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Effects of Gonadectomy and Sex Hormone Therapy on the Endotoxin-Stimulated Hypothalamo-Pituitary-Adrenal Axis: Evidence for a Neuroendocrine-Immunological Sexual Dimorphism*

EDUARDO SPINEDI, MARIA O. SUESCUN, RAFI HADID, TATIANA DANEVA, AND ROLF C. GAILLARD

Laboratory of Neuroimmunomodulation, Department of Neurosciences, Multidisciplinary Institute on Cell Biology (E.S., M.O.S.), 1900 La Plata, Argentina; and the Neuroendocrine Unit, Department of Medicine, University Hospital (R.H., T.D., R.C.G.), CH-1211 Geneva 4, Switzerland

ABSTRACT

Bacterial lipopolysaccharide (LPS) stimulates the hypothalamo-pituitary-adrenal axis by a mechanism involving the release of cytokines, which activate the CRH-ACTH system and, as a result, increase glucocorticoid secretion.

In the present study we investigated the possibility that endogenous sex hormones modulate the *in vivo* endotoxin-stimulated adrenal and immune responses in adult BALB/c mice. In preliminary experiments we determined that the maximal glucocorticoid release in response to LPS (50 μg, ip) administration was reached 2 h after treatment. The endotoxin effect on the adrenal and immune responses was then tested in male, randomly cycling female, 20-day-gonadectomized and 20-day-gonadectomized mice treated with either testosterone or estradiol.

In addition, *in vitro* experiments were performed to determine whether 1) LPS exerts any direct effect on basal and ACTH-stimulated corticosterone release, and 2) adrenal function is influenced by bilateral gonadectomy and sex steroid therapy.

Our results indicate that 1) randomly cycling female mice have significantly more pronounced corticosterone secretion than males 2 h after endotoxin injection, although the tumor necrosis factor responses were similar; 2) the response of the hypothalamo-pituitary-adrenal axis to endotoxin stimulation in female mice was invariable throughout the different stages of the normal estrous cycle; 3) gonadectomy leads to enhanced ($P < 0.05$) adrenal and immune responses to LPS stimulation compared to the responses in shams; 4) the endotoxin-elicited adrenal and immune overresponses observed in gonadectomized mice are reversed by testosterone treatment, regardless of sex; 5) LPS does not directly modify spontaneous and ACTH-stimulated adrenal corticosterone secretion; and 6) gonadectomy alone or combined with sex steroid therapy does not increase the *in vitro* adrenal response to ACTH stimulation.

Our findings further suggest an evident neuroendocrine-immunological sexual dimorphism during the acute phase of inflammatory processes. (*Endocrinology* **131:** 2430–2436, 1992)

THE DISCOVERY of a negative feedback loop between the immune system and the brain (1–3) is one of the most exciting advances in the field of neuroendocrine immunology. Immune cells can be stimulated by microorganism-derived toxins to secrete cytokines (4). In turn, cytokines may induce many host responses associated with endotoxemia (5) and characterized by fever, stress hormone release, mineral redistribution, and increased acute phase protein synthesis (6). Interleukin-1 (IL-1) (7) and tumor necrosis factor-α (TNF) (8) have been proposed as the most important mediators for the development of the above-mentioned pathophysiological responses.

Clinical and experimental evidence also indicates that gonadal steroids can modulate the immunological function. It has already been established that sexual dimorphism exists in the immune response to different noxa and that gonadectomy alters the immune response (9, 10). Skin allograft rejection time in mice is longer in males than in females, and orchidectomy significantly reduces this rejection time (11). Male F1 N2B/N2W mice are less susceptible to autoimmune lupus, but they will die if gonadectomized (12). In addition, the mitogen-driven plaque-forming cell response of B-lymphocytes *in vitro* is inhibited by androgens (13). This evidence is strongly supported by the fact that specific receptors for sex hormones are present in organs responsible for the immune response (14).

The aim of the present study was to determine whether endogenous sex hormones exert a modulatory effect on the *in vivo* hypothalamo-pituitary-adrenal (HPA) and immune axis responses to bacterial lipopolysaccharide (LPS) administration. For this purpose we studied neuroendocrine and immune status during the acute phase of endotoxin shock as well as the influence of gonadectomy and sex hormone therapy on the LPS-stimulated neuroendocrine-immune system.

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Address all correspondence and requests for reprints to: Dr. E. Spinedi, IMBICE, cc 403, 1900 La Plata, Argentina.

