Thermal Stress Reduces Serum Luteinizing Hormone and Bioassayable Hypothalamic Content of Luteinizing Hormone-Releasing Hormone in Hens

DAN J. DONOGHUE, BILL. F. KRUEGER, BILLY M. HARGIS, ANN M. MILLER, and MOHAMED EL HALAWANI

Departments of Poultry Science and Veterinary Microbiology, Texas A&M University and Texas Agricultural Experimental Station, College Station, Texas 77843, and Department of Animal Science, University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT

Studies were conducted to evaluate the effects of acute (24 h) thermal stress on anterior pituitary function in hens. Circulating levels of luteinizing hormone (LH) were measured and the ability of the pituitary to respond to luteinizing hormone-releasing hormone (LHRH) challenge was determined. Moreover, bioassayable hypothalamic LHRH content was assessed by using dispersed anterior pituitary cells.

In two separate experiments, circulating levels of LH were reduced in hens exposed to acute thermal stress (35°C). Injection of LHRH did not result in significant differences in release of LH between normothermic and hyperthermic hens. However, the hypothalamic content of bioassayable hypothalamic releasing activity from hyperthermic hens were significantly reduced compared with normothermic hens. Taken together, these data suggest that the reproductive decline in the acutely heat-stressed hen is mediated by reduced LH releasing ability of the hypothalamus.

INTRODUCTION

Exposure to thermal stress (acute or chronic) adversely affects ovulation rate and decreases reproductive performance in the laying hen (Miller and Sunde, 1975; DeAndrade et al., 1976, 1977; Jones et al., 1976; Wolfenson et al., 1979; Cowan and Michie, 1980; Tanor et al., 1984; Scott and Balnave, 1988a,b). However, the physiological mechanisms resulting in the reduced reproductive performance of hyperthermic hens are not clear at the present time. It has been suggested that the decline in ovulation rate may be an indirect consequence of reduced feed intake and subsequent body weight loss experienced by the hyperthermic hen (DeAndrade et al., 1976; Austic, 1985). Conceivably, however, the reduced reproductive performance in the hyperthermic hen is a direct consequence of exposure to elevated ambient temperatures. The possibility that thermal stress may directly disrupt neuro-endocrine mechanisms controlling ovulation prior to the decline in feed consumption and body weight has not been evaluated.

The focus of this investigation was to determine if acute thermal stress adversely affects the neuroendocrine control of luteinizing hormone (LH) release in the domestic hen. Acute heat stress was employed (24 h) to limit the confounding influence of reduced feed consumption and body weight loss.

MATERIALS AND METHODS

Four separate experiments were conducted in this study to evaluate the effects of acute thermal stress on the endocrine regulation of ovulation. In Experiments 1 and 2, circulating levels of LH were evaluated and in vivo pituitary responsiveness to luteinizing hormone-releasing hormone (LHRH) was determined in the hyperthermic hen. In Experiments 3 and 4, bioassayable
hypothalamic LHRH content was quantified in vitro in dispersed chicken pituitary cells.

**Experiment 1**

Twenty Single Comb White Leghorn hens, 46 wk of age, were separated into two equal groups and individually caged in two environmentally controlled chambers (10 birds per room). Both groups were acclimated to an ambient temperature of 23°C for a period of 2 wk. After the 2-wk acclimation period, one group was exposed to an ambient temperature of 35°C for 24 h (hyperthermic group). The ambient temperature of the hyperthermic group was increased slowly over a 1-h period from 23°C to 35°C. The relative humidity was 40% during the heat-stress period. The hens in the other environmentally controlled chamber were maintained at 23°C during this same 24-h period and served as controls (normothermic group). Immediately after this 24-h period, blood collection was initiated as described below. The two groups remained at their respective ambient temperatures throughout the blood sampling period.

To determine pituitary responsiveness during thermal stress, both temperature treatment groups were further subdivided into equal groups of five each and were injected i.v. with either LHRH dissolved in physiological saline (25 µg/kg body weight) or physiological saline alone. The hens were bled in the late afternoon to avoid sampling during the preovulatory LH surge. The hens were maintained on a lighting regime of 18L:6D (lights on at 0400 h, off at 2200 h). Standard layer mash containing 17% protein and 2718 metabolizable energy and water were provided ad libitum.

