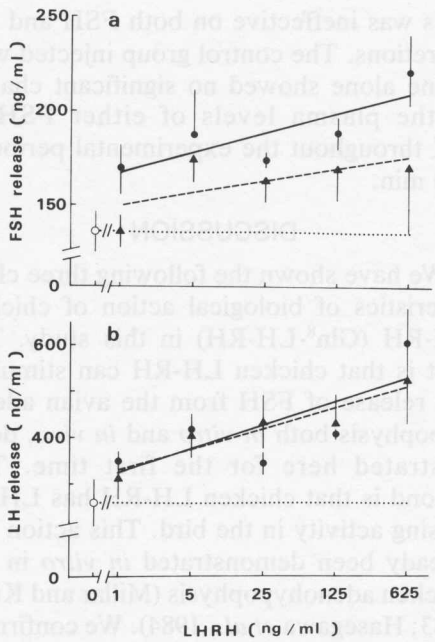

FIG. 1. Release of FSH (a) and LH (b) from adenohypophysial halves of Japanese quail *in vitro* in the presence of different concentrations of chicken (solid circles) or mammalian LH-RH (triangles). Doses of LH-RHs (ng/ml) are shown on the horizontal axis in a logarithmic scale, and the FSH and LH release on the vertical axis. A first-order regression line was calculated and drawn in each diagram. Each point represents the mean ± SEM of FSH and LH in four replicate incubation flasks. The saline control level (open circles) is indicated in the lower part of each figure. Amounts of FSH and LH are expressed in terms of the chicken FSH preparation, AGCHDS 111135A and chicken LH preparation, IRC-2(Gunma), respectively. Note the difference in the vertical axis scale between (a) and (b).

chicken LH-RH and mammalian LH-RH ($P > 0.05$).

Both chicken and mammalian LH-RH increased the release of LH significantly ($P < 0.05$) and dose dependently during the 2 hr of incubation (Fig. 1b). The maximum rate of increase of LH release was about two times the control level in both LH-RHs. The formula of the log dose–response line fitted was $Y = 73.2X + 324.6$ for chicken LH-RH and $Y = 65.5X + 329.8$ for mammalian LH-RH. There was no significant difference between responses to chicken LH-RH and mammalian LH-RH ($P > 0.05$).

Effects of Pulse Stimulation of Adenohypophysial Halves with Chicken and Mammalian LH-RH on the Release of FSH and LH in Vitro

Both chicken and mammalian LH-RH increased the release of FSH from adenohypophysial halves significantly ($P < 0.05$) at a dose level of 30 ng/ml (Fig. 2a). The release of the treated groups reached about 5.7 ng/ml as soon as the stimulation was given. Then, the release decreased and re-

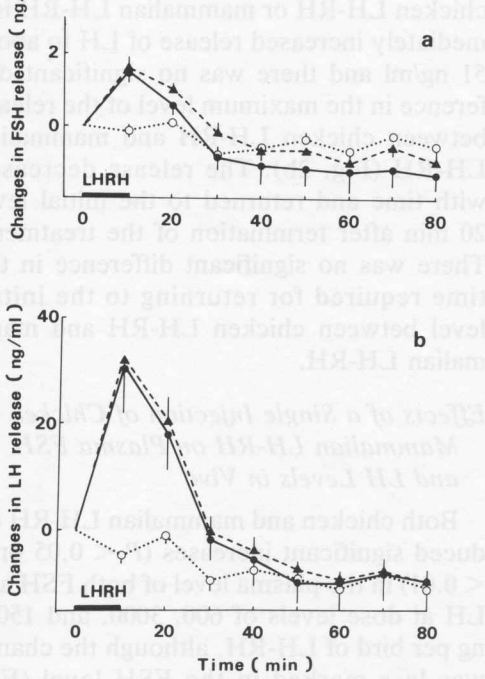

FIG. 2. Changes in the release of FSH (a) and LH (b) during 10 min from quail adenohypophysial halves which received a pulse stimulation with chicken or mammalian LH-RH (30 ng/ml) *in vitro*. The duration of the LH-RH pulse is indicated by a thick bar on the horizontal axis. The vertical axis shows the change in the FSH and LH release from the initial release. Each point represents the mean ± SEM of FSH and LH release in triplicate experiments. Chicken LH-RH (solid circles); mammalian LH-RH (triangles); saline (open circles).

turned to around the initial level of 4.06 ± 0.28 ng/ml (mean ± SEM) 20 min after the stimulation of LH-RH. The maximum enhanced level of the FSH release was 1.3 to 1.4 times the initial release in both chicken and mammalian LH-RH-treated groups, while the maximum level of the LH release was about 2.6 times (vide infra). Changes in the FSH release of the control group during the 80 min of the experimental period were within the range of random fluctuation.