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Materials and Methods

Experimental animals

All experiments were performed in adult BALB/c mice (7 weeks old) of both sexes. Animals were fed laboratory chow and water *ad libitum*

and maintained under controlled light (0700–1900 h) and temperature (20–22 C) conditions.

In vivo designs

Exp 1. Intact randomly cycling female (rcf) mice were divided by groups (six to eight mice per cage) the day before the experiment and maintained in the above-described housing conditions until the next morning. Animals were then injected ip (0800–0900 h) with 50 μl sterile saline solution (vehicle) alone or containing 50 μg LPS (Sigma Chemical Co., St. Louis, MO; L-3755) and killed by decapitation at different time intervals after treatment (30, 60, 120, and 240 min). Each pair of vehicle-injected animals was killed at the same times, and they represent the sample time zero. Trunk blood was collected, and plasma samples were frozen (−20 C) until measurement of corticosterone (B) concentration by a specific RIA (15).

Exp 2. Mice of both sexes were bilaterally orchidectomized (Odx), ovariectomized (Ovx), or sham operated under light ether anesthesia. Animals were then divided by sex and surgery and maintained in plastic cages (six to eight mice per cage). On day 1 after surgery, gonadectomized mice were injected sc (0900 h) with either testosterone propionate (Organon, Argentina; 20 μg/50 μl corn oil; Odx+TP and Ovx+TP) or estradiol benzoate (Sigma Chemical Co.; 2 μg/50 μl oil; Odx+EB and Ovx+EB). Sham-operated male (m) and rcf as well as Odx and Ovx mice were injected with oil alone. These injections were repeated on alternate days. The groups of female animals were followed by checking vaginal smears daily from the third day after surgery; Ovx and Ovx+TP mice attained almost a constant (during the last 15 days before death) vaginal smear similar to that observed at normal diestrus; on the other hand, Ovx+EB mice attained a constant vaginal smear similar to that at normal proestrus/estrus from 48 h after the beginning of sex steroid replacement treatment.

On day 20 after surgery, different groups of animals were injected (0800–0900 h) ip with either 50 μl sterile saline solution alone (veh) or containing 50 μg LPS. Two hours after treatment, mice were decapitated, and trunk blood was collected. Plasma samples were frozen (−20 C) until the determination of B, testosterone (T), and TNF concentrations. Plasma B and T concentrations were determined by specific RIAs (15, 16), and plasma TNF levels were measured by immunoradiometric assay (Medgenix, Fleurus, Belgium), as previously described (17). No significant differences were found in plasma T levels between m, Odx+TP and Ovx+TP groups (values ranging from 4–9 ng/ml), and no detectable plasma T concentrations were found in Odx and Ovx groups.

Exp 3. Intact adult female mice were classified in the following groups: a) rcf, b) on estrus, c) on metestrous, d) on diestrus, and e) on proestrus. Animals of groups b-e were selected accordingly to the results of the daily examination of vaginal smears for 3 consecutive weeks and 10–20 min before experimentation. Groups of six to eight mice were then injected with either 50 μl vehicle alone (basal) or containing 50 μg LPS and killed 2 h after treatments. Trunk blood was collected, and plasma samples were frozen (−20 C) until B concentration measurement.

In vitro experiments

Incubation of adrenal glands. Adrenal glands from different groups of mice were dissected free of adipose tissue; each gland was immediately transferred into a 13 × 53-mm polystyrene test tube containing 1 ml Earle's Balanced Salt Solution supplemented with BSA (2 g/liter), ascorbic acid (20 mg/liter), Trasylol (100 IU/ml), and antibiotics, pH 7.4 [incubation medium (IM)]. Glands were preincubated by shaking at 37 C for 30 min under a 95% O_2–5% CO_2 atmosphere. At the end of this period, media were discarded; then the glands were resuspended in 1 ml fresh medium alone (basal condition) or containing LPS (1–100 ng/ml), human ACTH (Peninsula Laboratories, San Carlos, CA; 0.022–2.2 nM), or different combinations of these substances and incubated for 2 h under 95% O_2–5% CO_2 atmosphere. In all experiments at least six tubes were used for each control or test group. After incubation, media were decanted and frozen at −20 C until measurement of B.

Analysis of data. Data were evaluated using analysis of variance, followed by Fisher's test for comparison of different mean values (18).

