0013-7227/97/$03.00/0
Endocrinology

Vol. 138, No. 10
Printed in U.S.A.

Endotoxin Inhibits the Reproductive Neuroendocrine Axis While Stimulating Adrenal Steroids: A Simultaneous View from Hypophyseal Portal and Peripheral Blood*

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ABSTRACT

This study was designed to test the hypothesis that systemic immune challenge with endotoxin inhibits the reproductive axis centrally by suppressing GnRH pulsatile release into hypophyseal portal blood. Using alert, normally behaving, ovariectomized ewes, we sampled hypophyseal portal blood at 10-min intervals beginning 4 h before and continuing 10 h after endotoxin (400 ng/kg, iv bolus, n = 6) or saline (vehicle, iv, n = 6). Simultaneous jugular samples for measurement of LH, cortisol, and progesterone were taken, and core body temperature was monitored by telemetry. Saline had no effect on any of the parameters in control ewes. In contrast, endotoxin dramatically inhibited the reproductive neuroendocrine axis coincident with stimulating the adrenal steroids, cortisol and progesterone, and elevating body temperature. Mean GnRH collection rate and GnRH pulse amplitude were suppressed (pre- *vs*. 7 h postendotoxin: collection rate 0.93 ± 0.31 *vs*. 0.34 ± 0.13 pg/min; amplitude 4.13 ± 1.33 *vs*. 1.30 ± 0.41 pg/min per pulse; $P < 0.05$ and $P = 0.01$). However, endotoxin did not have a significant effect on GnRH pulse frequency. Along with inhibited GnRH secretion, endotoxin significantly suppressed mean LH concentrations ($P = 0.001$) and LH pulse amplitude ($P < 0.05$). In addition, endotoxin suppressed LH pulse frequency ($P = 0.01$). Coincident with reproductive inhibition, endotoxin stimulated cortisol ($P < 0.001$), progesterone ($P < 0.01$), and core body temperature ($P < 0.001$). We conclude that the suppressive effects of endotoxin on the reproductive axis can be mediated centrally through an inhibition of GnRH and thus LH pulsatile secretion. The coincident stimulation of cortisol, progesterone, and temperature raises the possibility that the central inhibition of the reproductive system may be a consequence of any or all of these activated parameters. (*Endocrinology* **138:** 4273–4281, 1997)

IMMUNE stress has profound effects on female reproductive health, ranging from disruptions in cyclicity (1) to loss of pregnancy (2). To study how immune stressors cause reproductive dysfunction, bacterial endotoxins (*i.e.* lipopolysaccharides) have been used as a model of immune challenge. Endotoxins are the pathogenic moiety of gram-negative bacteria and, through a complex cascade of cytokines, neural afferents, and other mediators, induce pathophysiological responses throughout the body very similar to true bacterial infection (3). Dramatic alterations in neuroendocrine function are among these responses and include not only a well-documented activation of the stress axis but also an inhibition of the reproductive axis.

In a number of species, endotoxin and cytokines have been shown to inhibit tonic LH secretion (4–7) and block the preovulatory LH surge (1, 8, 9). A large body of evidence suggests this inhibition occurs at the level of the hypothalamus. Indeed, in the female rat, systemic endotoxin can inhibit the percentage of GnRH neurons expressing Fos protein on the afternoon of proestrus and can reduce the afternoon proestrous rise in GnRH primary transcript (10). Additionally, the cytokine interleukin-1 (IL-1) can decrease GnRH release *in vitro* from medial basal hypothalamic tissue (9) and, when given centrally, can suppress the normal increase in c-*fos* expression in GnRH neurons on the afternoon of proestrus (11). Push-pull perfusion studies have also shown that IL-1 given centrally can inhibit GnRH release into the median eminence in the proestrous rat (11, 12). In these *in vivo* studies directly assessing GnRH release, however, an anesthetized animal and acute surgical preparation were used, procedures that in and of themselves could be stressors that inhibit the reproductive axis.

At present, there is no direct evidence that a systemic immune stressor such as endotoxin actually inhibits GnRH secretion into the hypothalamic-pituitary portal blood in alert, normally behaving animals. In the sheep, such studies are possible; hypophyseal portal blood can be sampled from conscious and undisturbed animals at frequent intervals and for prolonged time periods. This approach thus allows direct assessment of hypothalamic neuroendocrine responses to immune challenge. Using the hypophyseal portal sampling methodology, we tested the hypothesis that endotoxin inhibits the reproductive axis centrally by suppressing pulsatile GnRH secretion.

Received April 1, 1997.

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* Preliminary reports have appeared in the abstracts of the Annual Meeting of the Society for the Study of Reproduction, 1995 and 1996 (Biol Reprod, 52[Suppl 1]:156, 1995; Biol Reprod, 54 [Suppl 1]:93, 1996). This work was supported by NIH Grants MH-11653 and HD-18337, and the Office of the Vice President for Research at the University of Michigan. Support was also provided by the Sheep Research, Standards and Reagents, Data Analysis, and Administrative Core Facilities of the P30 Center for the Study of Reproduction (NIH HD-18258).

Materials and Methods

Animals

Experiments were conducted on adult Suffolk ewes maintained at the Sheep Research Facility in Ann Arbor, MI. All ewes were ovariectomized to provide an animal model in which clear and robust GnRH and LH pulses occur, facilitating detection of endotoxin-induced suppression. Further, this model ensures that alterations in the pulse patterns do not result from changes in ovarian steroids. Ewes were accustomed to handling and were penned in pairs at least 1 day before experiments to create a calm, nonstressful sampling environment that minimized human contact. Peripheral blood was sampled by indwelling jugular catheters installed on the day of penning. Throughout the study, natural light/dark cycles were maintained. Ewes were fed before sampling and provided with food and water as needed during the experiment. Surgeries were performed aseptically under general anesthesia. All procedures were approved by the Committee for the Use and Care of Animals at the University of Michigan.

