Endotoxin inhibits the surge secretion of gonadotropin-releasing hormone via a prostaglandin-independent pathway

Kellie M. Breen, Heather J. Billings, Nathalie Debus, Fred J. Karsch

0013-7227/04/$15.00/0
Printed in U.S.A.

Endocrinology 145(1):221–227

doi: 10.1210/en.2003-1102

Endotoxin Inhibits the Surge Secretion of Gonadotropin-Releasing Hormone via a Prostaglandin-Independent Pathway

KELLIE M. BREEN, HEATHER J. BILLINGS, NATHALIE DEBUS, AND FRED J. KARSCH

Reproductive Sciences Program and Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan 48109

Immune/inflammatory challenges, such as bacterial endotoxin, disrupt gonadotropin secretion and ovarian cyclicity. We previously determined that endotoxin can block the estradiol-induced LH surge in the ewe. Here, we investigated mechanisms underlying this suppression. First, we tested the hypothesis that endotoxin blocks the estradiol-induced LH surge centrally, by preventing the GnRH surge. Artificial follicular phases were created in ovariectomized ewes, and either endotoxin or vehicle was administered together with a surge-inducing estradiol stimulus. In each ewe in which endotoxin blocked the LH surge, the GnRH surge was also blocked. Given this evidence that endotoxin blocks the estradiol-induced LH surge at the hypothalamic level, we began to assess underlying central mechanisms. Specifically, in view of the prior demonstration that prostaglandins mediate endotoxin-induced suppression of pulsatile GnRH secretion in ewes, we tested the hypothesis that prostaglandins also mediate endotoxin-induced blockade of the surge. The prostaglandin synthesis inhibitor flurbiprofen was delivered together with endotoxin and the estradiol stimulus. Although flurbiprofen abolished endotoxin-induced fever, which is a centrally generated, prostaglandin-mediated response, it failed to reverse blockade of the LH surge. Collectively, these results indicate endotoxin blocks the LH surge centrally, suppressing GnRH secretion via a mechanism not requiring prostaglandins. This contrasts with the suppressive effect of endotoxin on GnRH pulses, which requires prostaglandins as intermediates. (*Endocrinology* 145: 221–227, 2004)

IMMUNE/INFLAMMATORY CHALLENGES, such as bacterial endotoxin, potently suppress gonadotropin secretion (1–4) and disrupt the ovulatory cycle in a number of species (5–7). In terms of its effects on gonadotropin secretion, endotoxin inhibits both pulsatile LH release and the estradiol-induced LH surge. Considerable insight has been gained into mechanisms whereby endotoxin suppresses pulsatile LH secretion. For example, endotoxin disrupts both hypothalamic and pituitary function, inhibiting pulsatile GnRH secretion and pituitary responsiveness to GnRH (2, 8). Furthermore, endotoxin appears to suppress pulsatile GnRH and LH secretion indirectly, via a cascade of intermediates that include cytokines, such as IL-1 and TNF-α, as well as prostaglandins (3, 9, 10).

Surprisingly few studies have addressed mechanisms whereby endotoxin blocks the estradiol-induced LH surge, and those that have suggest the basis for inhibition of LH pulses and surges may not be entirely the same. For example, recent observations in ewes indicate that, unlike its acute suppression of pulses, which occurs within 1 h (2, 8, 9), endotoxin does not acutely inhibit LH release at the time of the surge. Rather, endotoxin acts to block the LH surge some 10–20 h in advance of the LH surge itself (7). During this early period in the surge induction process, the estradiol stimulus activates estrogen-sensitive neurons and the positive feedback signal is transduced by processes that ultimately lead to onset of the GnRH surge (11).

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

The present study consisted of two experiments to investigate mechanisms whereby endotoxin blocks the estradiol-induced LH surge in the ewe. First, we determined whether endotoxin blocks the LH surge centrally, interfering with the ability of estradiol to induce the surge of GnRH monitored in the hypophyseal portal circulation. Second, given prior evidence that prostaglandins are essential mediators of endotoxin-induced suppression of pulsatile GnRH and LH secretion in ovariectomized ewes (9), we tested the hypothesis that prostaglandins also mediate endotoxin-induced blockade of the LH surge. Our approach was to test whether the prostaglandin synthesis inhibitor flurbiprofen reverses the suppressive effects of endotoxin on the estradiol-induced LH surge.

Materials and Methods

General methods

Experiments were conducted from November, 1999, through July, 2001, on mature, ovariectomized Suffolk ewes maintained under standard husbandry conditions at the Sheep Research Facility in Ann Arbor, MI. All procedures were approved by the Committee for the Use and Care of Animals at the University of Michigan. This study consisted of two experiments. Experiment 1 was conducted during the nonbreeding season and experiment 2 during the breeding season. Our prior work has shown that estradiol induces a GnRH and LH surge in both the breeding and nonbreeding seasons and that there is no seasonal difference in sensitivity to the positive feedback action of estradiol (12, 13). In addition, endotoxin blocks both pulsatile and surge LH secretion in either season (Refs. 7, 9, and 14; and Breen, K., and F. Karsch, unpublished). Thus, interpretations in the present study are not compromised by having conducted the two experiments in different seasons. Furthermore, each experiment was internally controlled justifying conclusions made within each.