Results

In vivo experiments

Time-related effect of endotoxin on glucocorticoid release. This preliminary experiment was performed to determine the time-related effect of 50 μg LPS (ip injected) on plasma B levels in intact (rcf) female mice. For this purpose, different groups of animals (six to eight mice per group) were injected ip with 50 μl sterile saline solution alone or containing 50 μg LPS. Mice were killed 30, 60, 120, or 240 min after treatment. Pairs of vehicle-injected mice were killed at the different time intervals; they represent the sample time zero. Figure 1 shows that the administration of endotoxin significantly ($P < 0.02$ or less) enhanced plasma B levels over baseline values (sample time zero) in a time-related fashion. The maximal LPS effect on glucocorticoid secretion was observed 2 h after injection.

Effect of LPS administration on plasma glucocorticoid levels in gonadectomized and sham-operated mice of both sexes. Figure 2 shows the results of plasma B concentrations 2 h after the administration of 50 μl vehicle alone or containing 50 μg LPS in both m and rcf animals. LPS-treated m and rcf mice showed significantly ($P < 0.01$) higher plasma B levels than the respective control groups (veh). Furthermore, the increment in plasma B concentration induced by LPS injection was significantly ($P < 0.05$) higher in the rcf group than in the m group.

The effects of bilateral gonadectomy on basal and LPS-induced glucocorticoid secretion are also shown in Fig. 2. In both sexes gonadectomy did not modify basal (injection of vehicle alone) plasma B concentrations compared to basal values in shams (vehicle-injected m and rcf mice). The administration of LPS in gonadectomized mice significantly (*P*

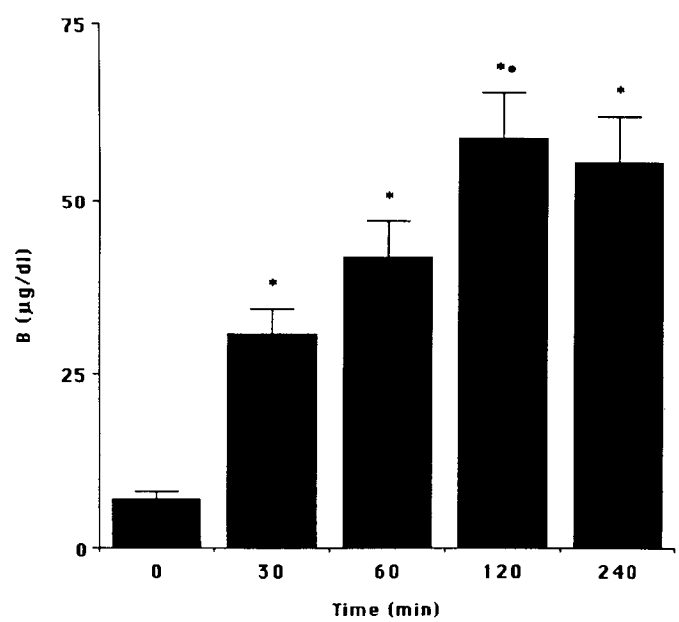

FIG. 1. Time-related effect of LPS (50 μg, ip) on plasma B levels in intact female mice. Each *bar* represents the mean ± SEM (n = 6–8 mice/group). *, $P < 0.02$ or less *vs.* sample time zero. ●, $P < 0.05$ *vs.* sample time 60 min.

