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Sexual Dimorphism of Neuroendocrine-Immune Interactions

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Introduction

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], characterized by fever, stress hormone release, mineral redistribution and increased acute phase protein synthesis [6]. Interleukin (IL)-1 [7] and tumor necrosis factor-α (TNFα) [8] have been proposed as being the most important mediators for the development of all these pathophysiological responses.

In the bidirectional interplay between the immune and endocrine systems, the communications most deeply investigated up to now have been those between the immune and the hypothalamo-pituitary-adrenal (HPA) and the HP-gonadal (HPG) [for review see, 9] axes. For instance, mitogen/antigen-activated immune cells secrete cytokines; in turn, these substances are able to stimulate the hypothalamus thus inducing the activation of the corticotropin-releasing hormone (CRH)-ergic function [10]. Once the CRH neuronal system has been activated, this peptide is able to locally (centrally) inhibit the HPG axis function [for references see, 11] and, via the corticotrope cells, to stimulate HPA axis function [12].

Evidence indicates that gonadal steroids modulate immunological function [13]. Our findings and those of other researchers suggest that while estrogens

enhance the immune response [14], androgens inhibit it [15] and that gonadectomy alters this response [16, 17].

Skin allograft rejection time in mice is longer in males than in females and orchidectomy significantly reduces the time for such rejection [16]. Male F1 N2B/N2W mice are less susceptible to autoimmune lupus, but will die if gonadectomized [17]. In addition, mitogen-driven plaque-forming cell response of B-lymphocytes in vitro is inhibited by androgens [18]. All this evidence is strongly supported by the presence of specific receptors for sex hormones in organs responsible for the immune response [13].

However, gonadal steroids seem to influence not only immune activity but also HPA axis function [19], in fact they can either positively (estrogen) [20, 21] or negatively (androgen) [22] modulate hypothalamic CRH production and, as a consequence, close the interactive circuit between the HPA and HPG axes [11].

A sex hormone basis for neuroendocrine-immunological sexual dimorphism has been described [13, 15]. The HPA axis function in rodents has been characterized as sexually dimorphic in both basal and post-stress conditions [15, 23]; moreover, sexual dimorphism in the HPA axis response holds true regardless of the type of stimulus since, for instance, both neuroendocrine [23] and immune [15] stresses induce a final release of glucocorticoid in plasma that is higher in female than in male adult animals.

Glucocorticoid secretion is crucial for metabolic adaptations of the organism to stress [24] and a glucocorticoid hormone basis tallies with survival of individuals in sepsis [24, 25].

Gender-Dependent Neuroendocrine-Immune Interactions

Young adult (8–10 weeks old) mice are the best laboratory animal model to study the influence of the gender background on the HPA axis response to immune challenge. A gender-dependent characteristic HPA axis response to endotoxin administration (2 mg/kg i.p.) is displayed in figure 1. As illustrated, the time course of plasma ACTH (fig. 1a) and corticosterone (fig. 1b) levels in response to lipopolysaccharide (LPS) stimulus is similar in both sexes; however, the intensity of the response is significantly greater in females than in males. Corticotrope resiliency [26] is completed 72 h after challenge and is identical in both groups of mice. Adrenal function, in basal and post-endotoxin conditions, displayed a very clear gender-dependent characteristic, female corticosterone plasma levels being at each time point significantly higher than male values. This pattern was also found in middle-aged (15-month-old) mice (fig. 1c, d), thus indicating the persistence of the gender-dependent difference in HPA axis function at more advanced ages. These characteristics were not only

