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Luteinizing Hormone Secretion from the Quail Pituitary in Vitro[1]

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ABSTRACT

An enzymatically dispersed pituitary preparation from Japanese quail (Coturnix coturnix) was used to study the dynamics of gonadotropin release. After an 18-h incubation, the cells were challenged with different luteinizing hormone-releasing hormones (LHRH) for 90 min. Using pituitary cells from mature males, mammalian and chicken LHRH I (Gln^8-LHRH) had approximately equal luteinizing hormone (LH)-releasing activity whereas chicken LHRH II (His^5, Trp^7, Tyr^8-LHRH) was 8–9 times more potent. The LHRH agonist (Trp^6, Pro^9-NEt-LHRH) had 15 times greater potency than chicken LHRH I. Pre-incubation with an LHRH antagonist (D-Phe^2, D-Trp^6-LHRH) significantly suppressed LH release. Acid extracts of median eminence released LH from pituitary cells, extracts from short-day and long-day males had equal activity, while tissue extracts from castrated males had significantly greater LH-releasing activity. Pituitary cells from sexually immature males released LH in response to chicken LHRH I in a similar profile to cells from mature males. These data indicate that the quail LHRH receptor in the male recognizes several different molecular species of LHRH and the response to LHRH is comparable between short- and long-day males.

Pituitary cells from ovulating females were variably sensitive to LHRH peptides, possibly due to changes in pituitary sensitivity during the ovulatory cycle. Pituitary cells from immature females did not release LH in response to chicken LHRH I. However, pituitary cells from immature females photostimulated for 1 wk displayed a response to chicken LHRH I and II similar to that of pituitary cells from males. These data may indicate that maturation of the LH-releasing mechanism occurs during photostimulation in the female quail whereas maturation in the male occurs prior to photostimulation.

INTRODUCTION

Luteinizing hormone-releasing hormone (LHRH), a decapeptide originally isolated from porcine and ovine hypothalami, is active in controlling gonadotropin secretion from the anterior pituitary of birds (Bonney et al., 1974; Davies and Bicknell, 1976) as well as mammals (Schally et al., 1973). Recent work has shown that the chicken hypothalamus contains two LHRH molecules, [Gln^8]-LHRH (chicken LHRH I, cLHRH I) (King and Millar, 1982a,b; Miyamoto et al., 1982, 1983) and [His^5, Trp^7, Tyr^8]-LHRH (chicken LHRH II, cLHRH II) (Miyamoto et al., 1984). These two peptides as well as mammalian LHRH (mLHRH) have been shown to be bioactive in releasing luteinizing hormone (LH) from chicken pituitaries, in vivo and in vitro (Millar and King, 1984). In contrast, rat pituitary cells display a limited response to the two forms of chicken LHRH. These data seem to indicate that the LHRH receptor in the chicken has a broader specificity than the mammalian receptor.

Since most studies of the avian pituitary have used the domestic hen, a relatively photo-insensitive species, we chose the Japanese quail, a photosensitive species, as an alternative model for understanding the regulation of avian gonadotropin secretion in vitro. This study describes a bioassay technique using dispersed quail pituitary cells developed to evaluate the relative potency of different LHRH peptides to release LH. In addition, possible alterations in response to LHRH due to photoperiod were evaluated.

MATERIALS AND METHODS

Animals

Japanese quail (*Coturnix coturnix japonica*) were raised from hatching under short-day conditions

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(8L:16D). To synchronize maturation, 6- to 8-wk-old birds were transferred to long days (16L:8D) and caged individually. After 4–6 wk of photostimulation, reproductive behavior and cloacal foam were observed in the males and regular oviposition was observed in the females, indicating maturation.

Pituitary Cell Dispersal and Incubation

Quail were decapitated 6–8 h after lights on (unless otherwise noted), trunk blood was collected, and pituitary glands were removed and transferred to minimum essential medium (MEM; Gibco Laboratories, Grand Island, NY), buffered with 4-(2-hydroxyethyl)-l-piperazineethanesulfonic acid (HEPES, 25 mM) containing 0.1% bovine serum albumin (BSA), L-glutamine (292 mg/l), penicillin (100 U/ml) and streptomycin (100 μg/ml). The glands were quartered with a razor blade, transferred with 10 ml MEM to a conical centrifuge tube and let stand vertically for 10 min. The supernatant was removed and replaced with 10–20 ml MEM containing 0.05% collagenase (type II, Sigma Chemical Co., St. Louis, MO). The tube was flooded with 95% O_2/5% CO_2, sealed, placed horizontally in a Dubnoff shaker at 37°C, and agitated at 100 cycles per min. After 30 min, the tissue fragments were gently drawn into and out of a sterile, siliconized Pasteur pipette, fire-polished to an approximate diameter of 1 mm, and the tube was placed vertically for 5 min to allow the fragments to settle. The medium containing cells was transferred to a clean tube and the fragments were exposed to 10–20 ml 0.25% trypsin (Sigma Chemical Co.) in MEM containing 5 mM ethylenediaminetetraacetate (EDTA). The tube was flooded with 95% O_2/5% CO_2 and returned to the shaker. After 30 min, the fragments were again triturated and 20 ml of MEM were added to dilute the trypsin. The cells were combined with those from the collagenase dispersal and spun at 100 × *g* with slow acceleration and no brake at room temperature for 15 min. The supernatant was removed and the cells were resuspended in 20–40 ml MEM and passed through nylon gauze into a siliconized beaker with a stir bar rotating at minimal speed. The cells were counted with a hemocytometer, diluted to 5.0×10^5 cells/ml and 1 ml delivered to 12 × 75 mm plastic tubes with caps. This cell concentration was chosen because baseline and stimulated LH value values fell on the most sensitive part of the radioimmunoassay (RIA) curve while values from a lesser cell concentration were not reliable. Cell viability of greater than 95% was consistently found after dispersal using trypan blue exclusion. This procedure produced approximately 1.2×10^6 cells per pituitary.