We used an artificial follicular phase model (13) that is well characterized in terms of estradiol signaling requirements for the GnRH and LH surge (11, 12, 15). To set up the model, ewes were ovariectomized aseptically under general anesthesia at least 4 wk before use. Then, an artificial luteal phase was simulated by treating ewes for 10 d with a 1-cm, sc, estradiol-filled Silastic implant (16) (Dow Corning Corp., Midland, MI) and two intravaginal progesterone-releasing devices (17) [controlled internal drug-releasing (CIDR), InterAg, Hamilton, New Zealand]. These treatments maintain luteal phase serum concentrations of estradiol and progesterone (~1 pg/ml and 2–4 ng/ml, respectively) (11, 13, 17). After 10 d (approximate length of progesterone elevation during the natural luteal phase), an artificial follicular phase was created by removing progesterone to mimic corpus luteum regression. Sixteen hours later, four 3-cm estradiol implants (peak estradiol implants) were inserted sc into the axillary region. These implants raise serum estradiol to the peak follicular phase level (~5–8 pg/ml) within 1–2 h and induce GnRH and LH surges beginning 18–24 h later (11, 12, 15). *Escherichia coli* endotoxin (*E. coli* lipopolysaccharide, serotype 055 B5; Sigma Chemical Co., St. Louis, MO) was dissolved in nonpyrogenic saline (20 μg/ml) and injected as a bolus (400 ng/kg, iv) 45 min in advance of the estradiol stimulus. Prior work indicates this endotoxin dose induces fever, provokes transient sickness behaviors (*e.g.* lethargy, labored breathing, and diarrhea), activates the hypothalamo-pituitary-adrenal axis, inhibits pulsatile GnRH and LH secretion, and can block the LH surge (1, 2, 7).

Experiment 1: does endotoxin disrupt the GnRH surge?

Eighteen ovariectomized ewes were surgically prepared for pituitary portal blood collection and sampled 2 wk later using the procedure of Caraty *et al.* (18), which permits sampling from fully conscious, minimally stressed animals. Before portal blood collection, ewes were set up in the artificial follicular phase model and given one of two treatments as illustrated in Fig. 1A: vehicle (n = 7) or endotoxin (n = 11). The peak estradiol implants were removed 12 h after their insertion to provide a stimulus close to the minimal duration needed to induce the LH surge in our animals (11). We selected this relatively short estradiol stimulus based on the logic that the GnRH/LH surge might be more susceptible to blockade than would be the case with a more prolonged (and presumably more powerful) estradiol stimulus. We previously observed that endotoxin blocks the LH surge in approximately 70% of ewes treated with a 12- to 14-h estradiol signal (Ref. 7; and Breen, K., and F. Karsch, unpublished). Jugular blood was sampled at 1-h intervals. Pituitary portal blood was withdrawn continuously, dispensed into tubes containing bacitracin to minimize GnRH degradation, and separated into hourly fractions from 15–36 h after insertion of peak estradiol implants. Rectal temperature was monitored hourly from 2 h before to 4 h after endotoxin to provide an independent assessment of efficacy of the endotoxin challenge. 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 lesion for sampling portal blood.

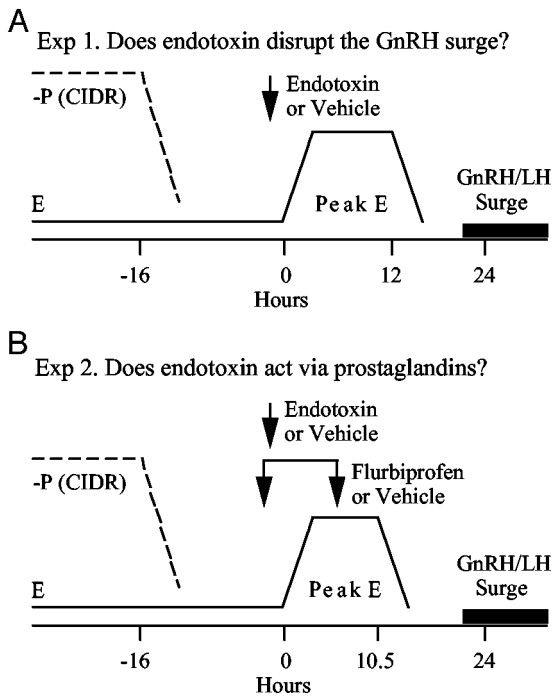

FIG. 1. Designs for experiment 1 (A) and experiment 2 (B). Time is depicted as hours relative to the insertion of the peak follicular phase estradiol (Peak E) implants. The *dashed and solid lines* designate the expected profile of serum progesterone (P) and estradiol (E) concentrations, respectively. *Arrows* indicate the time of endotoxin (400 ng/kg, iv bolus, given 45 min before estradiol) or its vehicle, and flurbiprofen (2 mg/kg, iv bolus given 30 min before endotoxin and 6 h later) or its vehicle. *Solid bars* indicate the expected period of the GnRH and LH surges in vehicle-treated ewes.

Experiment 2: does endotoxin disrupt the LH surge by a prostaglandin-dependent mechanism?

This experiment tested whether flurbiprofen, which blocks the cyclooxygenase-1 and -2 enzymes required for prostaglandin synthesis (19), would reverse endotoxin-induced inhibition of the LH surge. Flurbiprofen (Sigma) was dissolved in 95% ethanol (100 mg/ml) and injected as a bolus (2 mg/kg, iv, 1.0- to 1.5-ml injection volume) 30 min before endotoxin and again 6 h later (design in Fig. 1B). This dose blocks fever and reverses endotoxin-induced suppression of pulsatile GnRH and LH secretion in ovariectomized ewes (8, 9). The experiment was conducted according to a crossover design in which 10 ovariectomized ewes received each of three treatments in a random sequence during successive artificial follicular phases separated by 2 wk: 1) endotoxin, 2) endotoxin plus flurbiprofen, and 3) vehicle for both endotoxin and flurbiprofen. Estradiol implants were removed after 10.5 h (1.5 h earlier than in experiment 1) to increase the likelihood that endotoxin would block the LH surge. In this regard, results of experiment 1 indicated endotoxin was less effective than anticipated in blocking the LH surge, and we have evidence to suggest that shorter estradiol stimuli result in greater susceptibility to endotoxin-induced blockade of the surge (Ref. 7; and Breen, K., and F. Karsch, unpublished). Jugular blood for LH assay was sampled hourly by venipuncture from 0–38 h relative to insertion of peak estradiol implants. This encompassed the presurge period, when estradiol inhibits amplitude of GnRH and LH pulses (20, 21), as well as the period of the LH surge. Rectal temperature was taken hourly from 4 h before to 6 h after endotoxin to determine efficacy of the endotoxin challenge and confirm that flurbiprofen blocked fever, which is a prostaglandin-mediated response (22, 23). After the completion of sampling, progesterone-releasing devices were again inserted intravaginally to create a second and subsequently a third artificial luteal phase, and the experiment was repeated with the treatments crossed over.