Pilot studies

Pilot studies were conducted for two purposes: to establish a model in the ewe to study the effects of endotoxin on the reproductive and stress neuroendocrine axes, and to characterize basal, nonstress profiles of cortisol and temperature. Studies were carried out on seven ovariectomized ewes in the anestrous (n = 3) and breeding seasons (n = 4) (ovariectomy performed at least 1 week before experiments). Core body temperature was monitored with battery-operated, temperature-sensitive telemetry transmitters (MiniMitter, model CH-3, Sunriver, OR) implanted ip at the time of ovariectomy and tied to the broad ligament of the uterus. Jugular blood samples were taken remotely for measurement of LH and cortisol at 5- to 15-min intervals over 8–10 h. Following a baseline period of 2–4 h, either endotoxin (400 ng/kg, iv bolus) or an equivalent volume of saline vehicle was administered. Temperature and hormonal responses to endotoxin were compared with pretreatment values within the same ewe, determined either during the baseline period or on a preceding control day. [Statistical analyses were not employed to assess treatment effects because experimental conditions varied slightly among ewes.] In these pilot studies and in the main experiment, endotoxin (*Escherichia coli* 05:B55, Sigma, St. Louis, MO) was reconstituted in sterile saline to a stock concentration of 0.01 mg/ml. Final injection volume averaged 2.6 ml and was followed by a saline or heparinized saline (100 U/ml) flush of 6–8 ml. The dose of endotoxin (400 ng/kg, iv) was chosen based on a previous study in gonadectomized rams showing it induced reproductive and stress neuroendocrine alterations but did not cause severe adverse side effects (4).

Main experiment

The main experiment was conducted during the mid-breeding season (Oct.-Nov.) on 12 ewes, all ovariectomized and surgically fitted with the apparatus for hypophyseal portal blood collection 2–4 weeks before blood sampling. The surgical preparation and system for remote automated sampling of hypophyseal portal blood were modified from the technique of Caraty and Locatelli (13) and are described elsewhere (14). This procedure allows sampling in undisturbed, normally behaving ewes. Six ewes were randomly assigned to receive endotoxin (400 ng/kg, iv bolus; between 1730 and 2000 h); the remaining six were controls (saline vehicle, iv). In the endotoxin-treated ewes, MiniMitters were implanted ip at the time of ovariectomy to monitor core body temperature. Animals were sampled in pairs, one control and one endotoxin-treated. Portal and jugular blood were collected simultaneously (portal blood into ice-cold bacitracin 3×10^{-3} M) at 10-min intervals beginning 4 h before and continuing 10 h after endotoxin or saline. Technical difficulties prevented successful portal blood collection from one control and one endotoxin-treated ewe. Thus, to attain final group sizes of six, the protocol was repeated at least 2 weeks after the first sampling in two ewes (endotoxin and control treatments switched for the second sampling). We have previously shown that repeat sampling of the hypophyseal portal blood is possible and provides a valid description of GnRH secretion (14). After sample collection, ewes were killed with a barbiturate overdose (Beuthanasia, Schering-Plough Animal Health Corp., Kenilworth, NJ), and the pituitary was inspected to confirm appropriate placement of the cut for sampling hypophyseal portal blood.

Assays

GnRH was measured in portal plasma using a previously described RIA (15, 16). Briefly, a 750-μl aliquot of sample (~600 μl portal plasma and 150 μl bacitracin) was extracted in 2 ml of methanol, and duplicate aliquots of the reconstituted extract (equivalent to ~240 μl portal plasma) were assayed. To minimize the effect of between-assay variation, all samples from a given animal were measured in a single assay. Intraassay variation (eight assays), as determined by the median variance ratio of assay replicates (17), averaged 0.062, and assay sensitivity averaged 0.23 pg/tube.

LH was measured in duplicate aliquots of plasma (25–200 μl) using a modification (18) of a previously described RIA (19, 20) and is expressed in terms of NIH-LH-S12. All samples for a given ewe were measured initially at 100 μl in a single assay; reassays were done as necessary using smaller plasma volumes (25–50 μl) to assess peaks of LH pulses and using a larger volume (200 μl) to assess low LH concentrations. Mean intra- and interassay coefficients of variation (seven assays) were 7.8% and 7.7%, respectively, and assay sensitivity for 200 μl averaged 1.10 ng/ml.