The release of LH from the control adenohypophysial halves for the initial 10 min was 22.0 ± 4.1 ng/ml (mean ± SEM). It decreased slightly and gradually with time (Fig. 2b). The treatments with 30 ng/ml of chicken LH-RH or mammalian LH-RH immediately increased release of LH to about 51 ng/ml and there was no significant difference in the maximum level of the release between chicken LH-RH and mammalian LH-RH (Fig. 2b). The release decreased with time and returned to the initial level 20 min after termination of the treatment. There was no significant difference in the time required for returning to the initial level between chicken LH-RH and mammalian LH-RH.

Effects of a Single Injection of Chicken or Mammalian LH-RH on Plasma FSH and LH Levels in Vivo

Both chicken and mammalian LH-RH induced significant increases ($P < 0.05$ or $P < 0.01$) in the plasma level of both FSH and LH at dose levels of 600, 3000, and 15000 ng per bird of LH-RH, although the change was less marked in the FSH level (Fig. 3a,b). The maximum responses were observed 5 min after the injection in all cases, and there were no significant differences in the responses among the dose levels or between chicken and mammalian LH-RHs. However, the time required for return to the initial gonadotrophin level was greater in birds injected with higher doses of both chicken and mammalian LH-RH. The smallest dose (120 ng per bird) of both LH-RHs was ineffective on both FSH and LH secretions. The control group injected with saline alone showed no significant change in the plasma levels of either FSH or LH throughout the experimental period of 120 min.

DISCUSSION

We have shown the following three characteristics of biological action of chicken LH-RH (Gln^8-LH-RH) in this study. The first is that chicken LH-RH can stimulate the release of FSH from the avian adenohypophysis both *in vitro* and *in vivo*, demonstrated here for the first time. The second is that chicken LH-RH has LH-releasing activity in the bird. This action has already been demonstrated *in vitro* in the chicken adenohypophysis (Millar and King, 1983; Hasegawa *et al.*, 1984). We confirmed this action with the Japanese quail *in vivo* in addition to *in vitro*. The third characteristic is that chicken LH-RH enhances LH release more strongly than it does FSH release, when we compare in terms of the ratio between stimulated and unstimulated levels of each hormone.

We also confirmed that mammalian LH-RH could increase the release of both FSH and LH in the bird (Gledhill, 1977; Davies and Collins, 1979; Balthazart *et al.*, 1981 for FSH; Furr *et al.*, 1973; Bonney *et al.*, 1974; Bicknell and Follett, 1975; Davies and Bicknell, 1976 for LH). Mammalian LH-RH, as well as chicken LH-RH, was more potent as an LH releaser rather than as an FSH releaser in the bird.

As chicken LH-RH could stimulate the release of both FSH and LH in the Japanese quail as well as mammalian LH-RH does in mammals, we may designate Gln^8-LH-RH as avian LH-FSH-RH or gonadotrophin-releasing hormone. From the similarity in these characteristics of hormone actions to the avian adenohypophysis between chicken LH-RH and mammalian LH-RH, it appears that the substitution of an amino acid at the position 8 (Arg) of the mammalian LH-RH molecule with gluta-