The cells were preincubated overnight (18 h) at 39°C in a water-jacketed incubator (Forma Scientific, Marrietta, OH) receiving 95% air/5% CO_2. This 18-h preincubation was found to be necessary for responsiveness to cLHRH I since immediately after dispersal, the cells were insensitive. After preincubation, the tubes were spun at 100 × *g* for 10 min, the supernatant was poured off, saved, and replaced with 1 ml fresh medium containing test substances in triplicates or quadruplicates as indicated. The tubes were put in the Dubnoff shaker at 39°C and agitated at 100 cycles per min for a specified time interval, spun, and the supernatant was poured off and saved. The cells were lysed with 1 ml 0.05 M phosphate buffer (pH 7.4) and frozen at −20°C. After LHRH stimulation the amount of LH released overnight and the cellular content of LH were determined. Total LH, the sum of these values, was routinely calculated and found not to significantly vary within one experiment, indicating an equal number of cells were delivered to each tube.

Assay for LH

Fifty to 200 μl of medium were assayed in duplicate according to the method of Follett et al. (1972) using fraction AE-1 as the I^{125}-labeled hormone and standard. The antiserum (15/8) was used at a final dilution of 1:40,000. Antiserum and standard were kindly supplied by Dr. Colin Scanes, Rutgers University. Inter- and intraassay variance was 6.2 and 6.3%, respectively.

Test Substances

All peptides were purchased from Peninsula Labs. (Belmont, CA), except where noted. Mammalian LHRH (Beckman, Palo Alto, CA), LHRH I, LHRH II, salmon LHRH (sLHRH, Trp^7, Leu^8-LHRH), and an LHRH agonist ([Trp^6, Pro^9]-Net-LHRH; a gift of Dr. J. Rivier, Salk Institute, La Jolla, CA) were diluted in sterile saline and stored at −70°C. An LHRH antagonist (D-Phe^2, D-Trp^6-LHRH; a gift of Dr. J. Rivier, Salk Institute) was first diluted in propylene glycol and then in sterile saline. On the day of use, the LHRHs were thawed, diluted in MEM and used immediately.

Preparation of Hypothalamic Extracts

The anterior and lateral limits of the median eminence (ME) were defined by the optic chiasm and optic tracts and the posterior limit by the mammillary bodies. This tissue was held with fine forceps and excised to a depth of 2 mm with iris scissors. The fragments were weighed, homogenized in 0.1 N HCl (0.1 ml/ME) and frozen at −70°C. When ME extract for castrates was needed, animals were gonadectomized under anesthesia (xylaxine, 2.5 mg/kg and ketamine 6.7 mg/kg body weight) while in short-day and then transferred to long-day conditions for 4 wk. On the day of use, the homogenate was thawed and spun at 13,000 × *g* for 1 min in a microcentrifuge (Fisher, Pittsburgh, PA). The supernatant was neutralized with an equal volume of 0.1 NaOH, diluted in medium, and used immediately.

Statistics

Data from pituitary incubations were grouped by dose as well as by pre-incubation conditions, and analyzed by ANOVA. If significant differences ($p<0.05$) were found, the data were further analyzed by Duncan's multiple range test to ascertain where significant changes ($p<0.05$ and 0.01) occurred in LH release. The dose that induced half-maximal response (ED_{50}) was estimated by fitting the dose-response curves to a four-parameter logistic model (Rodbard, 1974).

RESULTS

A. Release of LH from Pituitary Cells from Males

1. Time course of LH release (Fig. 1). Pituitary cells from sexually mature males were exposed to medium alone or medium containing 10 ng cLHRH I for increasing lengths of time. The 90-min interval was shown to cause maximal increase in LH release (210% of baseline) over control in response to 10 ng cLHRH I (Fig. 1).