Cortisol concentrations were determined in duplicate 50-μl aliquots

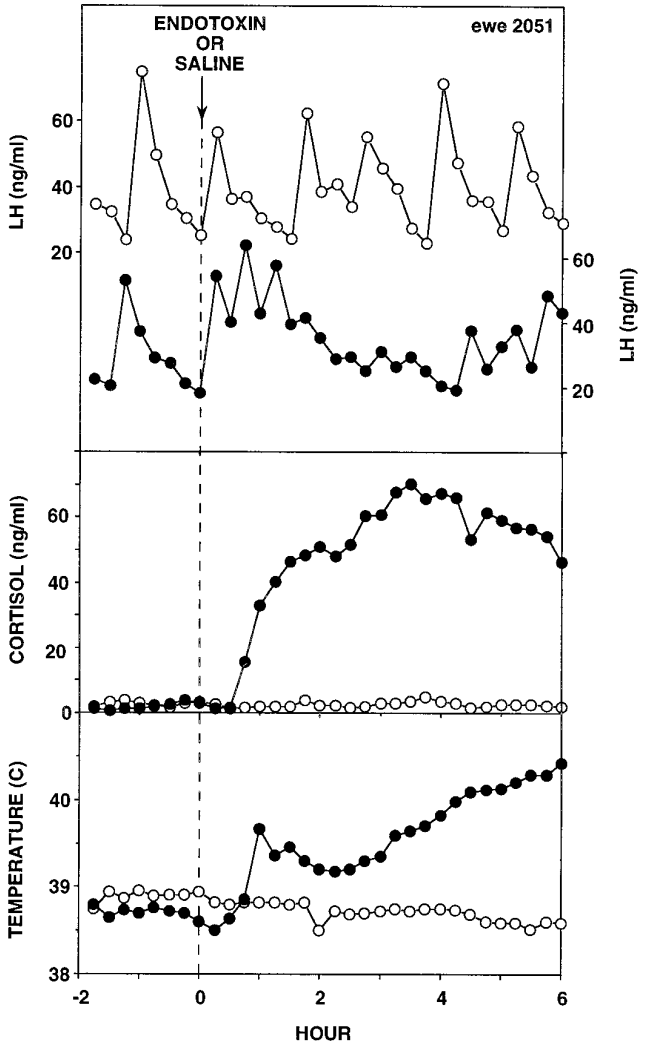

FIG. 1. Plasma LH, cortisol, and temperature profiles of a representative ewe (ovariectomized, anestrus) in pilot studies. Samples were taken over 8 h at 15-min intervals on a control day (○, saline iv) and following endotoxin-treatment day (●, 400 ng/kg, iv).

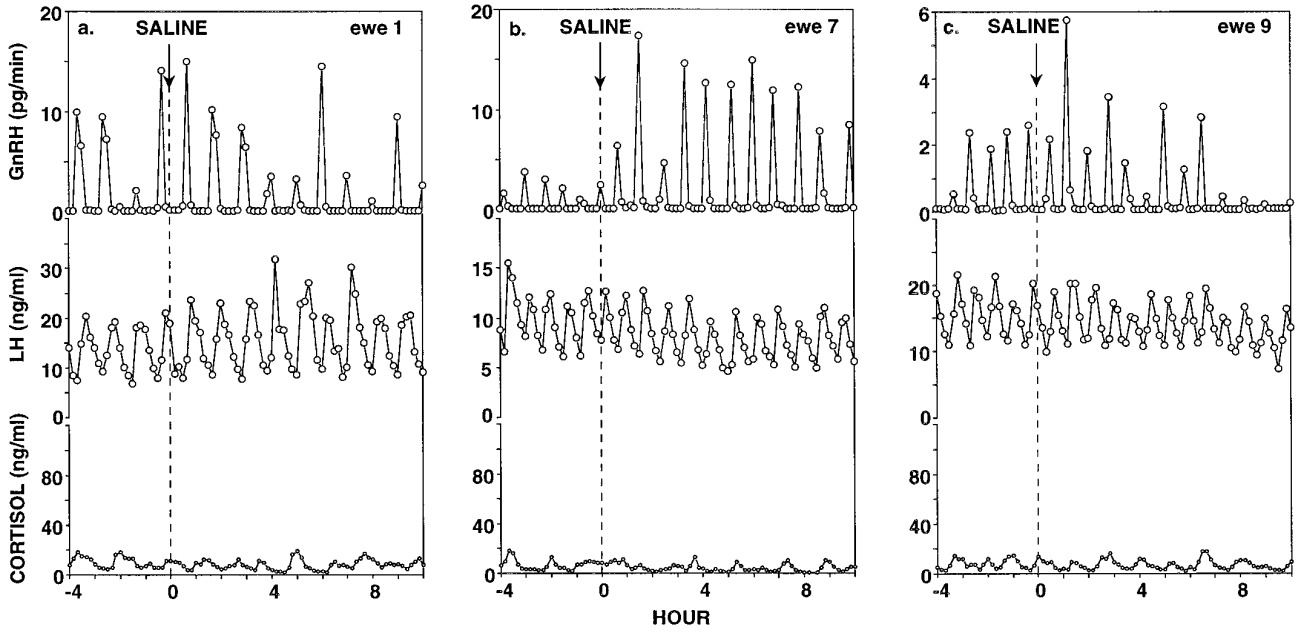

FIG. 2. Plasma GnRH, LH, and cortisol profiles in three representative control ewes receiving saline iv at time 0. Samples were taken at 10-min intervals simultaneously from portal (GnRH) and jugular (LH, cortisol) veins. c, Depicts a ewe exemplifying an abrupt fall in GnRH pulse amplitude observed after h 7 in 3/6 control ewes (suggested technical difficulties, see *Materials and Methods* for explanation). Note different scales among ewes for GnRH and LH.

of unextracted plasma by use of the Coat-A-Count Cortisol assay kit (DPC, Los Angeles, CA), validated in our laboratory for use in the sheep. Modifications of the kit protocol included adding a low point on the standard curve and increasing the incubation time with tracer to 1 h at 37 C. Samples from hypophysectomized ewes produced values below or at the sensitivity of the assay (0.86 ng/ml). Parallelism between serial dilutions of ovine samples and cortisol standard was confirmed. Accuracy averaged 96% when assay buffer was spiked with a known amount of cortisol. All samples from a given ewe were initially measured in a single assay. Mean intra- and interassay coefficients of variation (13 assays) were 15% and 9%, respectively, and assay sensitivity averaged 0.86 ng/ml.

Progesterone concentrations were measured in duplicate 100-μl aliquots taken from one sample every hour using the Coat-A-Count Progesterone kit (DPC) validated previously (21). In this study, we also determined that cortisol deoxycortisol, corticosterone and deoxycorticosterone did not appreciably cross-react. Mean intra- and interassay coefficients of variation (two assays) were 9.7% and 7.9%, respectively, and assay sensitivity averaged 0.08 ng/ml.

Data analysis

GnRH values are expressed as a collection rate (picograms per minute) rather than as a concentration to minimize error due to potential contamination of portal samples with peripheral blood or cerebrospinal fluid (judged minimal), or due to changes in flow of portal blood caused by positioning of the head. Pulses of GnRH and LH were identified using the pulse detection algorithm of Veldhuis and Johnson (22). Cluster sizes for peaks/nadirs were defined as 1/2 and 2/2 for GnRH and LH, respectively. The *t* statistics used to identify significant increases and decreases were 3.8/3.8 for GnRH and 2.6/2.6 for LH.