Hormone assays

LH concentrations were determined in duplicate aliquots (5–200 μl) of plasma using a modification (24) of a previously described RIA (25, 26). Values are expressed in terms of NIH-LH-S12. Mean intra- and interassay coefficients of variation were 6.0 and 5.8%, respectively, and assay sensitivity averaged 0.9 ng/ml. GnRH was measured in duplicate in methanol extracts of portal plasma (~250 μl of plasma extract per assay tube) using a previously described RIA (12, 27). Intra- and interassay coefficients of variation were 3.0 and 6.4%, respectively. Assay sensitivity averaged 0.14 pg/ml.

Data analysis

GnRH or LH surges were considered to have occurred if hormone values increased 3-fold above the mean of the presurge baseline and remained so for at least 5 h. The surge period was defined as the interval between the hour GnRH or LH concentration increased and remained above 3-fold the presurge baseline and the hour GnRH or LH concentration decreased and remained below 3-fold the presurge baseline.

Before statistical analysis, plasma hormone concentrations were log transformed to normalize variability across a broad range of values. Significance level was set at $P \leq 0.05$. In experiment 1, GnRH or LH peak height (maximal value during the surge period) and total integrated surge amount (sum of all values during the surge period) were analyzed by ANOVA. Technical difficulties precluded accurate GnRH assessment in one of the seven control ewes; data from this animal were excluded from further analysis. Experiment 2 was a crossover experiment in which each animal received three treatments: estradiol plus vehicle (positive control), estradiol plus endotoxin, and estradiol plus endotoxin plus flurbiprofen. The LH surge period (as defined above) during the positive control run was used to identify the expected surge period of each ewe during the other two runs when endotoxin was given to block the surge. Values for total integrated amount of LH and maximal concentration during the surge period, and LH concentrations during the presurge period (0–10 h relative to insertion of peak estradiol implants) were compared across treatments using repeated-measures ANOVA. In addition, Fisher's exact probability test was used to identify treatment effects on the proportion of ewes exhibiting the LH surge.

Results

Experiment 1: does endotoxin block the LH surge by preventing the GnRH surge?

Figure 2 presents GnRH and LH profiles during the surge period in vehicle- and endotoxin-treated ewes. All six vehicle-treated controls exhibited a robust LH surge accompanied by an unambiguous and sustained GnRH surge beginning approximately 20–22 h after onset of the estradiol stimulus and lasting 9–18 h (Fig. 2A, composite results). Endotoxin blocked the LH surge in 4 of 11 ewes, fewer than anticipated based on our prior studies (see *Discussion*). Of prime importance in terms of the experimental goals, endotoxin abolished the GnRH surge in all four ewes in which the LH surge was blocked (Fig. 2, B–E, depicts results in each of these four ewes). GnRH was invariably undetectable during the entire 22-h period of portal blood collection. The remaining endotoxin-treated ewes exhibited GnRH and LH surges comparable to those in control ewes in terms of time course, peak height, and total integrated hormone released during the surge period (Fig. 2F and Table 1). Endotoxin induced fever in all ewes (maximal rectal temperature: endotoxin, 40.4 ± 0.3 C; controls, 38.9 ± 0.2 C, $P < 0.01$). Maximal rectal temperature in response to endotoxin was no different among ewes that expressed the LH surge (40.4 ± 0.2 C) and those that did not (40.4 ± 0.4 C).

Experiment 2: does endotoxin disrupt the LH surge by a prostaglandin-dependent mechanism?

Representative LH profiles in ewes treated with vehicle, endotoxin, and endotoxin plus flurbiprofen are illustrated in

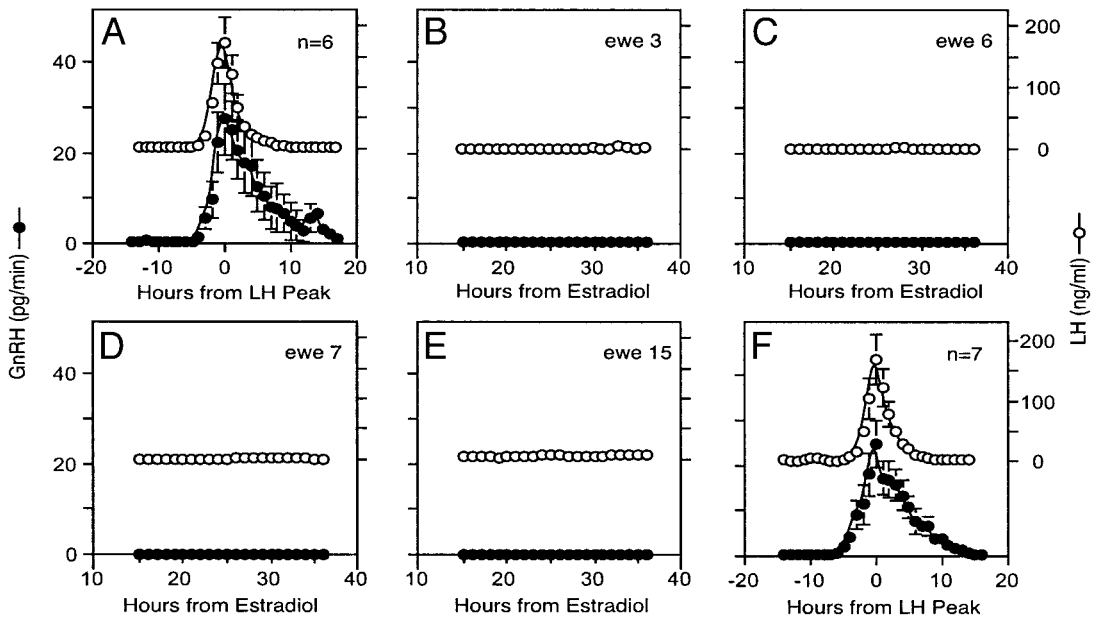

FIG. 2. GnRH in pituitary portal blood (*closed circles*) and LH in peripheral blood (*open circles*) in vehicle-treated control ewes (A) (mean ± SEM of six ewes), individual endotoxin-treated ewes in which the LH surge was blocked (B–E), and endotoxin-treated ewes in which the LH surge was not blocked (F) (mean ± SEM of seven ewes) in experiment 1. Data are normalized to the LH peak in A and F and to insertion of peak estradiol implants in B–E.