2. Luteinizing hormone-releasing activity of different LHRH molecules (Fig. 2, Table 1). Pituitary cells from mature males were used to determine the LH-releasing potency of mLHRH, cLHRH I, cLHRH II, sLHRH, and LHRH agonist over 90 min. A composite of the dose-response curves for cLHRH I and mLHRH were similar (Fig. 2), with comparable doses of each peptide releasing 50% of maximal release (ED_{50}, see Table 1). Maximal LH release occurred in response to 10 ng of either peptide; further increases in concentration were ineffective. Although cLHRH II was eight times more potent (ED_{50} = 0.096 nM) than cLHRH I (ED_{50} = 0.834 nM) in releasing LH, the maximum amount LH released was the same for both peptides. Based on two dose levels, sLHRH was more effective than cLHRH I in releasing LH ($p<0.05$ at 10 ng, $p<0.01$ at 1.0 ng). The agonist was more effective than cLHRH I in releasing LH, causing significant increases ($p<0.05$) in LH release over baseline at 0.05 ng agonist (not shown). Release was maximal at 1.0 ng; at doses greater than 1.0 ng, LH release was significantly depressed ($p<0.05$) compared to the 1.0 ng dose.

3. Effect of LHRH antagonist on the response to cLHRH I (Fig. 3). Cells from mature males were pre-incubated for 10 min with increasing amounts of the LHRH antagonist, and then challenged by adding 10 ng cLHRH I. All doses of the antagonist significantly suppressed cLHRH I-stimulated LH release. However, only 0.1 ng/ml antagonist was able to elimi-

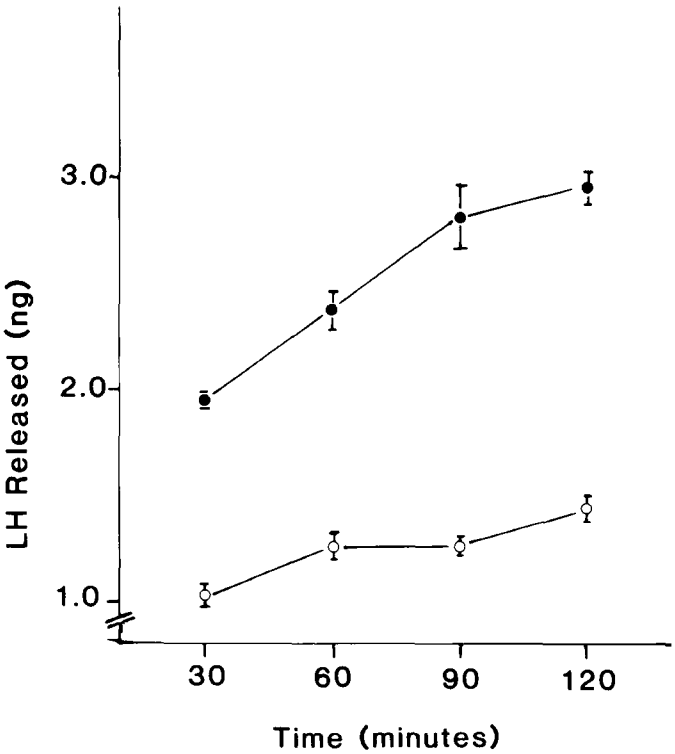

FIG. 1. Mean luteinizing hormone (LH) release (ng ± SEM, n=4) in baseline cells (*open circles*) and cells stimulated with 10 ng chicken luteinizing hormone-releasing hormone I (*closed circles*) with increasing *time* of incubation (minutes).