**Blood sampling protocol.** Six 2-ml samples of blood were taken from each hen by venipuncture from the brachial vein. The six blood samples were collected at −30, 0, 5, 15, 30, and 60 min relative to an i.v. injection with chicken LHRH-I (Peninsula Laboratories, Belmont, CA) dissolved in physiological saline. The zero time blood sample was taken immediately prior to injection with LHRH. The control hens received only an injection of physiological saline.

To partition the effect of thermal stress from handling effects on changes in serum corticosterone concentrations, the first blood samples were obtained within 30 s of removing the hens from their cages. Any effect of handling on serum corticosterone levels is not evident until 45 s after the initiation of handling (Beuving and Vonder, 1978). It was not possible to determine if blood samples taken subsequent to the first blood collection had elevated corticosterone levels due to either treatment effects or handling. Therefore, to properly assess the effects of thermal stress on corticosterone levels, only data from the first blood sample are presented in this paper.

After sampling, blood was allowed to clot, it was then centrifuged, and the serum was harvested and stored at −20°C. Rectal temperatures were taken after the last blood sample with a type J thermocouple thermometer (Cole-Palmer Instrument Co., Chicago, IL).

**Experiment 2**

Experiment 2 was performed to serve as a replicate for Experiment 1. This experiment was performed approximately 1 mo after Experiment 1 within the same environmental chambers. A different group of hens of similar age to those in Experiment 1 were subjected to the procedures described above.

**Experiment 3**

Forty-four Single Comb White Leghorn hens, 40 wk of age, were separated into two equal groups and placed two to a cage (22 birds per chamber) and maintained otherwise as described in Experiments 1 and 2. After exposure to either 23°C (control group) or 24 h of 35°C (hyperthermic group), the hens were killed; anterior pituitaries from the control group, and hypothalami from both groups were immediately removed and placed in ice-cold tissue culture medium or liquid nitrogen, respectively. Hypothalami removed from normothermic and hyperthermic hens were kept in separate containers.

Bioassayable hypothalamic LHRH content in normothermic and hyperthermic hens was assessed by the degree of LH release from dispersed pituitary cells following challenge with hypothalamic extract. The hypothalamic extract from normothermic and hyperthermic hens was prepared according to the methods of El Halawani et al. (1988). Dispersion of pituitary cells was accomplished by the method of Knapp et al. (1987). Dissociated pituitary cells were preincubated for 3 h at 40°C in 95% O₂/5% CO₂ in M199 supplemented with 10% heat-inactivated charcoal-stripped serum from 6-wk-old hens plus 50 U/ml penicillin, and 50 µg/ml streptomycin. After the 3-h preincubation, pituitary cells were centrifuged and resuspended in 1-ml aliquots of test incubation medium with or without hypothalamic extract. Incubation medium contained 50 U/ml penicillin, 50 µg/ml streptomycin, and 0.1% bovine serum albumin (fraction V, Sigma, St. Louis, MO).
Incubations were conducted with 8 tubes per treatment with \(4 \times 10^5\) viable cells per tube. The three treatments consisted of addition of medium alone, hypothalamic extract from normothermic hens, or extract from hyperthermic hens. The dose of hypothalamic extract used for challenge was 1.0 equivalents dose. Equivalents were determined by dividing the supernatant volume recovered after extraction divided by the number of hypothalami used. Preliminary experiments demonstrated significant LH release from dispersed pituitary cells with the 0.1 equivalents dose, whereas 1.0 equivalents gave a maximal response (data not shown). Cell viability was greater than 95% as determined on a live cell basis by the trypan blue exclusion procedure.

**Experiment 4**

Experiment 4 was performed to serve as a replicate for Experiment 3. This experiment was performed approximately 1 mo after Experiment 3 within the same environmental chamber. Hens of similar age to Experiment 3 were subjected to the procedures described above.

