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Lipopolysaccharide- and Tumor Necrosis Factor-α-Induced Changes in Prolactin Secretion and Dopaminergic Activity in the Hypothalamic-Pituitary Axis

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Key Words

Dopamine · Hypothalamus · Bacterial lipopolysaccharide · Pituitary · Prolactin · Tumor necrosis factor-α

Abstract

Bacterial lipopolysaccharide (LPS) affects pituitary hormone secretion, including prolactin release, by inducing synthesis and release of cytokines such as tumor necrosis factor-α (TNF-α). Since prolactin is mainly under tonic inhibitory control of dopamine, we investigated the effect of LPS and TNF-α on the hypothalamic-pituitary dopaminergic system. LPS (100–250 μg/rat, i.p.) decreased serum prolactin levels after 1 or 3 h. Sulpiride, a dopaminergic antagonist, increased serum prolactin and blocked the inhibitory effect of LPS. LPS increased hypothalamic dopamine and DOPAC concentrations and the DOPAC/dopamine ratio both in mediobasal hypothalamus and the posterior pituitary. LPS also enhanced dopamine and DOPAC concentration in the anterior pituitary. LPS elevated plasma levels of epinephrine, norepinephrine and dopamine but it did not modify the concentration of epinephrine or norepinephrine in the tissues studied. The administration of TNF-α (i.c.v., 1 h, 100 ng/rat) decreased serum prolactin but did not affect plasma catecholamine levels. TNF-α did not modify the DOPAC/dopamine ratio in hypothalamus or posterior pituitary but increased dopamine and DOPAC concentrations in the anterior pituitary. Incubations of hypothalamic explants showed that TNF-α did not modify in vitro basal dopamine release and reduced K^+-evoked dopamine release. On the contrary, incubations of posterior pituitaries showed that TNF-α significantly increased basal and K^+-evoked dopamine release. These results indicate that LPS and TNF-α increase dopamine turnover in the hypothalamic-pituitary axis. This increase in dopaminergic activity could mediate the inhibitory effect of LPS and TNF-α on prolactin release. Furthermore, the increase in dopaminergic activity elicited by LPS could be mediated by an increase in hypothalamic TNF-α during endotoxemia.

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Introduction

Administration of bacterial lipopolysaccharide (LPS) has long been known to activate the hypothalamic-pituitary-adrenocortical (HPA) axis [1, 2]. LPS induces the synthesis and release of cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor-α (TNF-α), which can also activate the HPA axis and therefore contribute to the activation by LPS [2, 3]. The secretion of cytokines is not restricted to injury, inflammation or infection since recent studies have indicated that IL-1 and IL-6 synthesis is altered during acute physical or psychological stress [2]. Previous studies have demonstrated the existence of stressor-specific circuits and the involvement of individual brain regions coordinating neuroendocrine responses to different stress factors [4].

The administration of LPS appears to increase the activity of some central neurotransmitters with some differences regarding the route of administration. The intraperitoneal administration of LPS has been shown to increase hypothalamic tryptophan concentration and the ratios of the serotonin (5-HT) metabolite, 5-hydroxyindoleacetic acid (5-HIAA)/5-HT and 3-methoxy-4-hydroxyphenylglycol (MHPG)/norepinephrine (NE), however with marked differences in time courses. Elevations in hypothalamic dihydroxyphenylacetic acid (DOPAC)/dopamine (DA) ratios were also reported, but they were smaller and less consistent [5]. Intracerebroventricular administration of LPS elicited similar neurochemical changes except for changes in DOPAC/DA ratios. This lack of dopaminergic response to LPS administered by this route suggested that other factors, such as cytokines secreted within the brain, were involved in neurochemical responses to LPS. The same authors reported that intraperitoneal administration of LPS elevated in vivo dialysate concentrations of NE and DA and their catabolites in the medial hypothalamus [6].

Although LPS and IL-1 have similar stimulatory effects on NE metabolism, an IL-1 receptor antagonist failed to block HPA and neurochemical changes induced by intraperitoneal administration of LPS, suggesting that IL-1 is not the sole mediator of the neurochemical responses to LPS and that it may contribute in only a minor way to the slower effects of LPS on the HPA axis [7]. However, other reports showed that antagonists of IL-1 or its receptors prevented neurochemical or HPA responses to LPS [8–10].