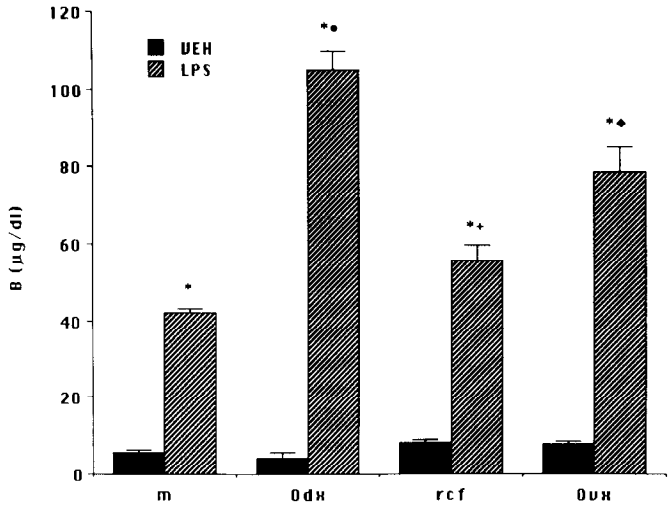

FIG. 2. Plasma B concentrations 2 h after the ip injection of vehicle alone (veh) or containing 50 μg endotoxin (LPS) in m, 20-day Odx, rcf, and 20-day Ovx mice. *Bars* represent the mean ± SEM (n = 6–8 mice/group). *, $P < 0.01$ *vs.* vehicle-injected mice. +, $P < 0.05$ *vs.* LPS-treated m mice. ♦, $P < 0.05$ *vs.* LPS-injected rcf mice. ●, $P < 0.05$ or less *vs.* LPS-treated m, rcf, and Ovx mice.

< 0.01) increased plasma B concentrations over the baseline values (veh). The LPS-elicited B secretion in Odx and Ovx mice was significantly higher ($P < 0.05$ or less) than that observed in sham-operated (m and rcf, respectively) mice. In addition, endotoxin administration induced significantly ($P < 0.05$) higher plasma B levels in Odx than Ovx animals.

Effects of sex steroid therapy on endotoxin-stimulated glucocorticoid release in gonadectomized mice. Gonadectomy alone and gonadectomy followed by sex steroid treatment (with either TP or EB) did not modify basal plasma glucocorticoid concentrations. Plasma B levels were (mean ± SEM; n = 6–8 mice/group) 5.3 ± 0.9, 5.6 ± 0.7, and 5.0 ± 1.0 μg/dl for the m, Odx+TP, and Odx+EB groups, respectively, and 8.1 ± 1.1, 7.9 ± 1.2, and 7.3 ± 0.7 for the rcf, Ovx+EB, and Ovx+TP groups, respectively.

In gonadectomized mice, sex hormone therapy completely prevented ($P < 0.02$ and $P < 0.05$ in Odx and Ovx mice, respectively) the enhanced adrenal response to LPS administration (see Figs. 3 and 4). The *in vivo* adrenal hyperresponse to endotoxin administration in Odx mice was prevented not only by restoring physiological T levels, but also by administering EB. Figure 3 shows that the LPS-stimulated B output in both Odx+TP and Odx+EB animals was similar to that induced by endotoxin in the m group. Similarly, Ovx mice treated with either estrogen (Ovx+EB) or T (Ovx+TP) had an adrenal response to LPS stimulation identical to that obtained in the rcf group (Fig. 4).

Effects of gonadectomy and sex hormone therapy on endotoxin-elicited TNF release in plasma. Plasma levels of TNF in basal conditions did not vary with the sex of the animals or with bilateral gonadectomy alone or followed by sex steroid treatment (mean ± SEM, 0.83 ± 0.21 ng/ml; n = 40 mice).

Endotoxin injection significantly ($P < 0.02$) enhanced TNF plasma levels over basal values in all groups of mice studied (see Figs. 5 and 6), with no sex-related differences between

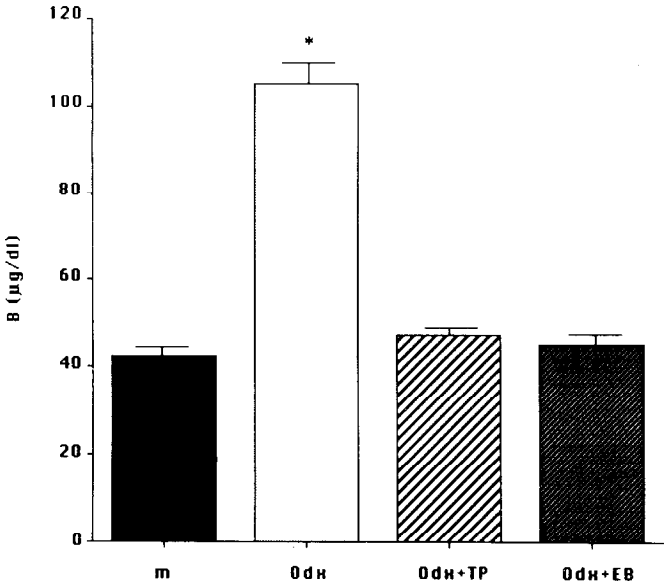

FIG. 3. Endotoxin (50 μg, ip)-induced B secretion in plasma in m and 20-day Odx mice, and effect of sex steroid (TP or EB) administration on LPS-stimulated B release in 20-day Odx mice. *Bars* represent the mean ± SEM (n = 6–8 mice/group). *, $P < 0.02$ *vs.* m, Odx+TP, and Odx+EB values.