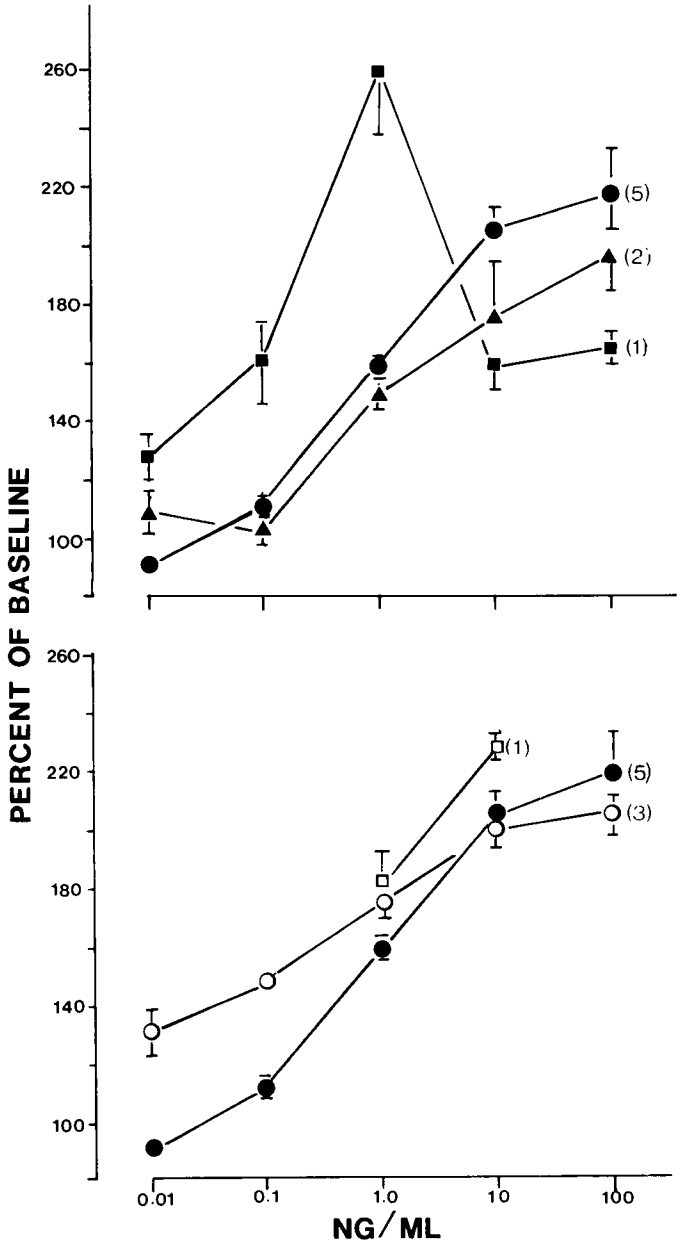

FIG. 2. Luteinizing hormone (LH) release from male pituitary cells expressed as *percent of baseline* (mean ± SEM) in response to increasing doses of luteinizing hormone-releasing hormone (LHRH) molecules shown as a composite of five cell preparations. *Numbers in parentheses* indicate different cell preparations used for each curve. Peptides used were chicken LHRH I (*closed circles*), mammalian LHRH (*closed triangles*), cLHRH II (*open circles*), agonist (*closed squares*), and salmon LHRH (*open squares*).

nate the response to cLHRH I. The other doses were unable to abolish a significant increase in LH release over baseline. The antagonist had no action on the unstimulated (baseline) LH release.

4. Effect of quail ME and cerebral cortical extracts on LH release (Fig. 4). Pituitary cells from mature males were challenged with increasing amounts of homologous ME, and the response was compared to that stimulated by an equivalent weight of cerebral cortical extract (control). Figure 4 shows that a dose of 0.05 ME equivalents stimulated a significant release ($p<0.05$) over control extracts, and the response plateaued at 0.1 ME equivalents. A further doubling of amount of ME (0.2 equivalents) caused no further release. Cerebral cortical extracts of equal weight had no effect on LH release.

5. Luteinizing hormone-releasing activity of ME from animals in different reproductive states (Fig. 5, Table 2). Pituitary cells from intact mature males were exposed to increasing doses of ME from 6- to 8-wk-old males kept in short-day conditions, 10- to 12-wk-old males photostimulated (long day) for 4 wk, 10- to 12-wk-old castrated males photostimulated for 4 wk and cLHRH I (Fig. 5). Short- and long-day ME were equipotent, but the extract of castrated male ME released 50% more at the maximum dose of 0.1 equivalents and was more effective at all doses than short-day or long-day ME extracts. The relative potencies of these extracts were determined by estimating the percentage of ME to release an amount of LH equivalent to 1.0 ng cLHRH (Table 2). Long-day male ME and short-day male ME were approximately equally potent, whereas the potency of ME from castrated males was 4- to 6-fold greater.

6. The effect of LHRH on LH release from pituitary cells of sexually immature quail. To determine the response of pituitary cells from immature quail to LHRH, pituitary cells from 8-wk-old male quail maintained in short-day conditions (8L:16D) were dispersed and exposed to increasing doses of cLHRH I. The profile of LH release from the cells in response

TABLE 1. Relative potencies of luteinizing hormone-releasing hormone (LHRH) peptides in releasing luteinizing hormone (LH) from male pituitary cells in vitro.

Peptide	ED_{50}[a]
Chicken LHRH I	0.96 ± 0.10 ng/ml (3) (0.834 nM)
Chicken LHRH II	0.12 ± 0.05 ng/ml (3) (0.097 nM)
Mammalian LHRH	0.94 – 1.8 ng/ml (2) (0.68 – 1.32 nM)
LHRH agonist	0.19 ng/ml (1) (0.057 nM)

[a] ED_{50} = dose of LHRH peptide that caused half-maximal LH release, mean ± SEM. Number in parentheses indicates experimental determinations from separate cell preparations.