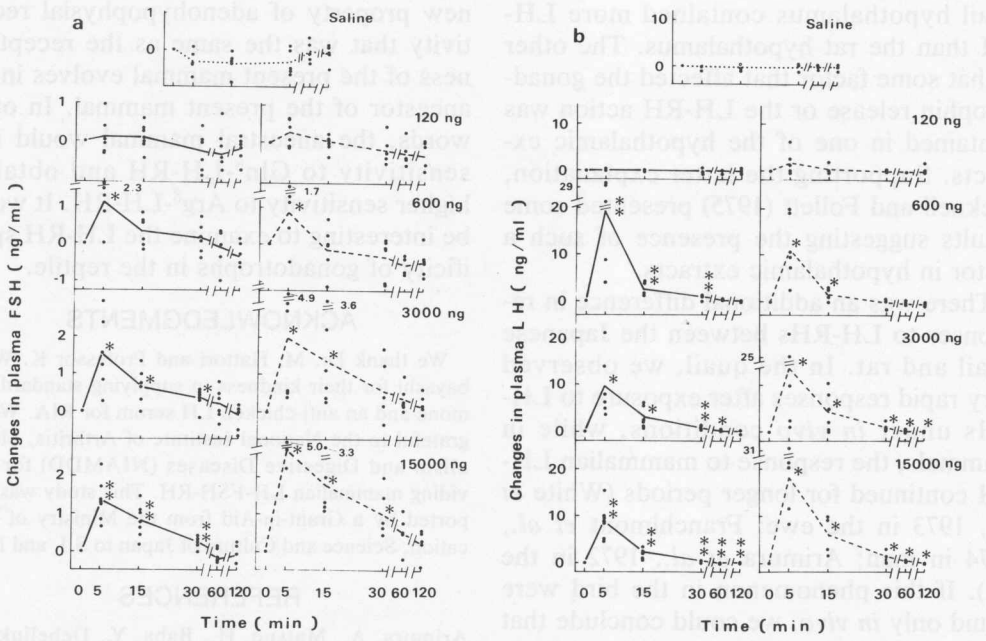

FIG. 3. Changes in plasma FSH (a) and LH (b) levels in male Japanese quail after a single injection of various doses of chicken or mammalian LH-RH. The vertical axis shows changes from the initial level in the concentration of FSH and LH in plasma after administration of chicken (left figures) or mammalian LH-RH (right figures). Each dot represents individual observations. In order to demonstrate large individual variations, we used individual values instead of means and SEM. The number of birds treated in each group is from five to eight. At several points, there may be less than five dots because of overlapping values. Solid and broken lines connect means of individual values. The injection of LH-RH or saline was performed at time zero. Note the difference in the scale on the vertical axis between (a) and (b). Statistical significance of the difference (tested by the two-way layout analysis of variance) in the response from that at time zero are indicated as follows: $*P \leqq 0.05$; $**P \leqq 0.01$.

mine (Gln) has no significant effect on gonadotrophin-releasing activity of LH-RH in birds. However, this is not true for mammals, as Yanaihara *et al.* (1972) and Miyamoto *et al.* (1982) reported that the gonadotrophin-releasing potency of Gln^8-LH-RH (chicken LH-RH) in the rat was only 4% of that of mammalian LH-RH. This shows that the hormone specificity of the receptivity of adenohypophysial gonadotrophs differs between mammals and birds. There have been other examples of such differences: des-Gly^{10}-LH-RH and Phe^5-LH-RH are more potent than Arg^8-LH-RH in the chicken (Bonney and Cunningham, 1977a), while the former two are less potent than the latter in the rat (Rivier *et al.*, 1972; Coy *et al.*, 1973; Yanaihara *et al.*, 1973). Hormone specificity of LH-RH receptivity of adenohypophysial cells may differ among vertebrate groups.

In a previous study comparing the LH releasing potency of a hypothalamic extract of quail with that of a rat hypothalamic extract, it was reported that the quail hypothalamic extract was more potent than the rat in stimulating LH release from the adenohypophysis of the Japanese quail *in vitro* (Hattori *et al.*, 1980). However, we could not find a significant difference in either FSH or LH releasing potencies between chicken and mammalian LH-RHs. This discrepancy may be explained by one of the following reasons. One is that the

quail hypothalamus contained more LH-RH than the rat hypothalamus. The other is that some factor that affected the gonadotrophin release or the LH-RH action was contained in one of the hypothalamic extracts. Supporting the latter explanation, Bicknell and Follett (1975) presented some results suggesting the presence of such a factor in hypothalamic extracts.

There was an additional difference in responses to LH-RHs between the Japanese quail and rat. In the quail, we observed very rapid responses after exposure to LH-RHs under *in vivo* conditions, while in mammals, the response to mammalian LH-RH continued for longer periods (White *et al.*, 1973 in the ewe; Franchimont *et al.*, 1974 in man; Arimura *et al.*, 1972 in the rat). If this phenomenon in the bird were found only *in vivo,* we could conclude that the half-life of circulating gonadotrophins in the bird was shorter than that in mammals. However, such a quick response is also observed *in vitro* in the quail. Accordingly, the responsiveness of gonadotrophs may be different between the bird and mammal. Davies and Collins (1979) reported that the release of FSH from the avian hypophysis stimulated by mammalian LH-RH lasted longer than the release of LH. Although we could not find such differences between FSH and LH secretions in the present experiments, we did find dose-dependent differences in the recovery time.

King and Millar (1980) reported that reptilian LH-RH was identical to avian LH-RH or more similar to avian LH-RH than to mammalian LH-RH. From this finding and the results of the present study, the following hypothesis may be proposed. In the course of the evolution of the mammal from the reptile, a point mutation that made Arg^8-LH-RH from Gln^8-LH-RH takes place, as already proposed by King and Millar (1982). We think that gonadotrophs of this ancestral animal would respond to both Gln^8-LH-RH and Arg^8-LH-RH equally as the present bird does. Then, a new property of adenohypophysial receptivity that was the same as the receptiveness of the present mammal evolves in the ancestor of the present mammal. In other words, the ancestral mammal would lose sensitivity to Gln^8-LH-RH and obtain a higher sensitivity to Arg^8-LH-RH. It would be interesting to examine the LH-RH specificity of gonadotrophs in the reptile.

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