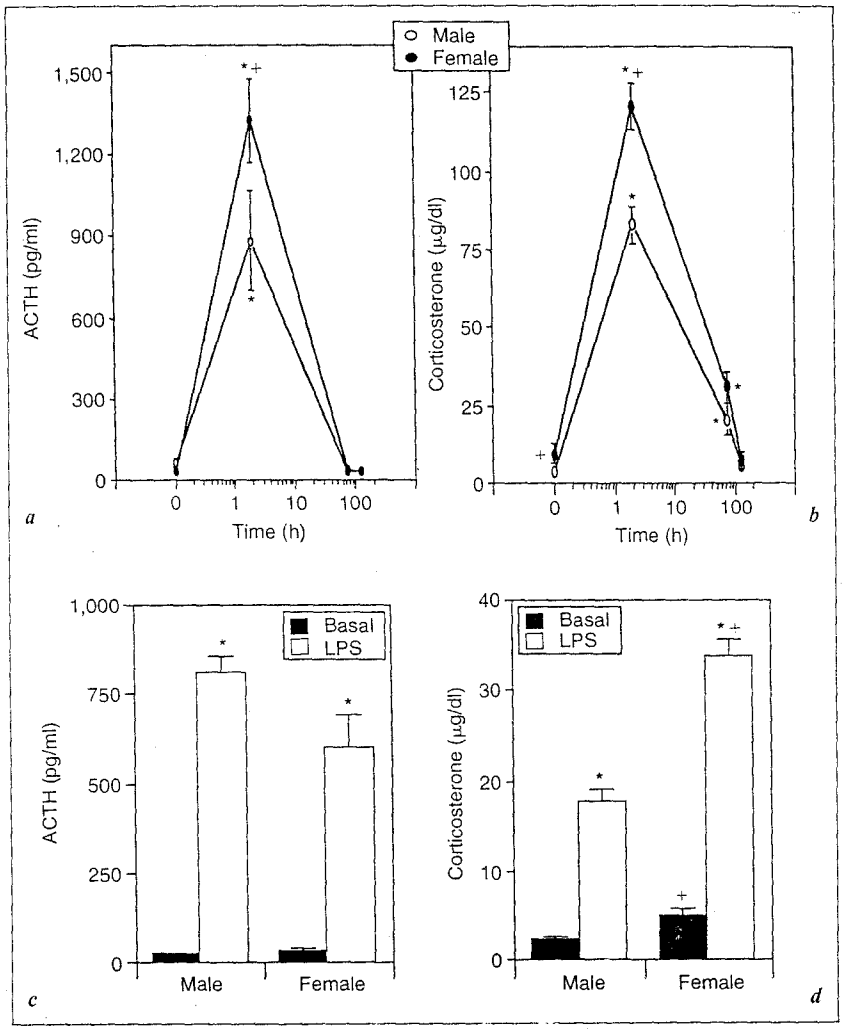

Fig. 1. Plasma ACTH (*a*) and corticosterone (*b*) concentrations before and after i.p. administration of endotoxin (2 mg/kg) in 2-month-old mice of both sexes. Circulating ACTH (*c*) and glucocorticoid (*d*) 2 h after i.p. injection of vehicle alone (basal) or containing LPS (2 mg/kg) in 15-month-old mice of both sexes. Mean ± SEM (n = 6–9 animals per group). *$p < 0.05$ vs. the respective basal values; $^{+}p < 0.05$ vs. males under similar conditions.