It has been reported that a constant infusion of IL-6 induced Fos expression in the parvocellular neurons of the paraventricular nucleus, indicating that circulating IL-6 can influence paraventricular nucleus activity [11]. In fact, IL-6 increases brain tryptophan and 5-HIAA concentrations [12], whereas it only produces either small [13] or no increases in hypothalamic NE turnover [14]. An IL-6 antibody attenuated the HPA response to LPS as well as the increased tryptophan and 5-HIAA/5-HT ratio, suggesting that IL-6 contributes to the HPA and indoleaminergic responses to LPS [15]. However, in knockout mice lacking genes for IL-1 [16] and for IL-6 [17], the HPA responses to LPS were not impaired.

TNF-α is considered one of the major mediators of endotoxic shock. TNF-α has been reported to stimulate corticotropin-releasing hormone release and to potentiate IL-1-induced release of arginine-vasopressin [2]. TNF-α injected intraperitoneally or intravenously into mice increased cerebral tryptophan concentration and the ratio of MHPG/NE in the hypothalamus, suggesting that TNF-α may contribute to the HPA, neurochemical and behavioral responses to LPS and other stimulators [18]. However, no statistically significant changes were reported in the concentration of NE, dopamine or their metabolites [18]. Also, pretreatment with an antibody to mouse TNF-α failed to modify the neurochemical and neuroendocrine responses to endotoxin [19]. Others, however, have reported inhibitory effects of TNF-α on the evoked NE release from the median eminence [20].

Prolactin plays a significant role in the regulation of the humoral and cellular immune responses in physiological as well as pathological states. Prolactin secretion is affected by stress although the prolactin-secretory response differs depending on the nature of the stressor and the time after the application of the stressor [21]. The net effect of cytokines seems to be inhibition of prolactin secretion [22]. DA is the principal hypothalamic neurohormone that tonically inhibits prolactin secretion [21]. Therefore, it is possible that DA may be implicated in the infection-induced effects on prolactin secretion. Tuberoinfundibular dopaminergic (TIDA) neurons project to the external zone of the median eminence, where DA is released to the long portal vessels, gaining access to the anterior pituitary. Also, DA can be released from the periventricular hypophyseal (PHDA) neurons and the tuberohypophyseal dopaminergic (THDA) neurons into the short portal vessels. In addition, DA can affect prolactin secretion by acting centrally or within the neurointermediate lobe. DA released from the nerve terminals of the PHDA neurons projecting into the intermediate lobe tonically inhibits the secretion of α-melanocyte-stimulating hormone from melanotrophs. DA released from the neuroterminals of THDA neurons pro-

jecting into the neural lobe has been shown to inhibit oxytocin release [21].

Considering that prolactin plays an important role in the maintenance of homeostasis, we investigated the involvement of catecholaminergic neurons in the control of prolactin secretion in an acute model of infection of animals receiving LPS. We also studied the effect of central administration of TNF-α on serum prolactin levels and the dopaminergic activity in the hypothalamus and pituitary.

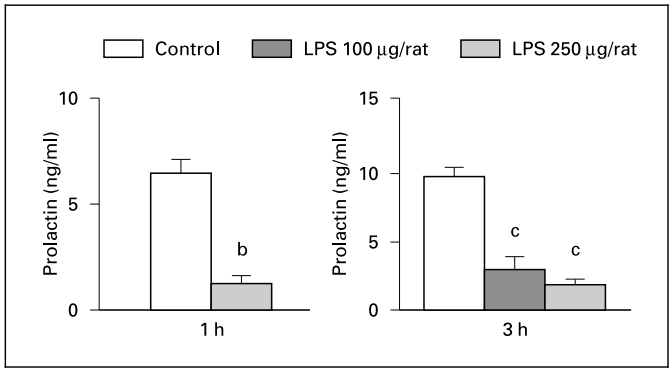

Fig. 1. Effect of LPS administration on serum prolactin levels. Values represent means ± SEM of 6–10 determinations per group. Data were evaluated by Student's t test or ANOVA followed by Dunnett's test. [b] $p < 0.01$, [c] $p < 0.001$, vs. control.