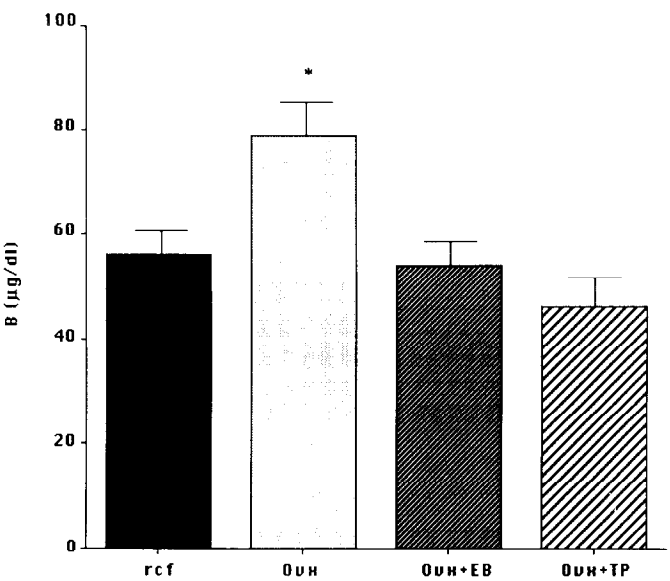

FIG. 4. Effects of LPS (50 μg, ip) on plasma B levels in rcf bilaterally Ovx mice and in Ovx mice treated with EB or TP. *Bars* represent the mean ± SEM (n = 6–8 mice/group). *, $P < 0.05$ *vs.* rcf, Ovx+EB and Ovx+TP values.

the sham-operated groups. Orchidectomy (Fig. 5) and ovariectomy (Fig. 6) alone significantly ($P < 0.05$) enhanced the effect of LPS on TNF release compared to values in sham-operated LPS-injected m and rcf mice, respectively. Priming gonadectomized mice with estradiol (Odx+EB) did not modify the effect of Odx alone, TNF values were still significantly higher than those in the m group (Fig. 5). Conversely, EB treatment in Ovx mice induced a TNF release response similar to that in rcf mice (Fig. 6).

On the other hand, the effect of gonadectomy on LPS-

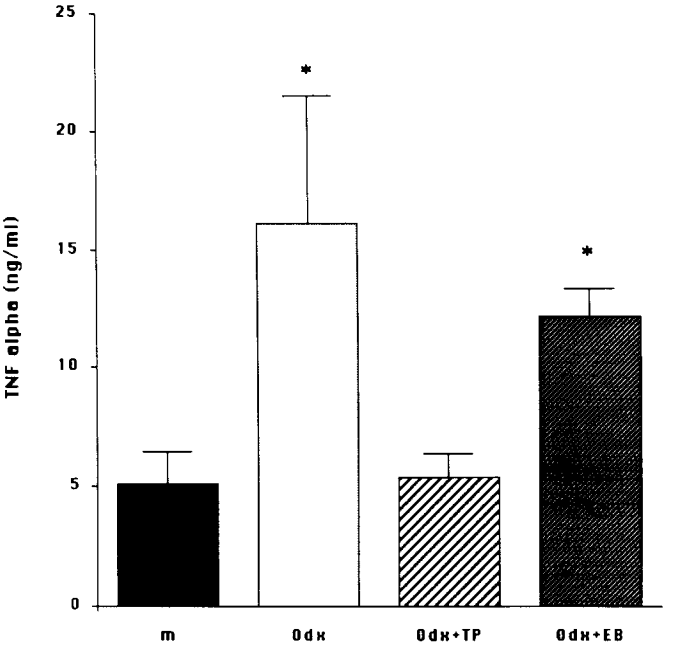

FIG. 5. Endotoxin (50 μg, ip)-elicited TNF secretion in plasma in m and 20-day Odx mice, and effect of sex steroid (TP or EB) treatment on LPS-stimulated TNF release in 20-day Odx mice. *Bars* represent the mean ± SEM (n = 6–8 mice/group). *, $P < 0.05$ *vs.* m and Odx+TP values.