For GnRH and LH, average pulse amplitude, pulse frequency (number of pulses per collection period), and mean concentration (for LH) or collection rate (for GnRH) were calculated for each ewe before and after endotoxin or saline injection. Similarly, mean cortisol, progesterone, and temperature values during the pre- and posttreatment periods were calculated for each ewe. Treatment effects, comparing values before *vs.* after endotoxin or saline, were identified by paired *t* test. Hormonal values were transformed before statistical analysis (log transformed for concentration, collection rate and pulse amplitude, and square root transformed for pulse frequency). Level of significance was set at $P \leq 0.05$.

Of note, the final 3 h of sampling (h 7–10) were excluded from statistical analyses due to suspected technical problems in the portal blood collection procedure. Specifically, three of the six control ewes exhibited an unexplained drop in GnRH pulse amplitude during this time period with no change in LH pulse amplitude. Further, in one endotoxin ewe, no clear GnRH pulses were exhibited during h 7–10, yet detectable LH pulses were evident. Adding to our suspicion of technical problems, we have obtained no indication of a diurnal change in GnRH pulse amplitude in ovariectomized ewes nor have we observed LH pulses that were not preceded by GnRH pulses (15, 23). Therefore, we excluded hormonal data between h 7–10 from statistical analyses in both the control and endotoxin groups. Likewise, data summaries in the *Results* compare the baseline period to the first 7 h after saline or endotoxin. Individual profiles for the full 10-h period, however, are illustrated graphically in the *Results*.

Results

Pilot studies

Figure 1 depicts plasma LH and cortisol concentrations and body temperature profiles during a control day (saline, ~8 ml iv) and the following endotoxin treatment day (endotoxin, 400 ng/kg iv) in an ovariectomized ewe sampled at 15-min intervals during the anestrous season. Among all ewes in the pilot studies, saline had no effect on plasma cortisol (before *vs.* after; 6.59 ± 3.82 *vs.* 6.89 ± 4.39 ng/ml) or core body temperature (before *vs.* after; 39.0 ± 0.2 *vs.* 38.9 ± 0.3 C). In contrast, endotoxin consistently induced marked increases in cortisol and body temperature (before *vs.* after: cortisol, 6.75 ± 4.74 *vs.* 56.84 ± 7.61 ng/ml; temperature, 38.8 ± 0.2 *vs.* 39.6 ± 0.3 C). Coincident with this stimulation, endotoxin inhibited mean LH levels by an average of 38% compared with pretreatment values (calculated as a percent rather than a mean due to marked variability among ewes in

LH concentrations before endotoxin, which ranged from 4–35 ng/ml). Also attesting to endotoxin efficacy, ewes developed transient sickness behaviors such as labored breathing, cough, diarrhea, shivering, and lethargy. These hormonal and pathophysiological responses to endotoxin in ovariectomized ewes confirmed observations of Coleman *et al.* (4) in gonadectomized rams and established a model system to study the effects of immune challenge on hypothalamic neurosecretion.

Main experiment

Figure 2 depicts the GnRH, LH, and cortisol profiles of three representative control ewes receiving saline iv at h 0. All control ewes exhibited regular pulsatile discharges of GnRH and LH typical of ovariectomized ewes (15, 23). Each pulse of GnRH corresponded to a pulse of LH. GnRH and LH pulse amplitude, pulse frequency, and mean collection rate (GnRH) or concentration (LH) were not significantly different when comparing the 4 h before to 7 h after saline (Fig. 3). Cortisol levels remained low throughout sampling (before *vs.* after saline; 11.53 ± 1.70 *vs.* 9.57 ± 1.60 ng/ml) and were within the same range as those in ewes not undergoing portal sampling (pilot studies). Cortisol followed a regular, low amplitude, oscillatory pattern in all control ewes; peaks of cortisol oscillations did not correspond with pulses of GnRH/LH. Although temperature was not monitored in these ewes (MiniMitters not implanted), control data were obtained in the ewes allocated for endotoxin treatment (MiniMitters implanted) on a day before treatment and at a corresponding time of day. In these ewes, saline had no effect on core body temperature (before *vs.* after saline; 39.5 ± 0.1 *vs.* 39.5 ± 0.1 C).

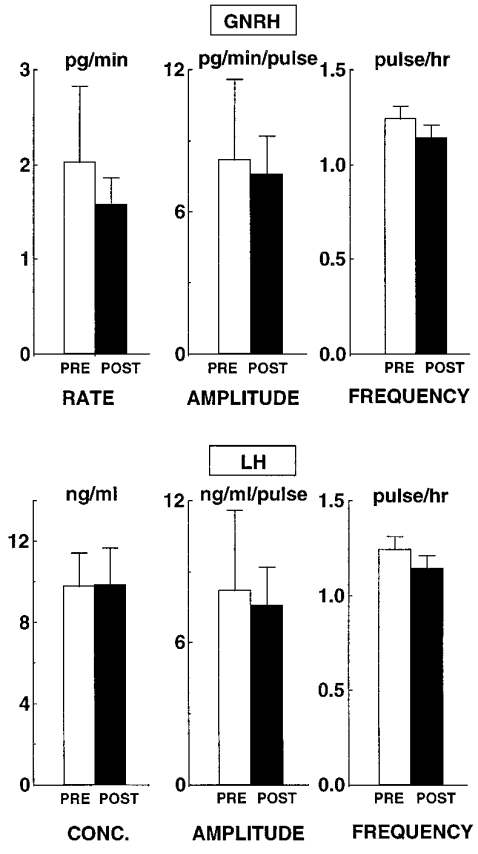

FIG. 3. Summary GnRH and LH responses in controls (n = 6), comparing baseline (*open bars*) to first 7 h after (*closed bars*) saline iv. Mean ± SEM is depicted for concentration (LH) or collection rate (GnRH), pulse amplitude, and pulse frequency. No significant effect of saline was observed for any of parameters as determined by paired *t* test.