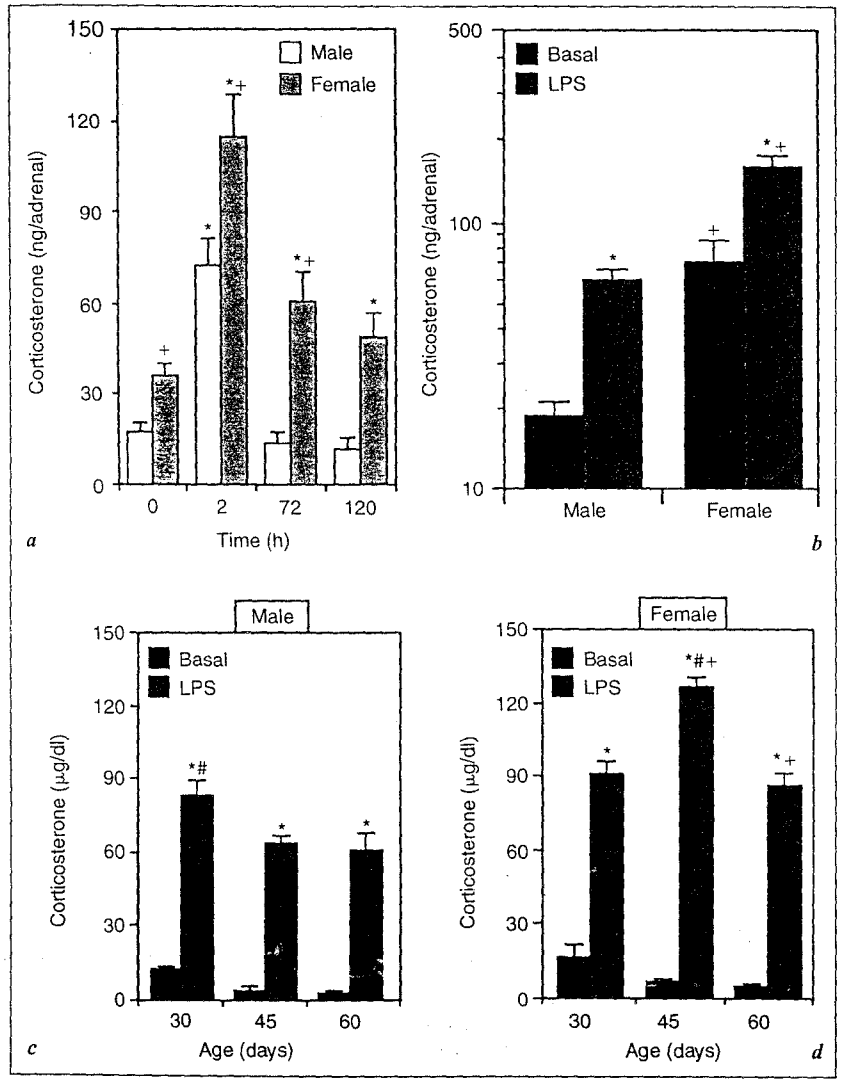

Fig. 2. Adrenal gland glucocorticoid content before and at several time points after i.p. administration of endotoxin (2 mg/kg) in 2-month-old mice of both sexes (*a*). Basal and LPS-stimulated (at 2 h) adrenal gland glucocorticoid content in middle-aged mice of both sexes (*b*). Circulating corticosterone under basal and post-LPS (2 mg/kg, i.p.) conditions in male (*c*) and female (*d*) mice of different ages. Mean ± SEM (n = 6–9 animals per group). $^{*}p < 0.05$ vs. the respective basal values; $^{+}p < 0.05$ vs. condition-matched males; $^{\#}p < 0.05$ vs. the remaining LPS values in mice of same sex.

observed in circulating plasma hormone levels, but also in adrenal glucocorticoid content (fig. 2a, b).

However, some parameters indicate that the enzymatic process could be involved in these patterns since, for instance, in 8-week-old mice, while females showed increased estrogen plasma concentrations 2 h after LPS (90.4 ± 5.3 vs. basal 35.4 ± 3.5 pg/ml), males developed a significant decrease in plasma testosterone levels (from 4.6 ± 1.9 ng/ml under basal conditions to 1.4 ± 0.3 ng/ml). This indicates that the gender-dependent differences in HPA axis function after immune challenge could have a sex-steroid hormone basis. An increased testicular aromatization of androgen to estrogen has been reported during endotoxemia [27]. Therefore, this increased aromatization in males, who are less immunoresponsive than females [15], could result in an enhanced immune response as an adaptation of the body's defense mechanisms shortly after injury. Because testosterone levels vary over development, and in order to determine whether physiological testosterone changes may modulate the HPA axis response to endotoxin, we measured the plasma corticosterone response to endotoxin administration in prepubertal (30-day-old) mice – a period of development with low testosterone levels (1.54 ± 0.04 ng/ml) – and in peri-postpubertal and adult mice (45 and 60 days old) – periods of development with normal testosterone levels (3.99 ± 0.11 and 3.81 ± 0.14 ng/ml, respectively). The corticosterone response to LPS was higher at 30 days of age, when testosterone levels are low, than in 45- and 60-day-old mice when testosterone levels are in the normal adult range (fig. 2c, d).

These results demonstrate the existence of an inverse correlation between the HPA axis response to LPS and the testosterone levels, confirming the inhibitory effect of androgens. Furthermore, the importance of circulating sex steroids in modulating the HPA axis response to LPS is also clearly suggested by the observation that the gender specific pattern of this response is absent in immature mice – both male and female mice showing the same response – whereas a clear sexual dimorphic pattern is apparent only after animals reached puberty, with female showing a greater response to LPS than males (fig. 2c, d).

However, these gender-related differences also depend on the strain of animal studied. It is known that while female Fischer 344/N rats are resistant, Lewis/N rats of the same sex are sensitive to developing arthritic syndrome after administration of group-A streptococcal cell wall fragments [28]; interestingly, LEW/N showed no gender-related difference in the corticotrope response to immune challenges, such as LPS (fig. 3a) and neurotoxin [29] (fig. 3b), while female Fischer 344/N rats were hyperresponsive, regardless of the stimulus (fig. 3a, b). The hyporesponsiveness of the corticotrope function in female Lewis/N rats was further confirmed when stimulated in vivo with CRH (0.5 μg per rat) but not with arginine vasopressin (5 μg per rat; fig. 3c, d). Considering