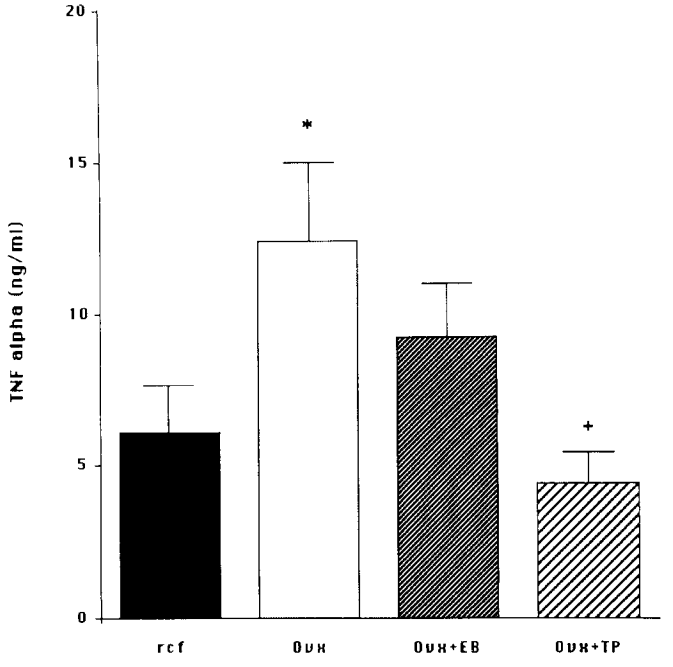

FIG. 6. Effects of LPS (50 μg, ip) on plasma TNF levels in rcf bilaterally Ovx mice and in Ovx mice receiving replacement therapy with EB or TP. *Bars* represent the mean ± SEM (n = 6–8 mice/group). *, $P < 0.05$ *vs.* rcf value. +, $P < 0.05$ *vs.* Ovx value.

stimulated TNF release was fully reversed by the treatment of both Odx- and Ovx mice with TP. Figures 5 and 6 show that the levels of TNF reached 2 h after LPS injection in Odx+TP and Ovx+TP were similar to those obtained with the same treatment in the m and rcf groups. However, in gonadectomized female mice, TP administration was more effective than EB treatment to reduce LPS-elicited TNF secretion in plasma (see Fig. 6).

Influence of the estrous cycle on basal and LPS-stimulated plasma B levels. These experiments were carried out to determine whether the endogenous sex steroid status, which characterizes each stage of the normal estrous cycle, influences basal and/or endotoxin-stimulated B secretion in plasma.

Our results indicated that there were not significant differences in basal and LPS-stimulated plasma B levels throughout the different stages of the normal estrous cycle (Table 1), with values ranging in an order of magnitude similar to that in rcf mice.

In vitro experiments

Effect of LPS on basal and ACTH-stimulated B secretion. In preliminary experiments we determined that the incubation of adrenal glands from intact male donors in the presence of ACTH significantly enhanced B release over the baseline (25.6 ± 6.1 ng/ml·adrenal gland; n = 18 flasks) in a concentration-related fashion (with B concentrations of 48.7 ± 7.1, 86.8 ± 10.3, and 143.2 ± 18.9 ng/ml·adrenal gland; for ACTH, 0.022, 0.22, and 2.2 nM, respectively; mean of three different experiments; six flasks per point/experiment).

To determine the possible existence of an adrenal site of action for LPS-stimulated glucocorticoid release, we evaluated the *in vitro* effects of different concentrations (1, 10, and 100 ng/ml) of LPS on spontaneous and 0.22 nM ACTH-induced B output. Table 2 shows that none of the different LPS concentrations tested affected B release by incubated adrenal glands, obtained from intact male mice, under basal conditions.

When the glands were incubated with ACTH (0.22 nM) alone, a significant ($P < 0.05$) increase in B production was found. The addition of LPS (1, 10, or 100 ng/ml) into the medium did not modify ACTH-elicited B release (see also Table 2).

Effects of gonadectomy alone or followed by sex steroid therapy on the adrenal response to ACTH. To determine whether gonadectomy alone and followed by sex hormone replacement therapy could influence the adrenal response, we investigated *in vitro* ACTH (0.22 nM)-stimulated B release by adrenal glands obtained from the different tested groups. We

TABLE 1. Basal and endotoxin-stimulated plasma B levels throughout the normal estrous cycle of the mice

Group	Plasma B conc. (μg/dl)	
	Basal	LPS
Random cycling	7.8 ± 1.6	55.3 ± 4.1[a]
Metestrus	6.9 ± 1.1	60.8 ± 5.2[a]
Diestrus	7.3 ± 1.2	54.6 ± 3.8[a]
Proestrus	8.1 ± 1.9	61.0 ± 7.1[a]
Estrus	6.3 ± 1.4	60.5 ± 6.7[a]

Values are the mean ± SEM (n = 6–8 mice/group). Values were obtained 2 h after the ip administration of vehicle alone (basal) or containing 50 μg endotoxin (LPS).