Material and Methods

Animals

Male Wistar rats weighing 200–250 g were used. The animals were fed laboratory chow and water ad libitum and kept under controlled conditions of light (12 h light/dark) and temperature (20–25°C). The animals were treated according to the NIH Guide for the Care and Use of Laboratory Animals.

Materials

All drugs, including bacterial LPS (*Escherichia coli* serotype 0111:B8), were purchased from Sigma (St. Louis, Mo., USA), except recombinant hTNF-α (Promega, Wisc., USA) and those indicated above.

Experimental Protocols

In vivo Experiments. Animals were injected intraperitoneally with LPS dissolved in pyrogen-free isotonic saline at a dose of 100 or 250 µg/rat and sacrificed by decapitation 1 or 3 h later. In some experiments, rats received an intraperitoneal injection of sulpiride sulfate (10 µg/rat) in isotonic saline or vehicle 20 min before sacrifice. For experiments involving intracerebroventricular administration of TNF-α, rats were anesthetized with tribromoethanol (35 mg/100 g body weight), implanted stereotactically (coordinates: A-P 0.6 mm, L –2 mm, D-V –3.2 mm) with steel cannulas in the lateral ventricle and placed in individual cages, 7 days before the experiment. On the day of the experiment, rats were injected through the cannula with TNF-α (100 ng/rat dissolved in 10 µl PBS) or vehicle. All animals were sacrificed by decapitation and trunk blood was collected either in empty tubes for serum prolactin determination or in tubes containing a drop of 342 m*M* sodium and potassium EDTA salts for plasma prolactin and catecholamine measurement. Serum and plasma were separated by centrifugation. After sacrifice, both the anterior and posterior pituitary glands as well as the brain were removed. A hypothalamic fragment that included the arcuate and periventricular nuclei and the median eminence was dissected by making a frontal cut just behind the optic chiasm extending dorsally 1.0 mm. A horizontal cut extended from this point caudally to just behind the pituitary stalk, where another frontal cut was made. Longitudinal cuts were made 1 mm lateral to the midline bilaterally. The tissues were immediately frozen on dry ice and stored at –70°C until catecholamine determination.

Incubation of Hypothalamic Explants and Posterior Pituitary

Two hypothalamic fragments or three posterior pituitary glands from nontreated rats were preincubated for 15 min in a Dubnoff shaker (60 cycles/min) at 37°C in an atmosphere of 95% O_2-5% CO_2 in 0.5 ml of Krebs-Ringer bicarbonate buffer (KRB; 118.46 m*M* NaCl, 5 m*M* KCl, 2.5 m*M* $CaCl_2$, 1.18 m*M* NaH_2PO_4, 1.18 m*M* $MgSO_4$, 24.88 m*M* $NaHCO_3$, pH 7.4) containing 10 µ*M* tyrosine, 10 m*M* glucose, 10 m*M* Hepes, 1 m*M* ascorbic acid, 0.1 m*M* bacitracin and 0.1% bovine serum albumin. Then, the medium was replaced with fresh KRB containing TNF-α (50 ng/ml) and the tissues were incubated for 60 min. After removal of medium (basal release), the tissues were incubated further for 30 min in KRB containing 40 m*M* K^+, balanced by reducing Na^+ concentration (K^+-evoked release), and TNF-α. At the end of each incubation period, the media were removed and collected in 0.1 *M* $HClO_4$ containing 0.65 m*M* $Na_2S_2O_5$, centrifuged at 26,000 *g* for 10 min and frozen on dry ice. Supernatants and tissues were stored at –70°C for no longer than 1 week.