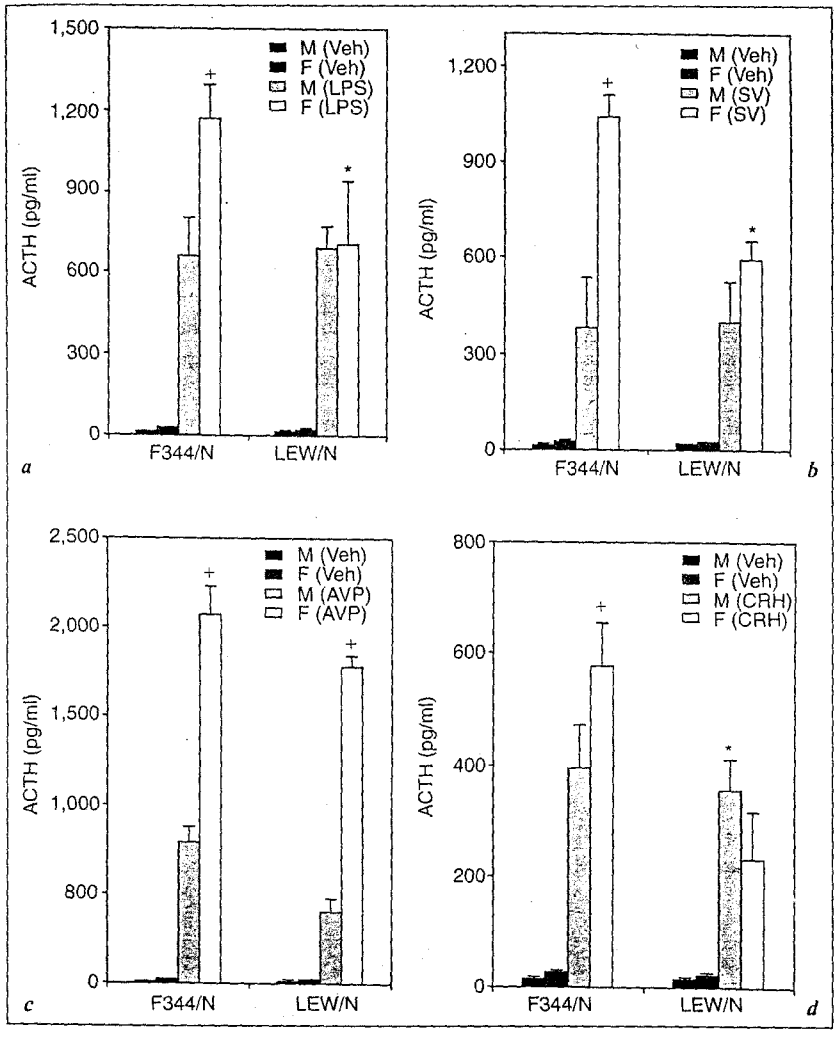

Fig. 3. Plasma ACTH levels in male (M) and female (F) adult F344/N and LEW/N rats under basal conditions (Veh) and after i.p. administration of either LPS (100 μg/rat; *a*), neurotoxin (SV, 100 μg/rat; *b*), vasopressin (5 μg/rat; *c*) or CRH (0.5 μg/rat; *d*). Mean ± SEM (n = 6–9 animals per group). All values post-stressor are significantly ($p < 0.05$) higher than the respective basal (Veh) values. *$p < 0.05$ vs. the respective values in F344/N rats; $^{+}p < 0.05$ vs. condition-matched males.

that cytokines released after immune system activation by either LPS or neurotoxin treatment result in enhanced CRH output [10, 29] and that CRH in turn stimulates corticotrope cells, the corticotrope hyporesponsiveness to CRH stimulation in female LEW/N rats indicates a decreased adrenal glucocorticoid response which is then less able to counteract the activated immune system and thus facilitates the development of the arthritic syndrome.

Evidence for a Sex Steroid Hormone Basis for Neuroendocrine-Immunological Sexual Dimorphism

To further characterize the modulatory effects of sex steroids on the responses of the HPA and immune axes during endotoxemia, we studied the influence of gonadectomy and sex hormone therapy on the LPS-stimulated neuroendocrine-immune system.

Adult BALB/c mice of both sexes were either sham operated or gonadectomized (Gnx) for 20 days (Odx = orchidectomized; Ovx = ovariectomized). Sex steroid replacement therapy in Gnx mice consisted of the administration of the homologous sex steroid on alternate days between days 1 and 19 after Gnx (20 μg testosterone propionate/50 μl corn oil, Odx+T; or estradiol benzoate, 2 μg/50 μl corn oil, Ovx+E). On day 20 after surgery, mice were injected intraperitoneally (i.p.) with vehicle alone (sterile saline solution) or containing LPS (2 mg/kg). In both sexes, gonadectomy did not modify basal corticosterone (B) levels, but significantly enhanced the LPS-induced corticosterone release. Moreover, the removal of endogenous androgens by orchidectomy induced higher plasma corticosterone release than when estrogens were removed by ovariectomy (fig. 4a, b). These results clearly suggest a predominant inhibitory role of testosterone on the HPA axis response to endotoxin. The importance of testosterone in modulating the HPA axis response to LPS was confirmed by the substitution therapy experiment because testosterone substitution completely prevented the enhanced adrenal response to LPS administration in Gnx animals of both sexes (fig. 4a, b). Surprisingly, however, the adrenal hyperresponse to LPS in Gnx mice of both sexes is not only prevented by testosterone, but also by the administration of estradiol (fig. 4a, b). Thus in the Gnx animals regardless of the sex, both estrogen and testosterone prevent the adrenal hyperresponse to LPS.