[a] $P < 0.01$ *vs.* basal value.

TABLE 2. *In vitro* effect of increasing concentrations of endotoxin (LPS) on spontaneous (IM alone) and 0.22 nM ACTH-stimulated B secretion by incubated adrenal glands from male mice

	B release
IM	24.3 ± 4.8
IM + LPS (1 ng/ml)	27.1 ± 5.3
IM + LPS (10 ng/ml)	22.9 ± 6.2
IM + LPS (100 ng/ml)	26.3 ± 4.2
ACTH	85.6 ± 10.8[a]
ACTH + LPS (1 ng/ml)	82.3 ± 9.7[a]
ACTH + LPS (10 ng/ml)	87.9 ± 11.2[a]
ACTH + LPS (100 ng/ml)	88.2 ± 12.1[a]

Values are expressed as nanograms of B secreted per ml medium/adrenal gland (mean ± SEM of three different experiments, with at least six flasks per point/experiment).

[a] $P < 0.05$ *vs.* values obtained in the absence of ACTH without or with different concentrations of LPS.

TABLE 3. Effects of gonadectomy (Gnx) and sex hormone therapy on the ACTH-induced B secretion by incubated adrenal glands from different groups

Group	Net B release	
	Male	Female
Sham	61.3 ± 9.1	50.7 ± 5.5
Gnx	87.1 ± 14.2	30.1 ± 7.3[a]
Gnx + TP	68.6 ± 18.1	49.7 ± 5.2
Gnx + EB	64.9 ± 9.2	45.4 ± 8.5

Values shown are levels of B released over the baseline after 2-h incubation with 0.22 nM ACTH; values are expressed as nanograms per ml medium/adrenal gland (mean ± SEM of three different experiments, with six flasks per point/experiment).

[a] $P < 0.05$ *vs.* sham female value.

found no significant differences in basal B release (22.8 ± 5.7 ng/ml·adrenal gland; mean of three different experiments, with six flasks per experiment) among the different groups.

Table 3 shows the 0.22 nM ACTH-induced B secretion over the baseline (net B release) by adrenal glands from different groups of male mice. As shown, gonadectomy alone or followed by treatment with different sex steroids did not affect ACTH-elicited glucocorticoid release. Conversely, in female mice, the adrenal response to ACTH stimulation was somewhat (although significantly, $P < 0.05$) decreased by ovariectomy, and such an effect was fully reversed by treatment with either TP or EB (see also Table 3).

Discussion

The present study demonstrates an evident neuroendocrine-immunological sexual dimorphism. Although it has been previously reported (19) that endotoxin stimulates the activity of the HPA axis, to our knowledge this is the first report indicating a modulatory role of endogenous sex hormones on the responses of both the HPA axis and the immune system after endotoxin administration. Our results indicate that during the acute phase response after LPS injection: 1) randomly cycling females release a higher amount of B, but not of TNF, than males; 2) LPS-stimulated B release in female mice is invariable throughout the different stages of the estrous cycle; 3) 20-day gonadectomized (Odx or Ovx) mice have glucocorticoid and TNF hypersecretion compared to sham animals; 4) the enhanced adrenal and immune responses seen in gonadectomized mice can be completely reversed by the administration of T regardless of the sex of the animals; 5) LPS does not directly modify spontaneous and ACTH-stimulated glucocorticoid secretion; 6) the HPA axis overresponse to endotoxin stimulation induced in gonadectomized animals was not due to the enhanced adrenal response as a consequence of gonadectomy. This sex-related immune-neuroendocrine response to endotoxin stimulation agrees with the immunological sexual dimorphism described by Grossman (10, 14).