TABLE 1. Effects of endotoxin or vehicle on the LH and GnRH surge

	Surge incidence		Peak height[a]		Total integrated[b]	
	LH	GnRH	LH (ng/ml)	GnRH (pg/min)	LH (ng/ml)	GnRH (pg/min)
Experiment 1						
Estradiol + vehicle	6/6	6/6	174.9 ± 52.2	27.8 ± 7.7	694.1 ± 151.1	181.6 ± 57.8
Estradiol + endotoxin	7/11	7/11	168.1 ± 38.2	24.7 ± 4.7	662.2 ± 144.9	167.8 ± 31.4

[a] Maximal value during the surge period (excludes values in animals not expressing GnRH/LH surges).
[b] Sum of all values during the surge period (excludes values in animals not expressing GnRH/LH surges).

Fig. 3. Composite results comparing LH surge incidence, maximal LH value, and total integrated LH during the surge period across treatments are depicted in Fig. 4. In this experiment, the duration of the estradiol stimulus was 1.5 h shorter than that in experiment 1, and endotoxin blocked the LH surge in eight of 10 ewes. Compared with the control run, endotoxin decreased ($P < 0.05$) the incidence of ewes responding with an LH surge, reduced ($P < 0.05$) the maximal LH value, and lowered ($P < 0.05$) total integrated LH during the surge period. Flurbiprofen did not reverse any of these inhibitory effects of endotoxin; values for all parameters were less than those in vehicle-treated ewes ($P < 0.05$) but not significantly different from those in ewes treated with endotoxin in the absence of flurbiprofen. Nevertheless, flurbiprofen prevented endotoxin-induced fever in each ewe, indicating prostaglandin synthesis was blocked (Fig. 5).

Plasma LH was monitored hourly during the *presurge* period (from 0–10 h after insertion of peak estradiol implants) when estradiol inhibits amplitude of GnRH and LH pulses (20, 21). In control ewes, LH concentrations declined steadily during this period (Fig. 6). This presurge decline was more pronounced in endotoxin-treated ewes ($P < 0.01$). Flurbiprofen failed to reverse this suppressive effect of endotoxin ($P < 0.01$ *vs.* controls). LH responses for all ewes during the three runs of the crossover were included in this analysis; values were not different between ewes that expressed the LH surge and those that did not.

Discussion

The present study permits two novel conclusions regarding mechanisms by which endotoxin blocks the estradiol-induced LH surge. First, endotoxin exerts this effect centrally, preventing the surge of GnRH released into the hypophyseal portal circulation. This conclusion is important in light of the recent demonstration that endotoxin also profoundly suppresses pituitary responsiveness to GnRH (8), a finding that provides a potential alternative mechanism whereby endotoxin could inhibit the LH surge. Second, the inhibitory effect of endotoxin on the LH surge does not require the synthesis of prostaglandins. This conclusion is highly interesting in view of evidence that prostaglandins mediate endotoxin-induced inhibition of the pulsatile mode of LH release in ovariectomized ewes. Collectively, these conclusions enhance understanding of the disruptive effects of immune/inflammatory challenge on reproductive neuroendocrine activity and ovarian cyclicity.

Our conclusion that endotoxin blocks the LH surge by suppressing GnRH secretion is reinforced by the finding that another type of immune/inflammatory challenge, the cytokine IL-1β, inhibited GnRH secretion and fos induction in GnRH neurons at the time of the preovulatory LH surge in rats (28). Nonetheless, it is puzzling why the LH surge was blocked by endotoxin in only four of the 11 ewes in experiment 1. Our earlier work (Ref. 7; and Breen, K., and F. Karsch, unpublished) indicated that endotoxin blocked the surge in approximately 70% of ewes receiving a 12- to 14-h estradiol stimulus, similar to the estradiol treatment in experiment 1. That the GnRH/LH surge was truly blocked in the four ewes of experiment 1 is substantiated by the absence of any trace of a GnRH or LH increase during the entire 22-h period of hourly portal blood collection (Fig. 2, B–E). Furthermore, that this blockade was due to endotoxin, rather than some unknown variable, is substantiated by our collective present and prior work in the artificial follicular phase