Figures 4 and 5 depict the hormonal and temperature profiles of all six endotoxin-treated ewes. In contrast to control animals, endotoxin profoundly inhibited pulsatile GnRH secretion. The pattern of the inhibitory response, however, differed among ewes. Four of the six ewes showed immediate GnRH suppression with a variable return in pulsatility (Figs. 4a-c, and 5a). One ewe showed a disruption in the regularity of GnRH pulses (Fig. 5b), and another showed a gradual drop in pulse amplitude with total inhibition late in the sampling (Fig. 5c). Endotoxin significantly suppressed mean GnRH collection rate ($P < 0.05$) and GnRH pulse amplitude ($P = 0.01$). Although GnRH pulse frequency was reduced in some of the ewes, an overall significant effect was not observed (Fig. 6).

As with GnRH secretion, endotoxin also inhibited pulsatile LH (Figs. 4 and 5). Mean concentration of LH ($P = 0.001$), LH pulse amplitude ($P < 0.05$), and LH pulse frequency ($P = 0.01$) were all significantly inhibited by endotoxin (Fig. 6).

Of note, in several of the endotoxin-treated ewes, there was an uncoupling of the GnRH and LH pulse patterns. For example, in ewes 2 and 10 (Fig. 5, b and c), LH levels declined precipitously during h 1–2 despite the continuation of robust GnRH pulses. Further, GnRH pulsatility escaped inhibition, whereas LH pulses remained suppressed in some of the animals. In particular, ewe 6 (Fig. 4c) showed a return of GnRH pulsatility before that of LH; ewes 10 and 13 (Fig. 5, a and b) showed transient bursts of GnRH pulses interspersed with periods of suppression, but no corresponding detectable LH pulses.

Coincident with the inhibition of GnRH and LH, endotoxin significantly stimulated cortisol and temperature in all ewes. Within 4 h of endotoxin, cortisol reached peak values 8- to 15-fold above baseline (before *vs.* after; 8.39 ± 0.81 *vs.* 75.10 ± 8.89 ng/ml, $P < 0.001$). Core body temperature also increased rapidly, although it rose more gradually than cortisol, and peaked 5 h after endotoxin (before *vs.* after; 39.4 ± 0.2 *vs.* 41.1 ± 0.2 C, $P < 0.001$). As in the pilot studies, animals exhibited transient sickness behaviors such as labored respiration, diarrhea, shivering, and lethargy, which generally subsided by the end of the experiment.

Progesterone, assessed in one 10-min sample every hour, was significantly stimulated by endotoxin (before *vs.* after; 0.11 ± 0.02 *vs.* 0.51 ± 0.14 ng/ml, $P < 0.01$) (Fig. 7). Peak response averaged 0.78 ± 0.22 ng/ml.

Discussion

In the present study, we demonstrate that systemic immune challenge with endotoxin profoundly inhibits the reproductive neuroendocrine axis coincident with stimulating the secretion of adrenal steroids and elevating core body temperature. Our results provide direct evidence that endo-