Catecholamine Determination

Catecholamines were extracted from incubation media and plasma samples with 2 *M* Tris HCl, pH 8.7, with 3,4-dihydroxybenzylamine hydrobromide (DHBA, 100 ng/ml) as internal standard and dehydrated alumina. After shaking for 10 min, samples were centrifuged at 19,000 *g* for 10 min. Pellets were washed 3 times with deionized water, centrifuged and 200 µl of 0.1 *M* H_3PO_4 was added. After shaking for 2 min, samples were centrifuged. Tissues were sonicated in 0.2 *M* $HClO_4$ containing 0.65 m*M* $Na_2S_2O_5$, 0.05% EDTA and 100 ng/ml DHBA. Aliquots of the homogenates were collected to determine protein concentration by the method of Lowry et al. [23] and the samples were centrifuged at 29,000 *g* for 15 min. Supernatants from plasma, media and tissue extracts were filtered and injected in an analytical column (Luna 5 µ C-18, 4.6 × 250 mm, Phenomenex) maintained at 37°C. Catecholamines were determined by high performance liquid chromatography with electrochemical detection and registered with an integrator. The mobile phase was prepared with 100 m*M* NaH_2PO_4, 1 m*M* heptanesulfonic acid, 0.5 m*M* EDTA and 6% acetonitrile (Baker), pH 3.0. Quantification and recovery calculation was performed using the Gilson 712 System Controller Software. The final concentration of catecholamines in media and tissues was expressed as ng/mg protein.

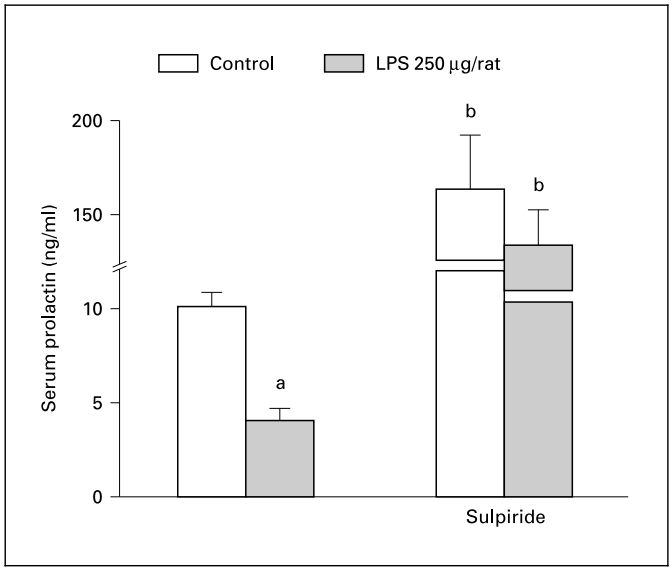

Fig. 2. Effect of LPS administration on serum prolactin levels 3 h after injection with or without intraperitoneal injection of sulpiride sulfate (10 μg/rat) 20 min before decapitation. Values represent means ± SEM of 8 determinations per group. Data were evaluated by two-way ANOVA. [a] $p < 0.01$ vs. respective control without LPS. [b] $p < 0.01$ vs. respective control without sulpiride.

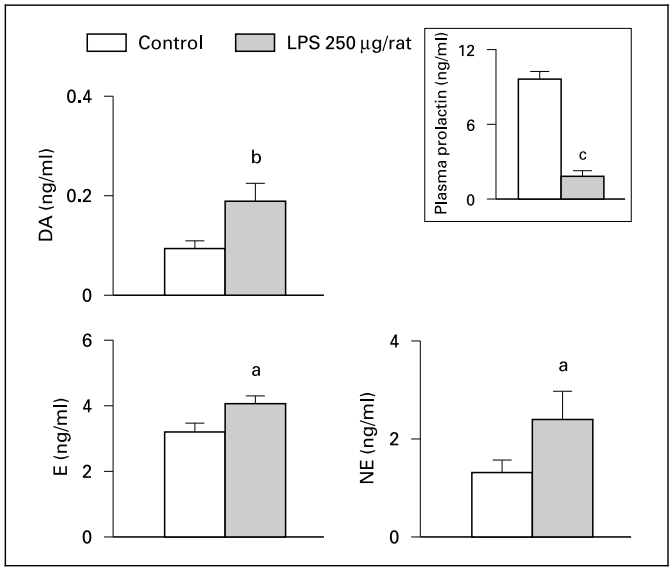

Fig. 3. Effect of LPS administration (i.p., 250 μg/rat, 3 h) on plasma levels of epinephrine (E), NE and DA. Insert shows plasma prolactin levels. Values represent means ± SEM of 8 determinations per group. Data were evaluated by Student's t test. [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$, vs. control.