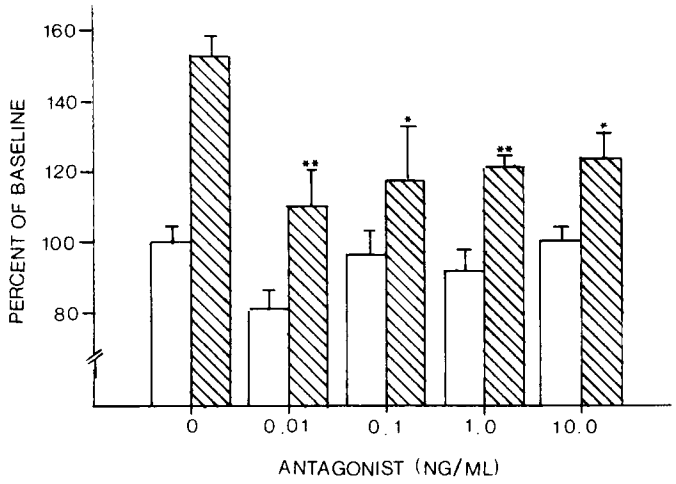

FIG. 3. Luteinizing hormone (LH) release (mean ± SEM, n=3) expressed as *percent of baseline* from male pituitary cells pre-incubated for 10 min with a luteinizing hormone-releasing hormone (LHRH) *antagonist* (D-Phe^2, D-Trp^6-LHRH) at doses indicated, and then challenged with medium alone (*white bars*) or medium containing 10 ng chicken LHRH I (*hatched bars*). Significant differences from cells with no antagonist are shown (*=$p<0.05$, **=$p<0.01$).

to cLHRH I was similar to that seen in cells from mature males. Exposure to 0.1 ng had no action on LH release, 1.0 ng caused a significant (148%, $p<0.05$) increase over baseline, and 10 ng cLHRH I released significantly more LH (190%, $p<0.05$). However, there was an approximately 25% lower baseline release and cellular content per 500,000 cells in these cells compared to cells from mature males.

B. Release of LH from Pituitary Cells from Females

1. Time course of LH release from pituitary cells from mature females (Fig. 6). Pituitary cells from regularly ovulating females killed at 7 h after lights-on were exposed to medium alone or medium containing 10 ng cLHRH I for increasing lengths of time. There was no difference between treatment at any time interval.

2. Effect of cLHRH I on pituitary cells from ovulating females at different times in the photoperiod (Table 3). To determine if time of death with reference to the 24-h cycle of lights-on influenced the response of pituitary cells from mature females to cLHRH I, animals were killed at 0, 3, and 7 h after lights-on. Pituitary cells from these groups were exposed to increasing concentrations of cLHRH I, and no response to this peptide was noted (Table 3). However, basal (control) LH release was significantly different ($p<0.05$), with the greatest amount being released at time = 0, and the least release at 7 h into

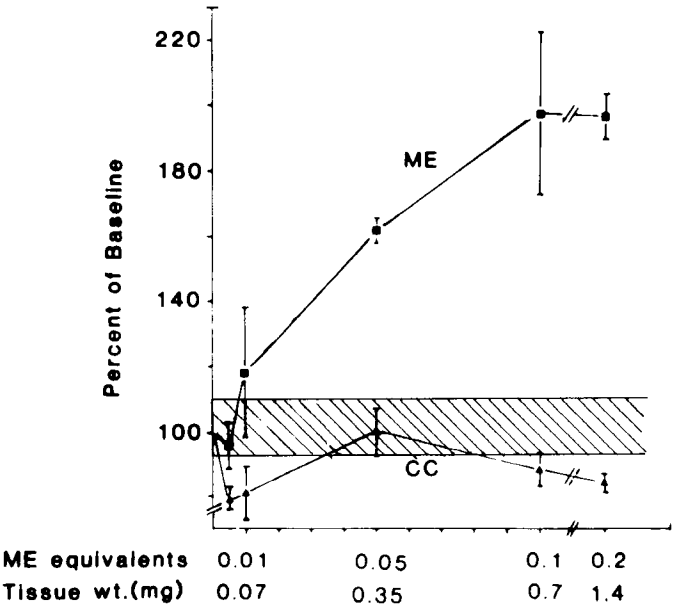

FIG. 4. Luteinizing hormone (LH) release, mean ± SEM, n=4, expressed as *percent of baseline* in response to median eminence (*ME*) extract (*square*) and equivalent weights of cerebral cortical (*CC*) extracts (*triangles*). *Shaded area* represents control (baseline) release ± SEM.

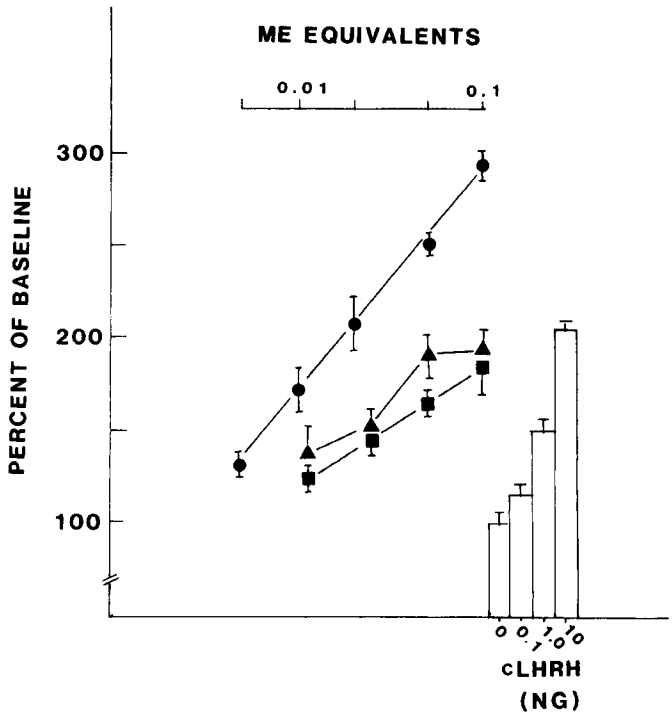

FIG. 5. Luteinizing hormone (LH) release, mean ± SEM, n=3, from male pituitary cells expressed as *percent of baseline*. Cells were challenged with increasing doses of chicken luteinizing hormone-releasing hormone I (*bars*), long-day median eminence (*ME*) extracts (*squares*), short-day ME extracts (*triangles*) and ME extracts from castrated males (*circles*).