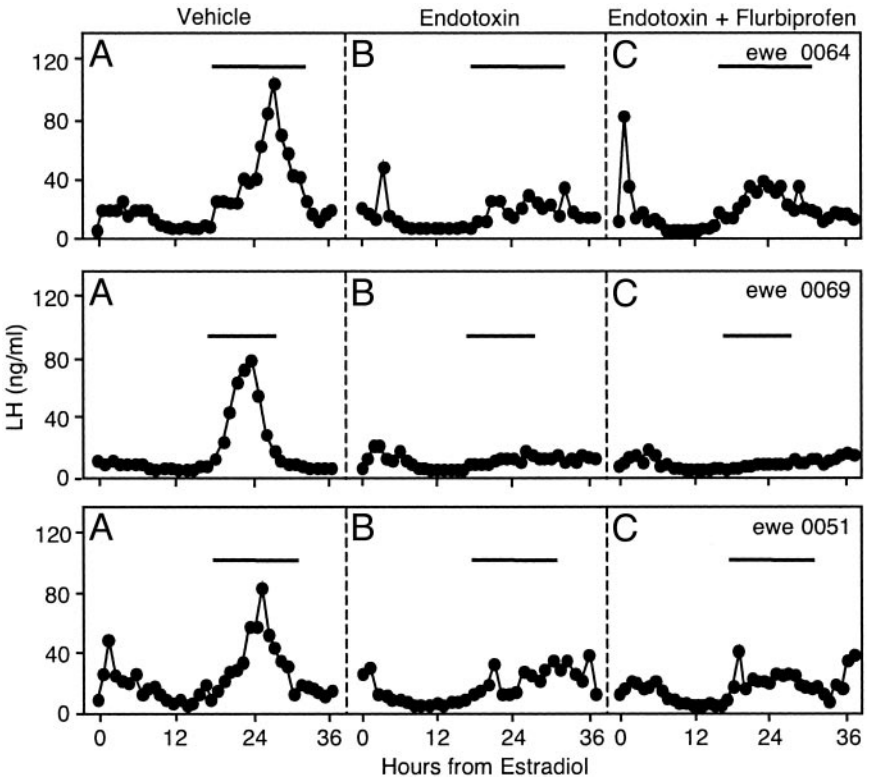

FIG. 3. Circulating LH concentrations in three representative ewes treated with vehicles for both endotoxin and flurbiprofen (A), endotoxin (B), and endotoxin plus flurbiprofen (C) in experiment 2. This was a crossover experiment in which each ewe received all three treatments. The *horizontal bar* in each panel depicts the surge period during the control run (see *Materials and Methods* for details). See Fig. 1 for design details.

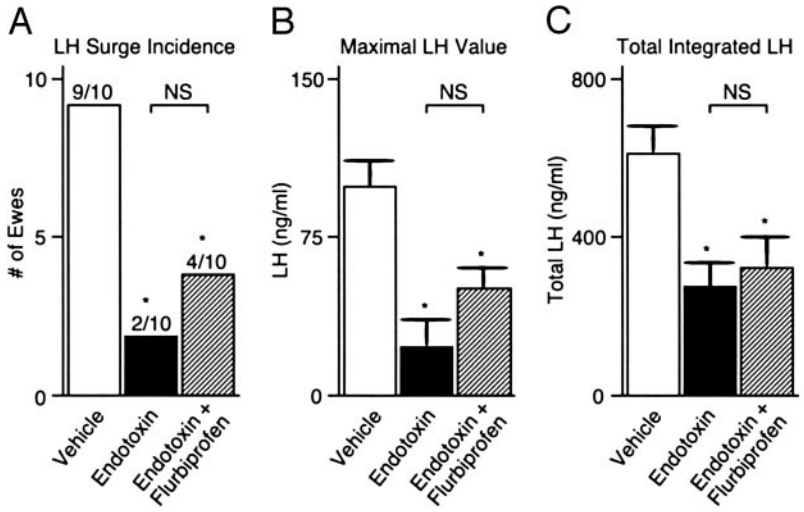

FIG. 4. Number of ewes expressing the LH surge (A), mean (± SEM) maximal LH value (B), and total integrated LH during the surge period (C) in vehicle, endotoxin, and endotoxin plus flurbiprofen ewes in experiment 2 (n = 10/treatment, crossover experiment). *, Significant differences between vehicle and endotoxin or between vehicle and endotoxin plus flurbiprofen-treated ewes ($P < 0.05$). Values between endotoxin and endotoxin plus flurbiprofen-treated ewes are not significantly different (NS).

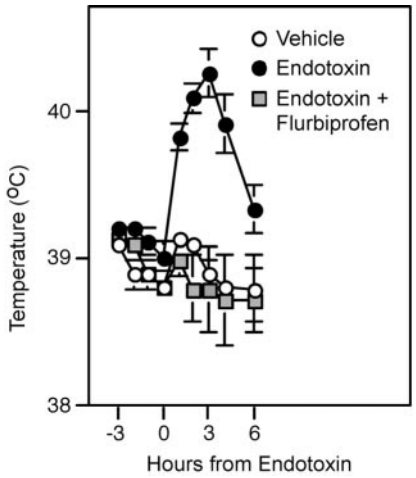

FIG. 5. Mean (± SEM; n = 10/treatment) rectal temperature values in response to vehicle (*open circles*), endotoxin (*closed circles*), or endotoxin plus flurbiprofen (*shaded boxes*) in experiment 2. Note that flurbiprofen blocked endotoxin-induced fever.

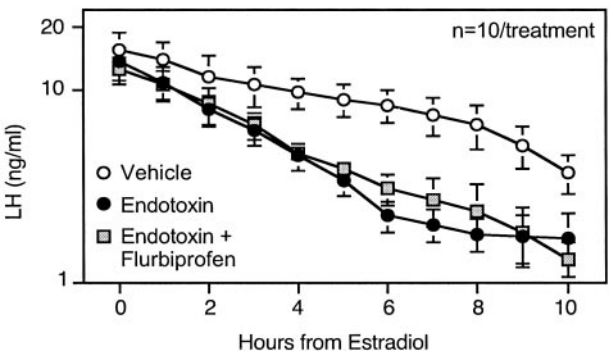

FIG. 6. Mean (± SEM; n = 10/treatment) plasma LH in ewes treated with vehicle (*open circles*), endotoxin (*closed circles*), or endotoxin plus flurbiprofen (*shaded boxes*) during the presurge period in experiment 2. LH is plotted on a logarithmic scale to facilitate illustration of the suppression of LH concentrations before the surge.