We also investigated whether gonadal steroids modulate the LPS-induced immune response by measuring the effect of endotoxin on the release of plasma TNFα in the same experimental design (see fig. 4c, d). Plasma TNFα levels were similar under all basal conditions. LPS administration significantly enhanced TNFα levels with no sex-related differences. Orchidectomy and ovariectomy significantly enhanced the effect of LPS on TNFα release. Replacement therapy

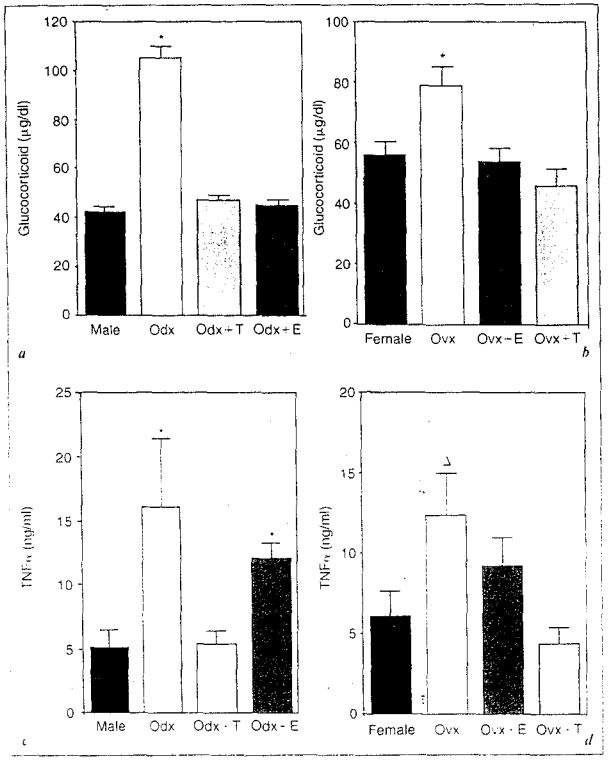

Fig. 4. Plasma glucocorticoid (*a*, *b*) and TNFα (*c*, *d*) 2 h after i.p. administration of LPS (50 μg mouse) in several groups of male (*a*, *c*) and female (*b*, *d*) 2-month-old mice. Mean ± SEM (n = 6–9 animals per group). All values post-stressor are significantly ($p < 0.05$) higher than the respective basal (vehicle-injected) values. *$p < 0.05$ vs. values in the remaining groups; $^{\Delta}p < 0.05$ vs. females and Ovx+T.

with testosterone completely reversed the enhanced LPS-elicited TNFα release in Gnx animals of both sexes. Estradiol treatment only partially blocked this effect in Gnx female mice and had no effect on Gnx male animals (fig. 4c, d). Thus, in both sexes, testosterone was more effective than estrogen treatment in decreasing the effect of gonadectomy on LPS-stimulated TNFα secretion, strongly supporting an endocrine-hormone basis for an immunological dimorphism.

Because sex steroids seem to play such an important modulatory role, we investigated whether the endogenous sex steroid status that characterizes each stage of the normal estrous cycle could influence basal and/or LPS-stimulated

Table 1. Experimental design

Treatment (previous-last)	Experimental day				
	D1	D2	D3	D4	D5
None-Veh	Δ, +				
None-LPS	*, +				
LPS2-Veh	*	*	Δ, +		
LPS2-LPS	*	*	*, +		
LPS4-Veh	*	*	*	*	Δ, +
LPS4-LPS	*	*	*	*	*, +

Mice employed in this experimental design were previously (1 week before) orchidectomized [receiving (Odx+T) or not (Odx) testosterone replacement therapy] or sham-operated (Sham).

Δ = i.p. injection of NaCl 0.9% (vehicle); * = i.p. injection of 25 μg of LPS; + = decapitation 2 h after the injection.

HPA axis function. Surprisingly, the estrous cycle had no effect on corticosterone release. Basal and LPS-stimulated corticosterone levels were not influenced by the normal estrous cycle. Therefore, in our mouse model acute changes in plasma estrogen levels throughout the 4-day cycle were unable to influence the HPA axis response to LPS. Others, however, have shown that, in female rats, the HPA axis is most sensitive to stress during the early portion of proestrus suggesting a facilitatory effect of estrogens on HPA axis function [30].

We extended the evidence for a sex hormone basis of immuno-neuroendocrine interaction by developing a model of tolerance to repeated endotoxin administration. In these experiments sham-operated (Sham) and 7-day Odx mice treated (Odx+T) or not (Odx) with testosterone propionate were tested. The dose of LPS administered i.p. was again 2 mg/kg, and the schedule of the tolerance model, which started 1 week after gonadectomy, was as follows: (a) on day 1 (D1) mice were injected with vehicle alone or containing LPS and animals were killed 2 h after treatment; (b) mice were injected on D1 and D2 with LPS and on D3 were injected with vehicle alone (LPS2-Veh) or containing LPS (LPS2-LPS), animals were then killed 2 h after the last treatment, and (c) mice were treated on D1, D2, D3 and D4 with LPS and on D5 were injected with either vehicle alone (LPS4-Veh) or containing LPS (LPS4-LPS) and killed 2 h after injection (for details see table 1). In the control group, Sham mice were injected every day with vehicle only.