TABLE 2. Relative potency of median eminence (ME) extracts from males of different reproductive states to release luteinizing hormone (LH).

Reproductive state	% of ME = 1 ng cLHRH I	cLHRH I equivalents per wt of ME (mg)
Long day (1 ME = 15 mg)	3.0% (0.45 mg)	2.2 ng/ml
Short day (1 ME = 11 mg)	2.5% (0.28 mg)	3.6 ng/ml
Castrate (1 ME = 10 mg)	0.7% (0.07 mg)	14.2 ng/ml

the photoperiod. There was no difference in total LH content between these groups.

3. Comparative effects of cLHRH I on cLHRH II. To evaluate the effect of cLHRH II, the more potent peptide, on LH release from ovulating females, pituitary cells from the birds killed 7 h after lights-on were exposed to increasing concentrations of cLHRH I and II. There was a significant increase (128%, $p<0.05$) in LH release over baseline in response to the two peptides (10 ng), though less than that seen in males. No dose-related effect was apparent.

4. Release of LH from pituitary cells from immature females. Pituitary cells from 6- to 8-wk-old females kept in short day (8L:16D) were exposed to increasing concentrations of cLHRH I. No increase in LH release was noted (data not shown).

5. Release of LH from pituitary cells from immature females (Fig. 7). Pituitary cells from 8- to 10-wk-old photostimulated (16L:8D, seven days) females were exposed to increasing doses of cLHRH I and II. These cells were extremely responsive to these peptides (Fig. 7). As in males, cLHRH II (ED_{50} = 0.193 nM) was more potent than cLHRH I (ED_{50} = 1.814 nM). However maximum release was comparable with both peptides.

DISCUSSION

These results demonstrate that isolated male quail pituitary cells respond to LHRHs and homologous ME in a manner similar to those of the domestic hen (Bicknell and Follett, 1975). The system thus provides an alternative model for studies of the dynamics of avian gonadotropin regulation in a smaller species than the domestic hen that is readily raised and maintained in the laboratory. The quail is also useful as an alternative to the hen because of its greater photosensitivity. The dose-response curves and the amount of gonadotropin released from the quail cells in response to a variety of LHRH peptides is remarkably similar to that reported by Bicknell and Follett (1975) for the hen. It is of interest to note that in contrast to pituitary cells from the quail, which need pre-incubation to be responsive, Bicknell

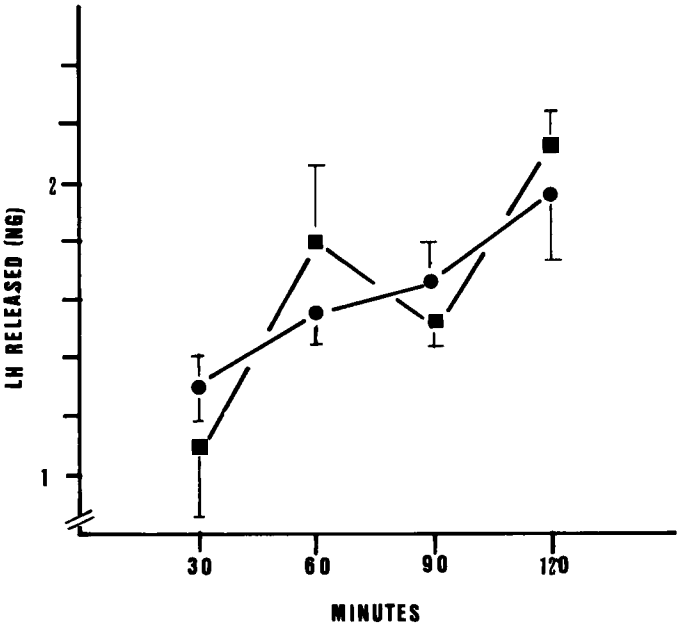

FIG. 6. Luteinizing hormone (LH) release (ng), mean ± SEM, n=3, from pituitary cells of ovulating females exposed to medium alone (*circles*) and 10 ng/ml chicken luteinizing hormone-releasing hormone I (*squares*) for increasing intervals of time (*minutes*).