**Assay Procedures**

LH levels were determined by a double antibody radioimmunoassay (RIA) according to the method of Burke et al. (1979) as validated for chickens by Goldsmith and Follett (1983). Corticosterone was assayed with double antibody RIA diagnostic kits (Radioassay Systems Laboratories, Carson, CA). Cross-reactivity for progesterone was less than 0.02% and less than 0.3% for all other steroids tested. In addition, a statistically parallel curve was demonstrated between known corticosterone standards and serum dilutions containing a total serum volume from 25 µl to 200 µl. The serum volume used in this assay was 100 µl. All samples for LH or corticosterone determination were assayed in duplicate within a single assay. For LH and corticosterone assays, respectively, the intraassay coefficients of variation were 12% and 2.6%, and the lower limits of assay sensitivity were 0.18 ng/ml and 0.19 ng/ml.

**Statistical Analysis**

Data were analyzed by analysis of variance (ANOVA) using the general linear model procedure of SAS (1982). Serial LH blood sample values were analyzed by ANOVA for repeated measures (SAS), and Tukey’s multiple range test was used to separate the means. Percentage data analysis is based on arcsin transformation.

**RESULTS**

**Experiments 1 and 2**

After exposure to 35°C for 24 h, hens had significantly elevated body temperatures (42.6°C and 42.7°C for Experiments 1 and 2, respectively) when compared with the 23°C controls (40.7°C and 40.8°C for Experiments 1 and 2, respectively). Injection of LHRH at the rate of 25 µg/kg body weight did not have a significant effect on body temperature (42.5°C vs. 42.6°C, 42.9°C vs. 42.7°C [Experiment 1], and 40.7°C vs. 40.7°C, 40.7°C vs. 40.8°C [Experiment 2] for injected and control hens, respectively).

Heat-stressed hens exhibited significantly reduced serum LH levels \((p<0.05)\) in both experiments compared with normothermic hens \((2.41 \pm 0.16 \text{ ng/ml vs. } 3.41 \pm 0.35 \text{ ng/ml; and } 2.10 \pm 0.23 \text{ ng/ml vs. } 3.19 \pm 0.43 \text{ ng/ml for hyperthermic vs. normothermic hens in Experiments 1 and 2, respectively; Fig. 1). These values represent data for the first blood samples collected prior to injection of LHRH. To determine the responsiveness of the pituitary to hypothalamic stimulation during thermal stress, LHRH was injected into the hens after the second blood sample was collected (Time 0). To clarify the response to
LHRH injection, serum LH concentrations are presented as a percent change between the mean of the first two blood samples and subsequent samples. This eliminates initial, basal differences of circulating LH concentrations (Fig. 1) in the hyperthermic hens vs. normothermic hens. Upon injection of LHRH (25 μg/kg body weight), circulating LH concentrations were not significantly different between normothermic and hyperthermic hens in Experiment 1 or Experiment 2 (Fig. 2).

Hens exposed to 24 h of thermal stress had significantly (p<0.05) elevated blood corticosterone concentrations compared with controls (3.37 ± 0.18 ng/ml vs. 4.74 ± 0.74 ng/ml normothermic and hyperthermic hens, respectively). Corticosterone data were combined between experiments since differences between experiments were not statistically significant (p>0.05).

Experiments 3 and 4

Challenge with 1.0 equivalents of hypothalamic extract prepared from normothermic and hyperthermic hens differed in ability to stimulate release of LH from pituitary cells. The hypothalamic extract from normothermic hens (p<0.05) increased LH release compared with cells receiving medium alone (62.0 ± 6.6 vs. 43.5 ± 2.4; Fig. 3). However, challenge with hypothalamic extract from the thermally stressed hens did not significantly stimulated LH release from pituitary cells (44.7 ± 2.0 vs. 43.5 ± 2.4; Fig. 3). In Experiment 4, from a new group of hens, 1.0 equivalents of hypothalamic extract from normothermic hens significantly increased LH release from pituitary cells compared with
cells receiving medium alone (56.2 ± 3.4 vs. 47.2 ± 2.8; Fig. 3). Challenge with extract from the thermally stressed hens did not significantly affect LH release (46.0 ± 2.6 vs. 47.2 ± 2.8; Fig. 3).