Figure 5 shows the decrease in body weight of the mice under repeated LPS treatment. Mean body weights were significantly decreased on day 2 of the

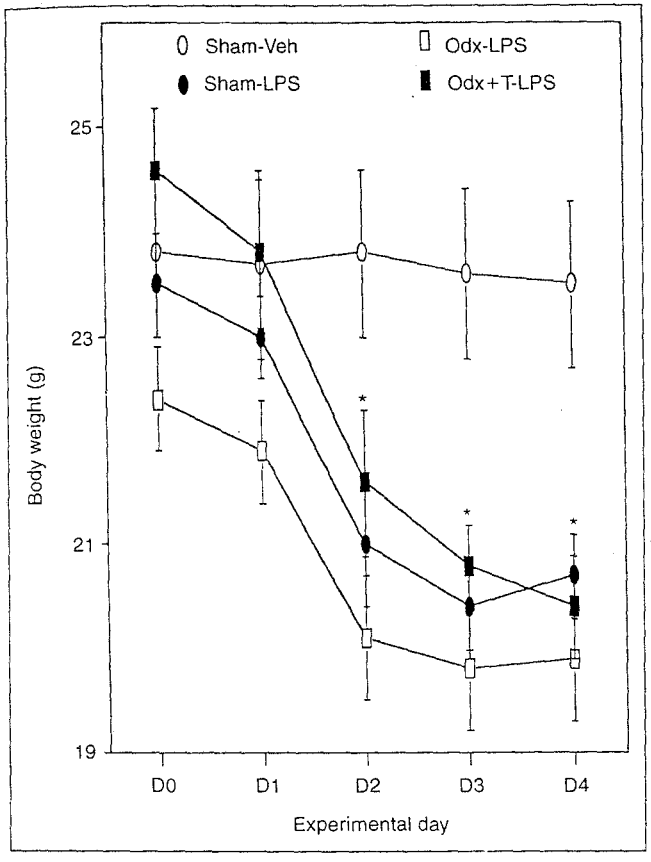

Fig. 5. Effects of i.p. injections of vehicle (Veh) or LPS (2 mg/kg) on the body weight of different groups of mice. Values registered 16 h before killing. Sham-Veh mice were killed on D5. Mean ± SEM (n = 6–11 animals per group). *p < 0.01 vs. Sham-Veh on same day.

experiment and remained low until the end of the experiment. The loss of body weight was similar in all groups (Sham, Odx and Odx+T mice). Figure 6a shows plasma ACTH levels in the different groups of mice. Animals killed 2 h after a vehicle injection without previous (Veh, D1) and with previous endotoxin treatments (LPS2-Veh, D3; LPS4-Veh, D5; for more details on the experimental design see table 1) showed plasma ACTH values within the non-stress range (10–70 pg/ml). These results indicate that previous treatments (either 2 or 4) with LPS did not modify the basal plasma ACTH levels measured 24 h after the last endotoxin administration. Uncastrated mice treated with LPS,

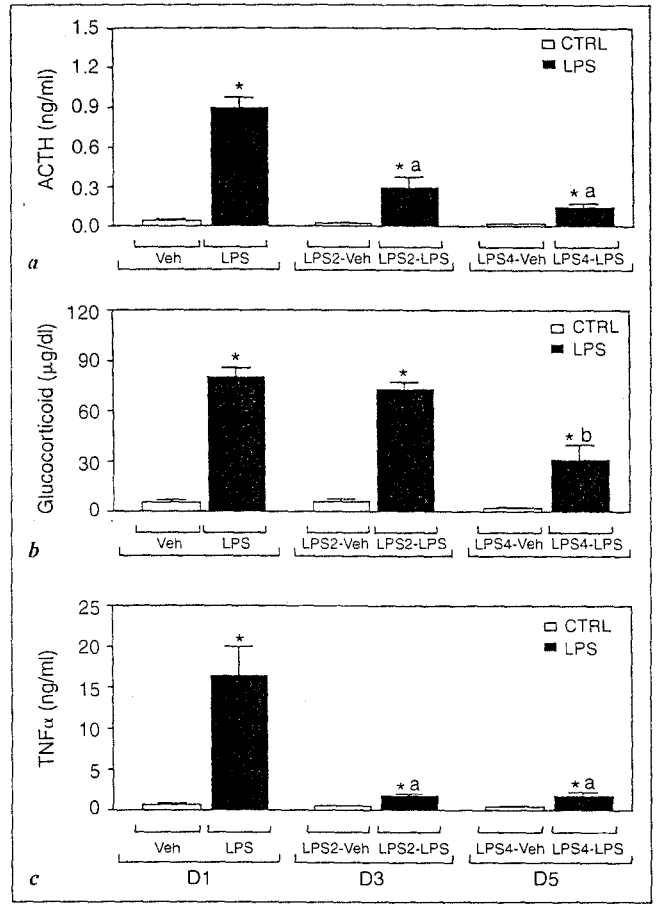

Fig. 6. Effects of single and repeated LPS (2 mg/kg) administration on plasma ACTH (*a*), glucocorticoid (*b*) and cytokine (*c*) levels 2 h after the last treatment with either Veh or LPS in sham male mice. Mean ± SEM (n = 6–11 animals per group). *p < 0.02 vs. the respective Veh; [a]p < 0.05 vs. LPS (D1); [b]p < 0.05 vs. LPS (D1) and LPS2-LPS (D3).