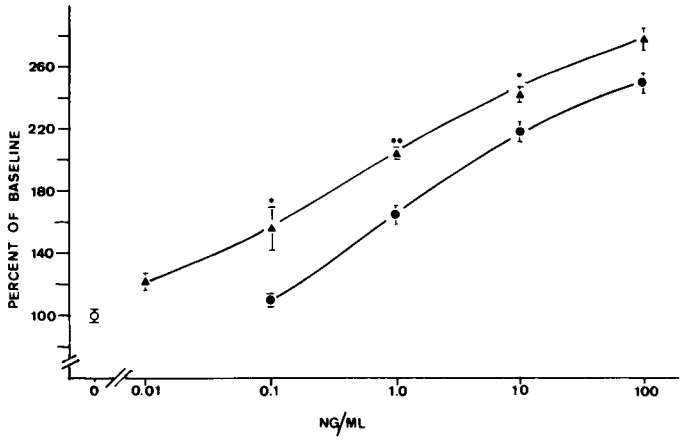

FIG. 7. Luteinizing hormone (LH) release from pituitary cells of females photostimulated for 1 wk expressed as *percent of baseline* (mean ± SEM, n=4) in response to medium alone (*open circle*), chicken luteinizing hormone-releasing hormone (cLHRH) I (*closed circles*) and cLHRH II (*triangles*). Significant differences between the action of the peptides at the same dose are shown with * (*=$p<0.05$, **=$p<0.01$).

TABLE 3. Luteinizing hormone (LH) released from pituitary cells of ovulating females killed at different times of the light cycle in response to chicken luteinizing hormone-releasing hormone I.

Time[a]	Control[bc]	0.1 ng/ml	1.0 ng/ml	10.0 ng/ml
0	3.34 ± 0.1*	2.88 ± 0.4	3.04 ± 0.06	3.16 ± 0.2
3	2.70 ± 0.2†	2.70 ± 0.2	2.84 ± 0.04	2.62 ± 0.1
7	1.81 ± 0.1††	2.38 ± 0.1	1.88 ± 0.1	2.20 ± 0.16

[a]Time after light-on.

[b]Cells exposed to medium alone; values with different symbols are significantly different ($p<0.05$).

[c]ng, mean ± SEM, n=4.

and Follett (1975) showed that cells from the hen lose responsivenss after preincubation.

In this study, cLHRH I and mLHRH were found to be equipotent in releasing LH from quail pituitary cells, as observed by Millar and King (1983) for dispersed chicken cells and Hattori et al. (1985) for whole quail pituitaries. In contrast, other studies have reported that cLHRH I is more potent than mLHRH in releasing LH from rooster (Johnson et al., 1984) and hen (Hasegawa et al., 1984) pituitary cells. These discrepancies may be due to differences in protein preparation or the physiological state of the pituitary cells. Chicken LHRH II was eight times more potent than cLHRH I, substantiating reports from other laboratories (Chou et al., 1985; Millar et al., 1986) using isolated chicken pituitary cells. The LHRH agonist was the most potent agent tested in the quail system, being nine times more potent than cLHRH I. In a mammalian system, in contrast, the agonist is 144 times more potent than mLHRH (Vale et al., 1977). To what extent these potency differences represent differences in experimental design, resistence of the LHRH to metabolic degradation, or receptor-ligand fit is of interest but cannot be resolved here. In the present study, the response to the LHRH agonist peaked at 1.0 ng/ml, and further increase in agonist caused a decline in LH release. This is not the response seen with rat pituitary cells where the maximum response is maintained with increasing agonist concentration (Vale et al., 1977). This indicates a possible difference in agonist action between mammals and birds. However, since cLHRH I and II are less potent than mLHRH in the mammalian pituitary cell system (1.5–3% and 30% of mLHRH, respectively; Miyamoto et al., 1983, 1984), and the quail cells also respond to avian, mammalian, and teleostean LHRH peptides, it would appear that the LHRH receptor in mammals is more selective than that of the quail. A situation similar to that of the quail exists in the goldfish pituitary, which responds equally well to a broad spectrum of LHRH molecules (mammalian LHRH = chicken LHRH I = salmon LHRH; Peter et al., 1985).

Millar and King (1984) have analyzed LHRH interaction with gonadotrophs of different vertebrates in terms of the theoretical molecular flexibility of the peptides in comforming to the receptor. This work has shown that changes in amino acid groups at certain points of the molecule prevent the activation of a given receptor. In mammalian systems, any alteration at position eight renders the peptide inactive as an LH-releasing agent, due to changes in the three-dimensional shape of the molecules. So far, all LHRH molecules sequenced from non-mammalian vertebrates have a different amino acid at position eight than mammalian LHRH and are less active than mammalian LHRH in releasing LH from mammalian pituitaries. Thus alterations in receptor structure that may occur in vertebrate evolution could prevent conformation of non-mammalian LHRHs to the mammalian receptor. This may explain the relative inactivity of non-mammalian LHRH molecules in mammals, which appear to have the most discriminating LHRH receptor and only one form of hypothalamic LHRH. In contrast, birds have at least two forms of hypothalamic LHRH, each of which activates the receptor. However, these two peptides may activate the receptor differently than since cLHRH II has been shown to be 15 times more potent than cLHRH I in releasing FSH from chicken pituitary cells (Millar et al., 1986).