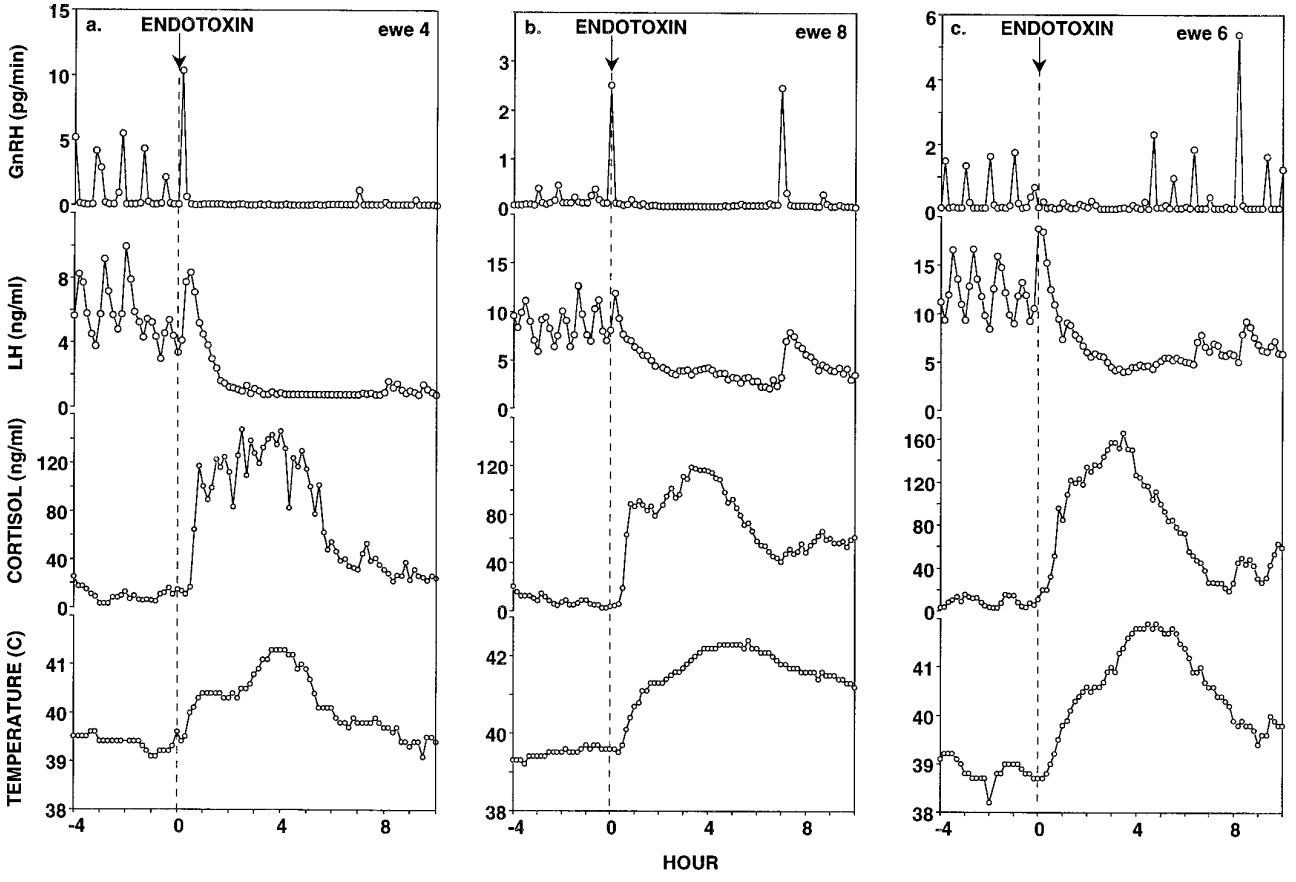

FIG. 4. Plasma GnRH, LH and cortisol profiles and core body temperature responses to endotoxin for three ewes showing immediate suppression of GnRH with a variable return in pulsatility. Endotoxin (400 ng/kg) was given iv at time 0, and samples were taken at 10-min intervals simultaneously from portal (GnRH) and jugular (LH, cortisol) veins. Body temperature was monitored at same interval by ip MiniMitter. Note different scales among ewes for GnRH and LH.

toxin can inhibit reproductive neuroendocrine function centrally by suppressing pulsatile GnRH release into hypophyseal portal blood. Indeed, within minutes of endotoxin, most ewes exhibited an interruption in the regular pulsatile rhythm of GnRH, and thus LH, pulsatile secretion.

Our study supports and expands on the work of Coleman *et al.* (4) showing that endotoxin suppresses LH pulsatile secretion in gonadectomized rams. Interestingly, in their study, endotoxin inhibited LH pulse frequency but did not affect LH pulse amplitude. In the present study, we observed a significant drop in both LH pulse frequency and amplitude. Although a reason for the different results is not known, it is noteworthy that Coleman *et al.* sampled peripheral blood intermittently at 10- to 15-min intervals, whereas we withdrew blood continuously and separated it into 10-min fractions. This difference in sampling procedure could alter the pattern of LH observed.

Although endotoxin inhibited all parameters of LH secretion, analysis of the GnRH data indicated a significant inhibition in the mean GnRH collection rate and GnRH pulse amplitude but not GnRH pulse frequency. The disparity in achieving comparable statistically significant inhibition of GnRH and LH pulsatility for all parameters points to two important issues. First, in physiological studies such as ours, it is important to pay particular attention to profiles in individual animals and to evaluate the time course of the responses. For example, each of our ewes did experience a period when no GnRH pulses were observed but the nature and timing of the disruption differed. Second, the LH response to a given treatment does not necessarily correspond to the effect on GnRH secretion. This issue becomes critical if it is presumed that pituitary hormone changes simply mirror changes in hypothalamic hormone secretion.

An uncoupling between GnRH and LH pulsatile secretion was clearly observed in at least half of the ewes treated with endotoxin. Importantly, all control ewes and all endotoxin ewes before treatment had robust rises in LH, which met the criteria for pulses and which occurred in response to detected GnRH pulses. This confirms our previous observations of a one-to-one relationship between GnRH and LH pulses in ovariectomized ewes (15, 23). Following endotoxin, however, this one-to-one relationship was no longer observed in all ewes. In two animals, LH declined during the first few hours after endotoxin despite continued robust GnRH pulses. Further, GnRH secretion escaped from inhibition before LH recovery in several of the ewes (Figs. 4 and 5). This provides circumstantial evidence that the pituitary response to GnRH may have been compromised. Our results thus raise the question whether endotoxin, and/or the mediators it induces, act at two levels within the hypothalamic pituitary