model. Specifically, in a total of 48 ewes not receiving endotoxin, an estradiol stimulus of 12 h or longer was 100% effective in eliciting the GnRH/LH surge (six of six in experiment 1 and 42 of 42 in Refs. 12, 15, and 29–31). Our best explanation for the lower incidence of surge blockade in experiment 1 is that the 12-h estradiol stimulus was sufficiently strong that, in some ewes, it overcame the suppressive effect of the endotoxin preparation used in the present experiment. This possibility is reinforced by the outcome of experiment 2, in which the estradiol stimulus was 1.5 h shorter (10.5 h in duration) and endotoxin blocked the LH surge in eight of 10 ewes. Another possible factor contributing to the variable surge blockade in experiment 1 relates to individual prior histories of infection and disease, and the consequent exposure to immune and inflammatory stimuli, all of which can lead to tolerance and desensitization (32). These histories were not known for our animals. Regardless of the explanation, our findings in experiment 1 indicate that endotoxin blocks the GnRH surge in ewes in which the LH surge is blocked. This provides strong evidence that endotoxin-induced blockade of the LH surge is mediated centrally, via inhibition of GnRH secretion.

The conclusion that endotoxin blocks the LH surge via a central mechanism does not, in itself, discount the possibility of an additional inhibitory effect at the pituitary gland. In this regard, prior work in ovariectomized ewes demonstrates that endotoxin reduces pituitary responsiveness to the brief (~5 min) stimulus of a GnRH pulse (8). Although the present study did not directly test the influence of endotoxin on pituitary responsiveness to the more powerful and prolonged (9–18 h) stimulus of the GnRH surge, our results provide strong evidence that the inhibitory effect of endotoxin on the LH surge cannot be explained by suppression at the level of the pituitary gland. The LH surge, when it escaped blockade by endotoxin, was comparable to that in vehicle-treated controls (see Table 1). This might reflect the finding that the vast amount of GnRH secreted during the surge greatly exceeds that needed to stimulate a full-amplitude LH surge (29), such that any suppressive effect of endotoxin at the pituitary level might be masked. Additional evidence that endotoxin does not block the LH surge at the pituitary level is provided by the earlier observation that endotoxin failed to attenuate the LH surge when treatment

was initiated just before the surge and continued throughout the period of LH release (7).

Our finding that the prostaglandin-synthesis inhibitor, flurbiprofen, failed to reverse the inhibitory effect of endotoxin on the estradiol-induced LH surge is keenly interesting in light of strong evidence that prostaglandins are obligatory mediators of other central responses to immune/inflammatory challenge, such as the generation of fever (22, 23). Of particular note, prostaglandins are essential for the suppressive actions of endotoxin on the pulsatile mode of GnRH and LH release (9). It is unlikely that the failure of flurbiprofen to reverse blockade of the LH surge was due to ineffective suppression of prostaglandin synthesis because the dose was the same as that used previously to reverse endotoxin-induced suppression of GnRH and LH pulses. Further, flurbiprofen prevented endotoxin-induced fever in the same ewes that it failed to reinstate the LH surge (Fig. 5), again suggesting prostaglandin synthesis was effectively blocked. The more likely explanation of our results, therefore, is that prostaglandins are not essential mediators of the suppressive effects of endotoxin on the estradiol-induced LH surge.

The foregoing discussion implies important differences exist between the mechanisms whereby endotoxin blocks the estradiol-induced LH surge and suppresses pulsatile LH secretion in ovariectomized ewes. With regard to pulses, both a mediatory role of prostaglandins and suppression of pituitary responsiveness to GnRH are important components of the inhibitory process (8, 9), but neither of these is needed to block the LH surge. Another fundamental difference is suggested by the timing of endotoxin effects on the two modes of gonadotropin secretion. Endotoxin inhibits LH pulses acutely, within 1 h (2, 8, 9), whereas its suppressive effects on the LH surge are delayed. In this regard, endotoxin blocks the surge when delivered at onset of the estradiol signal, which precedes the LH surge by approximately 20 h, but it is ineffective when given during the period that the LH surge is actually taking place (7). Thus, rather than inhibiting secretion *per se*, as is the case with LH pulses, endotoxin blocks the LH surge by preventing either the early activating effects of estradiol on estradiol-sensitive neurons or transduction of the positive feedback signal into subsequent surge secretion of GnRH and LH. Our findings, therefore, imply the neuroendocrine processes that mediate the regulatory input of this stress on GnRH pulses and surges are separable and fundamentally different.

Returning to the potential mediatory role of prostaglandins, it is pertinent to note that endotoxin stimulates production of several other inhibitors of gonadotropin secretion, notably endogenous opioid peptides, cytokines, and hormones of the hypothalamo-pituitary-adrenal axis (33–37). Whether or not prostaglandins are essential mediators under any given condition could be a function of these other inhibitory factors, and this, in turn, could depend on the gonadal steroid milieu. For example, ovarian steroids, most notably estradiol, enhance production of the inflammatory cytokines and the extent to which endotoxin stimulates cytokine synthesis (38, 39). Our present evidence that prostaglandins are not essential for endotoxin to block the LH surge was obtained in ovariectomized ewes treated with estradiol and progesterone to create an artificial follicular phase, whereas prior evidence that prostaglandins mediate the suppression of GnRH and LH pulses (9) was obtained in ovariectomized ewes devoid of gonadal steroids. Of interest, we observed here that flurbiprofen did not reverse endotoxin-induced suppression of LH secretion before the surge (Fig. 6), when GnRH and LH are released as pulses (20, 21). Perhaps under the endocrine milieu of falling progesterone and rising estradiol, the influence of other inhibitory intermediates prevails, such that prostaglandins are no longer essential for endotoxin to suppress reproductive neuroendocrine activity. Additional work is warranted to investigate this possibility and to identify mediators of endotoxin-induced suppression of gonadotropin pulses and surges within the endocrine setting of the follicular phase.