The LHRH antagonist (D-Phe^2, D-Trp^6-LHRH) was found to block cLHRH I action, indicating some structural and activation similarities between the mammalian and avian LHRH receptors. It is of interest to note that the antagonist did not show a dose-related action on suppressing LH release nor was it able to eliminate completely the response to

LHRH. Antagonists of LHRH do not appear to have a greater affinity for the LHRH receptor (Clayton and Catt, 1979), but rather may induce a conformational change in the LHRH receptor that makes LHRH stimulation impossible (Kuhl and Baumann, 1981).

In contrast to male quail pituitary cells, responses of cells from female birds were inconsistent, and killing birds at different times, with reference to lights-on, did not improve the response. Close observation of the hens in our colony revealed little synchronization of egg laying with respect to lights-on, and we believe the inconsistent results obtained here to be due to the pooling of pituitary glands from females at different physiological states, with reference to ovulation. Measurement of plasma steroids grouped according to the status of the ovulated egg in the oviduct has revealed significant changes in plasma steroid (progesterone, estradiol, and testosterone) (Connolly and Callard, unpublished results) that might influence the sensitivity of the pituitary cells to LHRH. Indeed we have demonstrated (Connolly and Callard, 1984) that testosterone decreases the response of male pituitary cells to LHRH. In another study, we have shown (Connolly and Callard, unpublished results) that pituitaries pooled from animals at the same stage of the ovulatory cycle, irrespective of lights on, exhibit distinct responses to LHRH that are least when plasma testosterone is high and most sensitive to LHRH when progesterone is rising. Thus, the poor LHRH responses of female pituitary cells described here are presumably due to the pooling of relatively few sensitive cells with many insensitive cells.

There is evidence from avian and mammalian studies showing that the sensitivity of the pituitary to LHRH varies throughout the reproductive cycle. Thus, ovulating chickens injected with 20 μg LHRH did not respond if the hormone was given within the 3 h prior to ovulation (Bonney et al., 1974), which is immediately after the preovulatory surge of LH. At the other times of the ovulatory cycle, a 2-fold increase in plasma LH in response to LHRH was observed.

Other work in the chicken has also found maximal sensitivity to LHRH during maturation, which diminishes and disappears just prior to the first ovulation (Bonney et al., 1974). This decrease in sensitivity occurs as plasma LH and plasma gonadal steroids (progesterone, testosterone, and estradiol) increase. Work with the rat has shown that LHRH receptor numbers increase during sexual maturation (Duncan et al., 1983), and that during puberty the male and female pituitary is most sensitive to exogenous LHRH, releasing the greatest percentage of increase in plasma LH during that time (Debeljuk et al., 1972). In the rat, the responsiveness to LHRH is minimal on the day following ovulation (Diestrus 1), begins to increase on the second day of diestrus, is clearly augmented on proestrus and reaches a peak at the time of the preovulatory LH surge (afternoon of proestrus) (Zeballos and McCann, 1975). Dispersed pituitary cells taken during these different time periods in the rat estrous cycle retain sensitivity differences (Speight and Fink, 1981), and changes in responsiveness during the menstrual cycle of the human have also been shown to follow a similar pattern (Yen et al., 1975).

The present study indicates that in contrast to mature females, immature females recently moved from short-day to long-day conditions for 1 wk provide cells with adequate gonadotropin content and sensitivity to LHRH to allow a response in the absence of possible inhibitory plasma steroids. In contrast to these photostimulated immature females, pituitary cells from short-day, immature females did not respond to LHRH, possibly due to the absence of LHRH receptors. Since LHRH is thought to induce its own receptors (Duncan et al., 1983), low LHRH levels in immature females could be responsible for LHRH insensitivity.

Follett (1984) has shown that movement of male quail from short-day to long-day conditions will bring about an immediate increase in plasma LH. This immediate increase in gonadotropins probably reflects available LHRH for secretion since median eminence from short- and long-day male quail contained approximately equal quantities of bioactive LHRH. This is supported by our observations. The calculated concentration of bioactive LHRH in the median eminence of the male quail (2.2 ng/mg or 33 ng/ME), based on the pituitary cell bioassay, is comparable to that found by RIA in the infundibulum of the chicken (69 ng; Johnson and Advis, 1985). These levels of LHRH in the median eminence would provide adequate quantities of LHRH to release LH at an ED_{50} of 1 ng/ml. The increase observed in the ME of the castrate has been previously shown in the quail (Bicknell and Follett, 1975), though to a lesser extent.

In conclusion, quail pituitary cells, in vitro, have been shown to release LH in response to several forms of LHRH. This suggests that the avian LHRH receptor

is not as discriminating as that of mammals. The response in cells from males did not vary with sexual maturation. In contrast, pituitary cells from females showed no response at sexual immaturity, maximal response during photostimulation, and minimal or no response at maturity. The basis for the minimal response at maturity may be due to fluctuations in sensitivity to LHRH during the avian ovulatory cycle that are retained in dispersed pituitary cells.

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