in single (LPS, D1) or repeated (LPS2-LPS, D3 and LPS4-LPS, D5) doses, had significantly higher plasma ACTH levels (2 h after the last endotoxin administration) than those observed in control groups (killed 2 h after vehicle injection on D1, D3 and D5, respectively). However, the LPS-induced plasma ACTH secretion on D1 (2 h after one injection) was significantly higher than values found 2 h after the third (D3) and fifth (D5) LPS injections (fig. 6a) demonstrating the occurrence of a kind of tolerance to repeated LPS administration.

Similar to the ACTH values, plasma B levels in control groups (those killed 2 h after a last administration of vehicle with or without previous LPS treatment) were also within the non-stress range (1–10 μg/dl; fig. 6b). Two hours after the last LPS administration a significant increase over control values in adrenal B release took place, regardless of the day of treatment. However, after repeated LPS administration, the endotoxin-elicited B secretion decreased; it was significantly lower 2 h after the fifth (D5) than after both the first (D1) and the third (D3) LPS administrations (fig. 6b). In this paradigm, plasma TNFα values also show a tolerance in response to repeated LPS administration, similar to the one described for plasma ACTH levels (fig. 6c). Finally, while on D1 of treatment there is a high correlation between plasma ACTH ($r = 0.8$) or B ($r = 0.7$) and TNFα levels after LPS administration, this correlation was significantly lower on D3 and D5 of treatment ($r = 0.3$ for ACTH and $r = 0.4$ for B). This observation clearly suggests that the activation of the HPA axis function under repeated LPS treatment is independent of TNFα secretion in plasma and that other cytokines released after LPS administration could be responsible for the HPA axis response.

Although hypothalamic CRH and pituitary ACTH remained unchanged after single or repeated LPS injection, compared to control animals, adrenal B content was significantly enhanced 2 h after the first, third and fifth endotoxin injections (fig. 7). To determine whether sex steroids could influence the HPA axis and immune functions in our design, we studied these responses in 7-day-orchidectomized mice substituted (Odx+T) or not (Odx) with testosterone propionate. As it can be seen in figure 8a, Odx and Odx+T had no significant effect on plasma ACTH levels in controls (animals killed 2 h after vehicle administration on D1, D3 and D5, respectively). However, Odx was able to significantly enhance the LPS-elicited ACTH secretion after the first LPS injection, and this effect was only partially prevented by testosterone therapy (Odx+T). Surprisingly, Odx and Odx+T had no such effects after the third and fifth LPS administration. Although in the paradigm using 7-day Gnx animals, Odx had no effect on other parameters such as hypothalamic CRH content, pituitary ACTH content, plasma corticosterone and TNFα levels, it enhanced the LPS-induced increase in adrenal B content (fig. 8b). Indeed, the LPS effect was significantly enhanced by orchidectomy 2 h after the first (LPS, D1), third (LPS2-LPS, D3) and fifth (LPS4-LPS, D5) endotoxin administrations compared to the respective sham-operated animals (treated with LPS in a similar fashion). Furthermore, testosterone replacement therapy (Odx+T) completely abolished this effect induced by orchidectomy, regardless of the experimental day, suggesting again an inhibitory effect of the androgens on adrenal glucocorticoid synthesis under endotoxemia (fig. 8b).

This observation tallies with data showing a modulatory effect of sex steroid hormones on glucocorticoid metabolism under other stress conditions