In summary, this study allows two novel conclusions regarding mechanisms whereby endotoxin blocks the estradiol-induced LH surge. First, endotoxin blocks the LH surge centrally, preventing the GnRH surge in hypophyseal portal circulation. Second, the suppressive effect of endotoxin on the LH surge does not require prostaglandin synthesis. Beyond these conclusions, we have gathered initial evidence that the inhibitory effect of endotoxin on pulsatile gonadotropin secretion before the surge may also not require prostaglandins. This contrasts with our earlier finding (9) that prostaglandins are essential for endotoxin to suppress pulsatile GnRH and LH secretion in ewes devoid of ovarian steroids. Collectively, our findings prompt additional work to identify stress-related regulatory inputs to neuroendocrine processes that generate GnRH pulses and surges, where these inputs converge, and how the changing endocrine environment of the ovarian cycle influences pathways inhibiting reproductive neuroendocrine function during immune/inflammatory stress.

Acknowledgments

We sincerely appreciate Doug Doop and Gary McCalla for their expertise in animal care. We also thank Emily Adams, Dr. Graham Barrell, Marty Brown, Emily Wessinger, and April Yasunaga for their endless hours of experimental support and Dr. Tom Harris for his contribution to the design of this study. Finally, we are grateful to Drs. Gordon D. Niswender and Leo E. Reichert, Jr., for supplying RIA reagents.

Received August 22, 2003. Accepted September 29, 2003.

Address all correspondence and requests for reprints to: Fred J. Karsch, Reproductive Sciences Program, University of Michigan, 300 North Ingalls Building, Room 1101 SW, Ann Arbor, Michigan 48109-0404. E-mail: fjkarsch@umich.edu.

This work was supported by NIH-HD-30773, T32-HD07048, and the Office of the Vice President for Research at the University of Michigan. N.D. was supported by la Fondation pour la Recherche Médicale (SE000738-01) (France).

Present address for H.J.B.: Department of Cell Biology, Neurobiology and Anatomy, University of Cincinnati College of Medicine, P.O. Box 670521, Cincinnati, Ohio 45267-0521.

Present address for N.D.: UMR868, Elevage des Ruminants en Régions Chaudes, 2 place P, Viala, 34060, Montpellier Cedex 1, France.

A preliminary report has appeared in Biology of Reproduction (Vol. 64, Supplement 1, 2001 Abstract 29).

References

1. **Coleman ES, Elsasser TH, Kemppainen RJ, Coleman DA, Sartin JL** 1993 Effect of endotoxin on pituitary hormone secretion in sheep. Neuroendocrinology 58:111–122