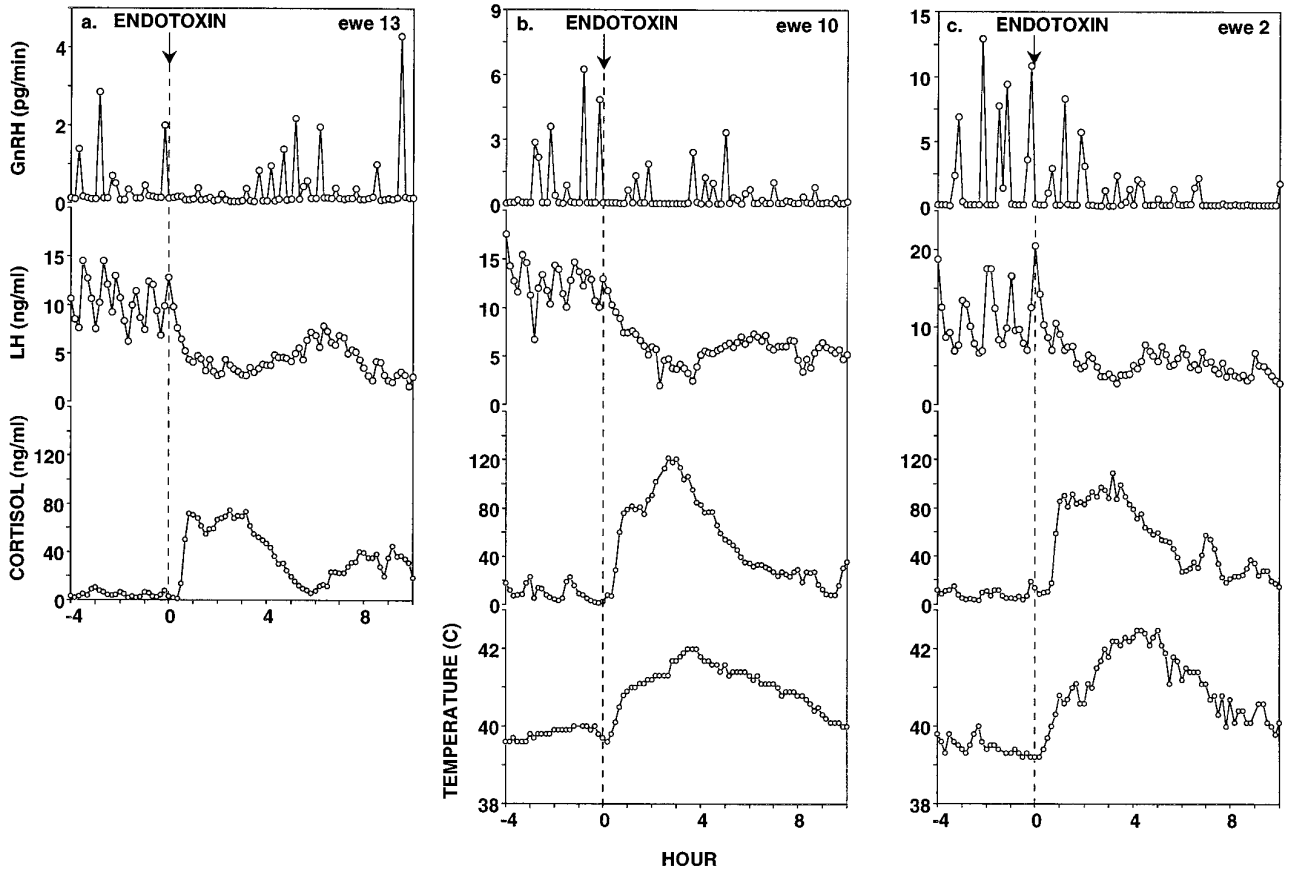

FIG. 5. Plasma GnRH, LH, and cortisol profiles and core body temperature responses to endotoxin in two ewes that showed disruption in regularity of GnRH pulses (a and b) and one ewe that showed a gradual drop in GnRH pulse amplitude with total inhibition late in sampling (c). See legend of Fig. 4 for further details. Note, ewe 13 was an animal that underwent repeat portal sampling. Temperature data are not available, because the ewe was originally designated as a control animal (ewe 7). Also, note different scales among ewes for GnRH and LH.

axis: centrally to inhibit GnRH release, and at the pituitary to inhibit the response to GnRH. Of interest in this regard, systemic endotoxin can reduce GnRH receptor gene expression in the anterior pituitary of female rats (10), although *in vitro* studies suggest cytokines can act at the pituitary to stimulate LH secretion (24, 25). Clearly additional studies are necessary to test for suppression at the pituitary level in the ewe. Such an effect could, however, help to explain the significant inhibition of LH pulse frequency but not GnRH pulse frequency we observed.

In addition to the profound suppressive effects of endotoxin on the reproductive neuroendocrine axis, our data show coincident elevation of body temperature and stimulation of the steroids, cortisol and progesterone. Of note, the elevated progesterone was presumably of adrenal origin, because the ewes were ovariectomized. Such a rise in progesterone has also been observed in ovariectomized monkeys following central delivery of the cytokine IL-1 (26). Because the temperature and adrenal hormone responses were temporally associated with reproductive suppression, it is of interest to consider whether any or all may mediate the suppressive effects of endotoxin on GnRH secretion. With regard to temperature, we are not aware of studies that have systematically examined its potential influence on GnRH and LH secretion. It is of interest, however, that subpyrogenic doses of endotoxin in rodents can still activate the neuroendocrine stress axis, as indicated by ACTH and corticosterone responses (27). Such doses could be useful in teasing apart a role for fever as a mediator of reproductive suppression.

Although the potential influence of temperature remains to be systematically examined, an interaction between the stress and reproductive axes has been studied extensively. A large body of evidence suggests that the reproductive axis can be suppressed by hormones from all levels of the stress axis (7, 28–34). For example, the adrenal glucocorticoid cortisol has been shown to inhibit LH and FSH secretion in the monkey (28). Such suppression, however, takes days to become evident, suggesting a more chronic role for this adrenal steroid. In contrast, the hypothalamic hormones CRH and arginine vasopressin (AVP) appear to play a pivotal role in reproductive suppression in the acute setting. CRH and AVP administered centrally can inhibit tonic LH secretion in several species (7, 29, 30). In addition, CRH given into the lateral ventricle can inhibit GnRH release into the portal blood on the afternoon of proestrus in the anesthetized rat (31). Finally, anatomical evidence suggests that, in the monkey, AVP-immunoreactive boutons synapse with GnRH-immunoreactive neurons (32) and, in the rat, CRH-immunoreactive elements contact GnRH-immunoreactive elements (33).

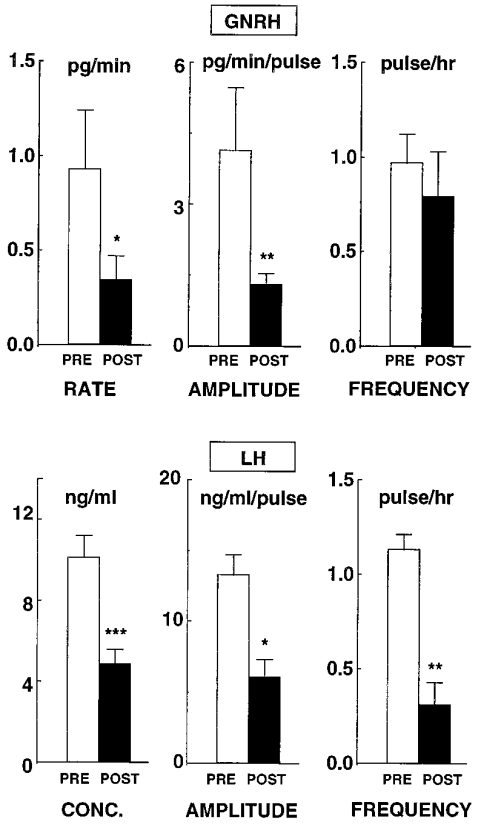

FIG. 6. Summary GnRH and LH responses in endotoxin-treated ewes (n = 6), comparing baseline (*open bars*) to first 7 h after (*closed bars*) endotoxin (400 ng/kg, iv bolus). Mean ± SEM is depicted for concentration (LH) or collection rate (GnRH), pulse amplitude, and pulse frequency. Endotoxin significantly inhibited all parameters except GnRH pulse frequency as determined by paired *t* test. *, $P < 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