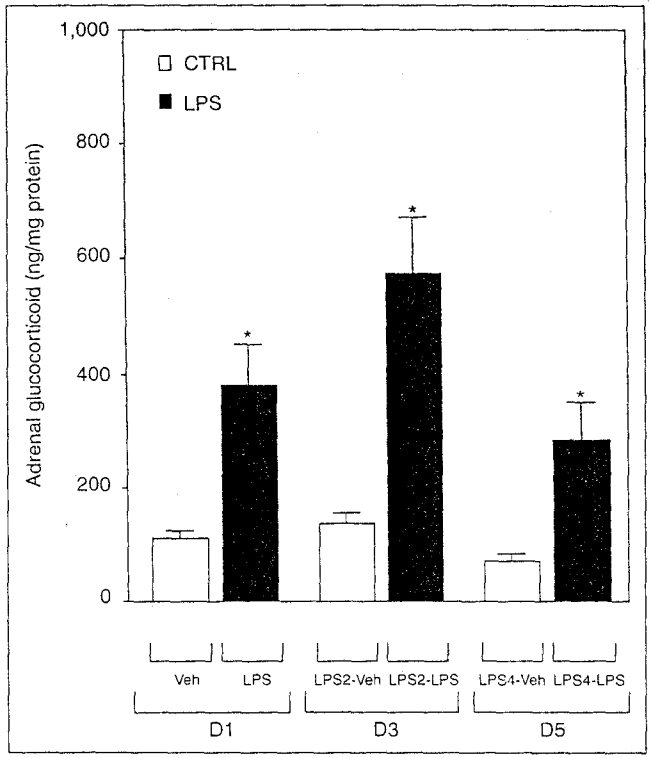

Fig. 7. Effects of single and repeated LPS (2 mg/kg) administration on adrena glucocorticoid content 2 h after the last treatment with either vehicle (Veh) or LPS in shan male mice. Mean ± SEM (n = 6–11 animals per group). *$p < 0.01$ vs. the respective Veh.

[31, 32]. Thus, our results indicate a clear inhibitory effect of the androgen o adrenal glucocorticoidogenesis.

Transient hypogonadism has been described in severely ill patients and suc an effect has been attributed to an increased testosterone to estradiol conversio due to an endotoxin-enhanced aromatase activity [33], thus, a decrease in peripl eral androgen concentration may represent a body's defense mechanism for su vival after injury. These observations are in agreement with the data reported i this chapter regarding the enhanced HPA axis response seen in orchidectomize mice during the acute phase of endotoxic shock. However, the mechanism(involved in the lack of a modulatory effect of testosterone on the HPA ax response under repeated LPS administration remains to be determined.

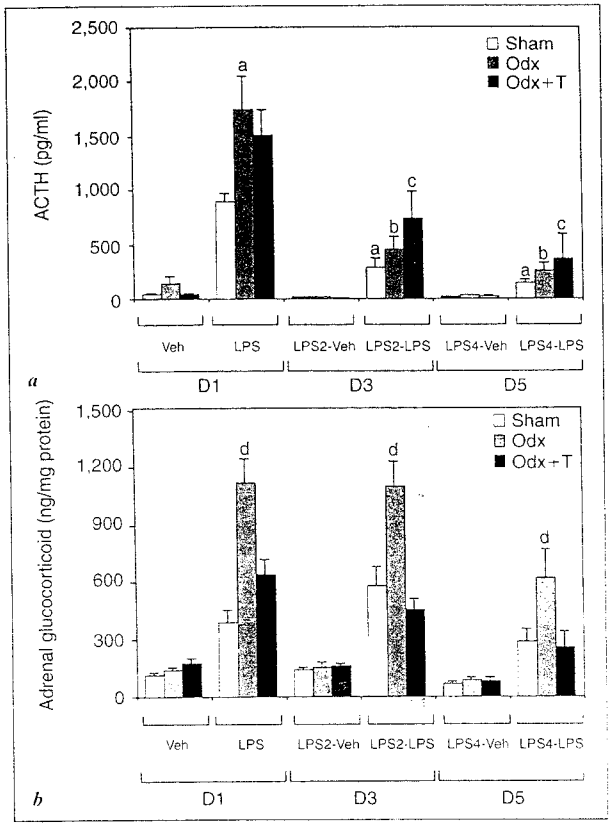

Fig. 8. Effects of orchidectomy alone or combined with testosterone therapy on plasma ACTH levels (*a*) and adrenal gland glucocorticoid content (*b*) 2 h after the last vehicle (Veh) or LPS (2 mg/kg) injection. Mean ± SEM (n = 6–11 animals per group). LPS values on D1, D3 and D5 were significantly ($p < 0.02$) higher vs. respective controls. [a]$p < 0.05$ vs. LPS D1 shams; [b]$p < 0.05$ vs. LPS D1 Odx; [c]$p < 0.05$ vs. LPS D1 Odx+T; [d]$p < 0.05$ LPS shams and Odx+T on same day.

Conclusion

Acute inflammation represents a threat to the integrity of the organism, which requires metabolic changes such as increased secretion of glucocorticoids [24] for survival after injury. The results presented indicate that sex hormones play an important modulatory role in the HPA axis response after inflammation and further suggest that these molecules blunt the effect of inflammation. In conclusion, in addition to gender difference [34], all the data strongly support a sex steroid basis for neuroendocrine-immunological sexual dimorphism. Because inflammation and other purely neuroendocrine stressors probably stimulate different subtypes of hypothalamic CRH neurosecretory terminals or CRH receptors, it could be extrapolated that these neuronal subpopulations undergo a different process of maturation during development and that such sex-related characteristics persist up to middle age. The sexual dimorphism in the response of the HPA axis to immune signals may represent an important factor in the understanding of reciprocal interactions between the two systems in physiological and pathological conditions.

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