In vivo administered LPS is able to activate not only immune (8) but also other cell populations. It has been reported that endotoxin induces IL-6 output from incubated hypothalamic fragments (20) and enhances anterior pituitary IL-1 mRNA levels (21). Cytokines, in turn, could stimulate hypothalamic CRH (21–24) as well as pituitary (25–27) and extrapituitary (28) ACTH secretion. In addition, activation of the HPA axis by other substances secreted shortly after endotoxin must not be discounted. In fact, serotonin (29), epinephrine (30), bradykinin (31), and prostaglandins (32) released during the acute phase response of the endotoxic shock are able to stimulate the HPA axis. Whether the amount released and the effects of these substances on HPA axis function are dependent on the sex steroid environment remains unknown.

On the other hand, LPS has no direct effect on mice (Spinedi, E., and R. C. Gaillard, unpublished observation) or rat (33) ACTH secretion, and as reported in this study, it is unable to directly modify glucocorticoid output from the adrenal gland. These observations, therefore, suggest that the sexual dimorphism in endotoxin-stimulated HPA axis function may be due to a regulatory effect of sex steroids on LPS-stimulated immune and neuroendocrine cells (20, 21).

In the present study we found no differences between T and estradiol in preventing the enhanced LPS-stimulated glucocorticoid release in gonadectomized animals (regardless of the sex of the mice). However, our results suggest a predominant inhibitory role of T on the HPA-immune axis response to endotoxin injection. In fact, physiological levels of the androgen (m group) lead to a lower B release in plasma compared to that in female mice (rcf). Moreover, the removal of endogenous androgens by Odx induces higher plasma B secretion than when estrogens are removed by Ovx. In addition, LPS-stimulated HPA axis function in female mice was not influenced by the normal estrous cycle, indicating that acute changes in plasma estrogen levels throughout the different stages of the 4-day cycle are not able to modify the neuroendocrine response to endotoxin administration. Although bilateral gonadectomy (Odx and Ovx) also induces increased LPS-elicited TNF secretion, we found no differences between plasma TNF levels reached after LPS in Odx and Ovx mice; similar results were obtained when comparing m and rcf animals. Whereas T therapy completely reversed the enhanced LPS-elicited TNF release in gonadectomized mice of both sexes, estradiol treatment only blocked this

effect in gonadectomized female mice and had no effect on gonadectomized male animals. However, in both sexes T was more effective than estrogen treatment in decreasing the effect of gonadectomy on LPS-stimulated TNF secretion, strongly supporting an endocrine-hormone basis for immunological dimorphism (9, 10, 14). The latter could indicate that estradiol (in the dose employed in this study) is less effective than T on endotoxin-stimulated TNF-producing cells. However, we cannot rule out the possibility that sex steroids might also affect the release of IL-1 after LPS administration; this substance, in turn, stimulates HPA axis function (3, 22).

TNF has been shown to have both stimulatory and inhibitory effects on the HPA axis. TNF stimulates *in vivo* ACTH secretion (34) by a mechanism probably due to the release of CRH from the hypothalamus (24, 35). However, it has recently been shown that TNF inhibits *in vitro* anterior pituitary hormone release by hypothalamic releasing factors (36) and stimulates adrenal steroid synthesis (37) and secretion (38). The stimulatory effect of TNF suggested in the present study fits the above-mentioned observations. In fact, the inhibitory action of TNF was only observed *in vitro* after several hours of incubation, whereas the stimulatory effect was present after 1–2 h, as seen in this study.

Our results also show that the *in vitro* adrenal response to ACTH remained unaltered in all groups of male mice. Conversely, the adrenal response to ACTH stimulation was somewhat decreased after Ovx, and this was restored to normal when Ovx mice received sex hormone therapy. These data clearly rule out the possibility that the *in vivo* enhanced HPA axis response to endotoxin could be due to an overresponse of the adrenal itself as a consequence of Ovx. Whether the impaired adrenal response observed *in vitro* after Ovx is due to a sex steroid hormone modulation of ACTH receptors in the adrenals of female mice is still unknown.

The different HPA axis responses to endotoxin stimulation observed *in vivo* cannot be explained by changes in hypothalamic CRH and anterior pituitary ACTH content or *in vitro* basal and CRH-stimulated ACTH release, since neither parameter was affected by chronic gonadectomy and sex steroid therapy (data not shown). It remains to be determined whether there is any sex-related change in the hypothalamo (CRH)-pituitary (ACTH) system response to cytokine stimulation.

Acute inflammation represents a threat to the integrity of the organism that requires metabolic changes (39), such as increased secretion of glucocorticoids (40), for survival immediately after injury. Our results strongly support the hypothesis that sex hormones blunt the effect of inflammation.

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