**DISCUSSION**

We have demonstrated that acute exposure to 35°C reduces circulating levels of LH in the mature laying hen (Fig. 1). The decline in serum LH levels is indicative of the diminished reproductive performance in the hyperthermic hen. This relationship has been reported for other stressful conditions, i.e. reduced calcium and feed availability (Luck and Scanes, 1979; Richard-Yris et al., 1987) and under conditions of elevated corticosterone (Etches et al., 1984; Petitte and Etches, 1988).

The decline in LH apparent in this study was not due to a direct effect of thermal stress at the level of the pituitary. Intact pituitary integrity was evident in the hyperthermic hen upon evaluation of serum LH concentrations following injection of LHRH (Fig. 2). The lack of change in LH released after LHRH injection demonstrates pituitary responsiveness in the hyperthermic hen.

The decline in serum LH, without a change in pituitary responsiveness to exogenous LHRH, could indicate reduced output of endogenous LHRH from the hypothalamus. If thermal stress increases degradation or reduces synthesis and subsequent output of LH, then a resultant decline in circulating levels of LH could occur. It is suggested that this is what happens in the heat-stressed hen; thus hypothalami removed from hyperthermic hens had greatly reduced LHRH content as assessed by bioassay (Fig. 3). In total, these data indicate hypothalamic hypofunction as a possible mediator of the reproductive decline during thermal stress.

In mammals, thermal stress has also been reported to reduce serum LH concentrations in domestic cattle (Madan and Johnson, 1973; Miller and Alliston, 1974; Vaught et al., 1977; Roa and Pandey, 1983). To our knowledge, hypothalamic LHRH content has not been determined during thermal stress in mammals. The decline in serum LH for rats subjected to chronic immobilization stress, however, has been suggested to be mediated by a reduction in hypothalamic LHRH content or release (Briski et al., 1984; Gambacciani et al., 1986; Nikolarakis et al., 1988).

The cause for the reduction in hypothalamic LHRH content in the thermally stressed hen is not clear. The avian brain may be adversely affected by elevated levels of circulating corticosterone occurring during thermal stress. It is known that high concentrations of corticosterone depress circulating LH levels in Aves (Deviche et al., 1979; Etches et al., 1984) without affecting pituitary sensitivity to LHRH. Infusion of corticosterone (Petitte and Etches, 1988) or hypothalamic implants of corticosterone (Wilson and Follett, 1975) block the photo-stimulated increase in LH in Aves, presumably due to inhibition of LHRH output. Although the time-course relationship between elevated corticosterone and reduced hypothalamic LHRH content was not established in this study, it is conceivable that the increase in serum corticosterone occurs prior to and then promotes the decline in hypothalamic LHRH content. Moreover, the increase in circulating corticosterone levels that occurred in this study are consistent with previously reported elevated corticosterone levels occurring during acute thermal stress (Edens and Siegel, 1975; Ben Nathan et al., 1976; Beuving and Vonder, 1978; Siegal and Gould, 1982; Pardue et al., 1985).

Although available evidence supports the role of corticosterone in reducing hypothalamic function during thermal stress, other possibilities can not be ruled out. Specifically, altered brain monoamine concentrations—the result of elevated body temperature—may inhibit hypothalamic function. Acute exposure of birds to elevated ambient temperatures has been reported to alter brain monoamine levels and turnover rate (El Halawani and Waibel, 1976; Braganza and Wilson, 1978a,b). Since monoamines are putative hypothalamic regulators of gonadotropin secretion (El Halawani et al., 1982) altered levels would be indicative of a functional change in hypothalamic activity. Alternatively, corticosterone can also affect monoamine levels in the brain (see review, Meyer, 1985).

In conclusion, this is the first report that exposure to acute thermal stress alters hypothalamic/hypophysal regulation of reproductive function in the laying hen. Specifically, reduced circulating levels of LH may be the result of hypothalamic hypofunction in the thermally stressed hen. Moreover, the pituitary retains the ability to synthesize and release LH, as demonstrated by LHRH injection, during thermal stress.

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