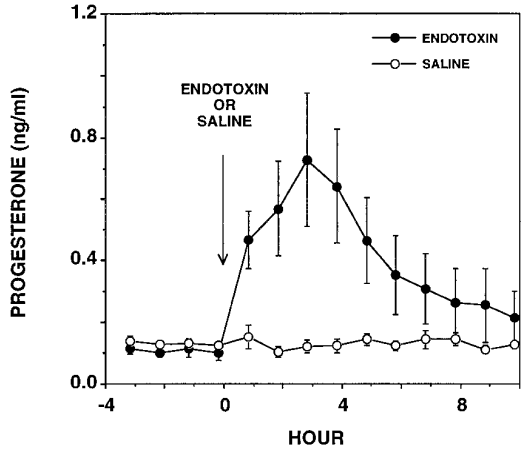

FIG. 7. Mean ± SEM for plasma progesterone concentrations in control (○) and endotoxin ewes (●). Values were determined in one 10-min sample every hour. Endotoxin significantly stimulated progesterone ($P < 0.01$).

Despite this strong evidence that the central hormones of the stress axis can acutely suppress the reproductive neuroendocrine axis, it is unclear whether CRH and/or AVP specifically mediate the suppression of pulsatile GnRH and LH secretion induced by immune stress. We have observed that endotoxin markedly stimulates CRH and AVP in hypophyseal portal blood of the ewe coincident with the GnRH and LH suppression reported in the present studies (35). Thus, terminals of GnRH neurons in the median eminence may be exposed to elevated CRH and AVP following endotoxin. It is important to recognize, however, that this does not necessarily apply to the region of GnRH perikarya. In fact, in the rat, the evidence points away from a mediatory role, at least for CRH, because CRH antagonists and antibodies fail to prevent suppression of LH following central delivery of IL-1 (11, 36). Additionally, in the male rat, ablation of the paraventricular nucleus, a major source of neuroendocrine CRH and AVP, does not prevent the suppression of LH secretion following central delivery of IL-1 (37). This argues against a mediatory role for CRH and AVP of paraventricular origin, at least in this rodent species. In ovariectomized rhesus monkeys, however, antagonists to either CRH or AVP can overcome the suppression of pulsatile LH secretion induced by central delivery of IL-1 (7, 38). Finally, it is important to note that, in sheep, CRH given centrally may actually stimulate LH secretion under some conditions (39, 40). Collectively, these findings suggest species differences and warrant further investigations to assess a possible role for CRH and/or AVP in mediating the reproductive effects of endotoxin in the sheep.

In addition to the potential inhibitory actions of CRH and AVP, the stimulation of progesterone may also contribute to suppression of GnRH and LH pulsatile secretion induced by endotoxin. Strong evidence demonstrates that progesterone inhibits GnRH and LH pulsatile secretion in the ewe (41). However, to suppress GnRH and LH in ovariectomized sheep in the breeding season, as in our study, progesterone must reach higher concentrations than those we observed in response to endotoxin (42, 43). Additionally, progesterone is known to inhibit pulse frequency not pulse amplitude (43, 44), the latter of which we observed in this study. Thus, progesterone alone cannot account for the GnRH and LH suppression we observed. However, in conjunction with other responses to the immune stress (*e.g.* cortisol, CRH, AVP), progesterone may well contribute. Of interest, endotoxin has been shown to stimulate progesterone secretion in ovariectomized cows, much as we observed in the sheep, and this stimulation was coincident with tonic LH suppression (5).

As a final note, two procedural issues must be addressed in conducting and interpreting studies such as ours. First, the absolute amount of GnRH quantified by our method can vary with the location and extent of the portal vasculature cut (14), which is difficult to standardize among sheep. This makes it problematic to compare absolute amounts of GnRH among animals. In fact, in the present study, the amount of GnRH collected per minute during the baseline period appeared to be higher, on average, in the control ewes *vs.* the endotoxin-treated ewes. This caveat, however, does not weaken our overall conclusions because we used each animal as its own control, comparing values before and after treatment. The second issue is whether the experimental procedure of portal blood collection could be a stressor that inhibits the reproductive axis in and of itself. This is unlikely in our study for three reasons. First, GnRH and LH secretion

were not inhibited in vehicle-treated controls. Second, cortisol levels remained within the range of basal values we observed in our pilot studies in which portal samples were not taken. Finally, cortisol in the control animals showed a clear rhythmic oscillation of its own, a pattern characteristic of glucocorticoid secretion in the basal state (45).

In conclusion, we have shown that systemic immune challenge with endotoxin dramatically inhibits GnRH and LH pulsatile secretion coincident with stimulating adrenal cortisol and progesterone secretion and elevating body temperature. The temporal association between the stress and reproductive responses provides a basis to consider if activation of the adrenal axis contributes to reproductive inhibition induced by this immune challenge in the ewe. Of additional interest, the suppressive effects of endotoxin on pulsatile GnRH and LH secretion may have implications for female cyclicity. Indeed, we have found that endotoxin interrupts the normal progression of the natural follicular phase of the ewe, interfering with the preovulatory estradiol rise and delaying the LH surge (46). This prompts an intriguing question: Can the suppression of GnRH pulsatile secretion induced by this immune stress, as described in the present study, disrupt the follicular phase of the estrous cycle?

Acknowledgments

We are sincerely indebted to Doug Doop and Gary McCalla for their dependable and skillful assistance with the animal experimentation, and to Barbara Glover for her expertise in conducting and analyzing GnRH RIAs. We would also like to thank Laura Morrison for assistance in conducting the preliminary studies, Vasantha Padmanabhan and Judy Van Cleeff for their help in conducting and interpreting the main experiment, Drs. Gordon D. Niswender and Leo E. Reichert, Jr. for supplying RIA reagents, and Dr. Johannes D. Veldhuis for providing the Cluster pulse detection algorithm.

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