2. **Battaglia DF, Bowen JM, Krasa HB, Thrun LA, Viguié C, Karsch FJ** 1997 Endotoxin inhibits the reproductive neuroendocrine axis while stimulating adrenal steroids: a simultaneous view from hypophyseal portal and peripheral blood. Endocrinology 138:4273–4281
3. **Rivest S, Rivier C** 1993 Centrally injected interleukin-1β inhibits hypothalamic LHRH secretion and circulating LH levels via prostaglandins in rats. J Neuroendocrinol 5:445–450
4. **Feng YJ, Shalts E, Xia LN, Rivier J, Rivier C, Vale W, Ferin M** 1991 An inhibitory effect of interleukin-1α on basal gonadotropin release in the ovariectomized rhesus monkey: reversal by a corticotropin-releasing factor antagonist. Endocrinology 128:2077–2082
5. **Xiao E, Xia-Zhang L, Barth A, Zhu J, Ferin M** 1998 Stress and the menstrual cycle: relevance of quality in the short- and long-term response to a 5-day endotoxin challenge during the follicular phase in the rhesus monkey. J Clin Endocrinol Metab 83:2454–2460
6. **Rivest S, Lee S, Attardi B, Rivier C** 1993 The chronic intracerebroventricular infusion of interleukin-1β alters the activity of the hypothalamic-pituitary-gonadal axis of cycling rats. I. Effect on LHRH and gonadotropin biosynthesis and secretion. Endocrinology 133:2424–2430
7. **Battaglia DF, Beaver AB, Harris TG, Tanhehco E, Viguié C, Karsch FJ** 1999 Endotoxin disrupts the estradiol-induced luteinizing hormone surge: interference with estradiol signal reading, not surge release. Endocrinology 140: 2471–2479
8. **Williams CY, Harris TG, Battaglia DF, Viguié C, Karsch FJ** 2001 Endotoxin inhibits pituitary responsiveness to gonadotropin-releasing hormone. Endocrinology 142:1915–1922
9. **Harris TG, Battaglia DF, Brown ME, Brown MB, Carlson NE, Viguié C Williams CY, Karsch FJ** 2000 Prostaglandins mediate the endotoxin-induced suppression of pulsatile gonadotropin-releasing hormone and luteinizing hormone secretion in the ewe. Endocrinology 141:1050–1058
10. **Yoo MJ, Nishihara M, Takahashi M** 1997 Involvement of prostaglandins in suppression of gonadotropin-releasing hormone pulse generator activity by tumor necrosis factor-α. J Reprod Dev 43:181–187
11. **Evans NP, Dahl GE, Padmanabhan V, Thrun LA, Karsch FJ** 1997 Estradiol requirements for induction and maintenance of the gonadotropin-releasing hormone surge: implications for neuroendocrine processing of the estradiol signal. Endocrinology 138:5408–5414
12. **Moenter SM, Caraty A, Karsch FJ** 1990 The estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 127:1375–1384
13. **Goodman RL, Legan SJ, Ryan KD, Foster DL, Karsch FJ** 1981 Importance of variations in behavioral and feedback actions of oestradiol to the control of seasonal breeding in the ewe. J Endocrinol 89:229–240
14. **Debus N, Breen KM, Barrell GK, Billings HJ, Brown M, Young EA, Karsch FJ** 2002 Does cortisol mediate endotoxin-induced inhibition of pulsatile luteinizing hormone and gonadotropin-releasing hormone secretion? Endocrinology 143:3748–3758
15. **Evans NP, Dahl GE, Caraty A, Padmanabhan V, Thrun LA, Karsch FJ** 1996 How much of the gonadotropin-releasing hormone (GnRH) surge is required for generation of the luteinizing hormone surge in the ewe? Duration of the endogenous GnRH signal. Endocrinology 137:4730–4737
16. **Karsch FJ, Dierschke DJ, Weick RF, Yamaji T, Hotchkiss J, Knobil E** 1973 Positive and negative feedback control by estrogen of luteinizing hormone secretion in the rhesus monkey. Endocrinology 92:799–804
17. **Van Cleeff J, Karsch FJ, Padmanabhan V** 1998 Characterization of endocrine events during the periestrous period in sheep after estrous synchronization with controlled internal drug release (CIDR) device. Domest Anim Endocrinol 15:23–34
18. **Caraty A, Locatelli A, Moenter SM, Karsch FJ** 1994 Sampling of hypophyseal portal blood of conscious sheep for direct monitoring of hypothalamic neurosecretory substances. Methods Neurosci 20:162–183
19. **Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, Gildehaus D, Miyashiro JM, Penning TD, Seibert K, Isakson PC, Stallings WC** 1996 Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature 384:644–648
20. **Karsch FJ, Foster DL, Bittman EL, Goodman RL** 1983 A role for estradiol in enhancing luteinizing hormone pulse frequency during the follicular phase of the estrous cycle of sheep. Endocrinology 113:1333–1339
21. **Evans NP, Dahl GE, Glover BH, Karsch FJ** 1994 Central regulation of pulsatile gonadotropin-releasing hormone (GnRH) secretion by estradiol during the period leading up to the preovulatory GnRH surge in the ewe. Endocrinology 134:1806–1811
22. **Saper C** 1998 Neurobiological basis of fever. In: Kluger MJ, Bartfai T, Dinarello CA, eds. Molecular mechanisms of fever. New York: New York Academy of Science; 90–94
23. **Coceani F, Akarsu ES** 1998 Prostaglandin E_2 in the pathogenesis of fever: an update. In: Kluger MJ, Bartfai T, Dinarello CA, eds. Molecular mechanisms of fever. New York: New York Academy of Science; 76–82
24. **Hauger RL, Karsch FJ, Foster DL** 1977 A new concept for control of the estrous cycle of the ewe based on the temporal relationships between luteinizing hormone, estradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. Endocrinology 101:807–817
25. **Niswender GD, Midgley Jr AR, Reichert Jr LE** 1968 Radioimmunologic studies with murine, ovine and porcine luteinizing hormone. In: Rosenborg E, ed. Gonadotropins. Los Altos, CA: GERON-X; 299–306
26. **Niswender GD, Reichert Jr LE, Midgley Jr AR, Nalbandov AV** 1969 Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84: 1166–1173
27. **Caraty A, Locatelli A, Schanbacher B** 1987 Augmentation by naloxone of the frequency and the amplitude of LH-RH pulses in hypothalamo-hypophyseal portal blood in castrated ram. C R Acad Sci III 305:369–374
28. **Rivest S, Rivier C** 1993 Interleukin-1β inhibits the endogenous expression of the early gene c-*fos* located within the nucleus of LH-RH neurons and interferes with hypothalamic LH-RH release during proestrus in the rat. Brain Res 613:132–142
29. **Bowen JM, Dahl GE, Evans NP, Thrun LA, Wang Y, Brown MB, Karsch FJ** 1998 Importance of the gonadotropin-releasing hormone (GnRH) surge for induction of the preovulatory luteinizing hormone surge of the ewe: dose-response relationship and excess of GnRH. Endocrinology 139:588–595
30. **Evans NP, Dahl GE, Mauger DT, Padmanabhan V, Thrun LA, Karsch FJ** 1995 Does estradiol induce the preovulatory gonadotropin-releasing hormone (GnRH) surge in the ewe by inducing a progressive change in the mode of operation of the GnRH neurosecretory system? Endocrinology 136:5511–5519
31. **Moenter SM, Brand RC, Karsch FJ** 1992 Dynamics of gonadotropin-releasing hormone (GnRH) secretion during the GnRH surge: insights into the mechanism of GnRH surge induction. Endocrinology 130:2978–2984
32. **Greisman SE** 1983 Induction of endotoxin tolerance. In: Nowotny A, ed. Beneficial effects of endotoxin. New York: Plenum; 149–178
33. **Carr DB, Bergland R, Hamilton A, Blume H, Kasting N, Arnold M, Martin JB, Rosenblatt M** 1982 Endotoxin-stimulated opioid peptide secretion: two secretory pools and feedback control *in vivo*. Science 217:845–848
34. **Heijnen CJ, Kavelaars A, Ballieux RE** 1991 β-Endorphin: cytokine and neuropeptide. Immunol Rev 119:41–63
35. **Tilders FJ, DeRijk RH, Van Dam AM, Vincent VA, Schotanus K, Persoons JH** 1994 Activation of the hypothalamus-pituitary-adrenal axis by bacterial endotoxins: routes and intermediate signals. Psychoneuroendocrinology 19: 209–232
36. **Givalois L, Dornand J, Mekaouche M, Solier MD, Bristow AF, Ixart G, Siaud P, Assenmacher I, Barbanel G** 1994 Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. Am J Physiol 267:R164–R170
37. **Rivier C** 1995 Influence of immune signals on the hypothalamic-pituitary axis of the rodent. Front Neuroendocrinol 16:151–182
38. **De M, Sanford TR, Wood GW** 1992 Interleukin-1, interleukin-6 and tumor necrosis factor-α are produced in the mouse uterus during the estrous cycle and are induced by estrogen and progesterone. Dev Biol 151:297–305
39. **Zuckerman SH, Bryan-Poole N, Evans GF, Short L, Glasebrood AL** 1995 *In vivo* modulation of murine serum tumor necrosis factor and interleukin-6 levels during endotoxemia by oestrogen agonists and antagonists. Immunology 86:18–24

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**