Prolactin Determination

Prolactin was measured by a double antibody radioimmunoassay utilizing the RP3 reference preparation and the anti-rPRL-S-9 serum provided by the National Hormone and Pituitary Program (Torrance, Calif., USA). The intra- and interassay coefficients of variation were both less than 9%.

Statistics

The results were expressed as means ± SEM. The significance of the differences between means was determined by Student's t test or one-way analysis of variance (ANOVA) followed by Dunnett's test or by a two-way ANOVA with interaction terms. Differences were considered significant when $p < 0.05$. All experiments were performed at least twice. Figures represent results of individual experiments.

Results

Effect of LPS Administration on Serum Prolactin Levels

Serum prolactin levels were significantly decreased 1 h after the intraperitoneal administration of LPS (250 μg/rat) and remained significantly decreased 3 h after injection (100 and 250 μg/rat; fig. 1).

Sulpiride sulfate (10 μg/rat, i.p.) injected 20 min before sacrifice increased serum prolactin levels in both control and LPS-treated (250 μg/rat) animals. In the presence of this dopaminergic antagonist, the inhibitory effect of LPS on serum prolactin levels was not observed (fig. 2).

Effect of LPS Administration on Plasma Catecholamine Levels

Three hours after LPS administration (250 μg/rat), plasma levels of epinephrine, NE and DA were significantly increased (fig. 3).

Effect of LPS Administration on Catecholamine Concentration in Hypothalamic and Pituitary Tissues

In the hypothalamic fragments, DA and DOPAC concentrations were significantly increased 3 h after LPS administration (250 μg/rat). The DOPAC/DA ratio was also significantly increased. However, LPS did not modify the concentrations of epinephrine or NE content (fig. 4).

In the posterior pituitary, LPS did not significantly affect DA or DOPAC concentrations although the DOPAC/DA ratio was increased. Also, no differences were observed in epinephrine or NE concentrations after the injection of LPS (fig. 5). In the anterior pituitary, LPS administration significantly increased DA and DOPAC concentrations (fig. 6).

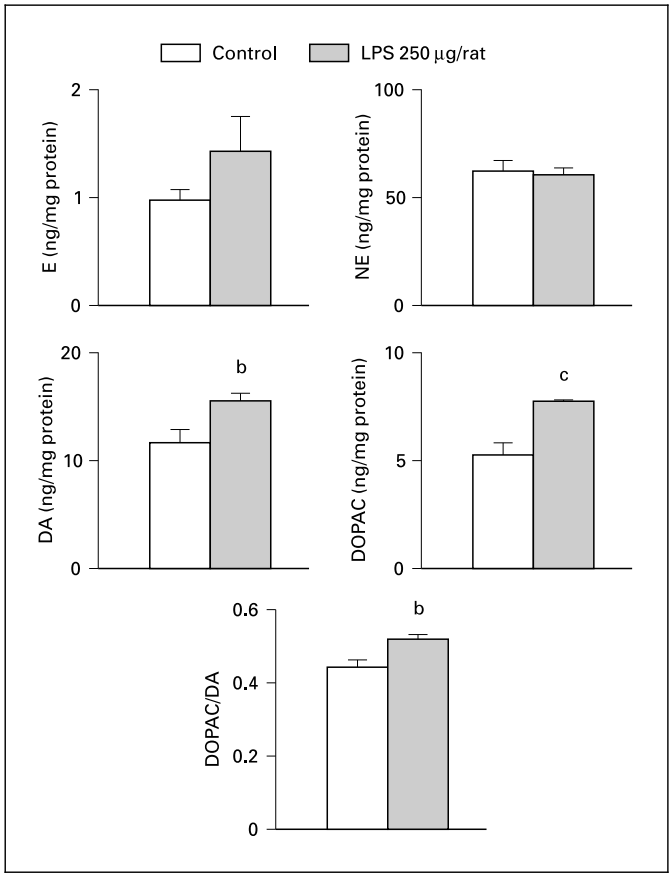

Fig. 4. Effect of LPS administration (i.p., 250 μg/rat, 3 h) on epinephrine (E), NE, DA and DOPAC hypothalamic concentrations and DOPAC/DA ratio. Values represent means ± SEM of 7–10 determinations per group. Data were evaluated by Student's t test. [b] $p < 0.01$, [c] $p < 0.001$, vs. control.

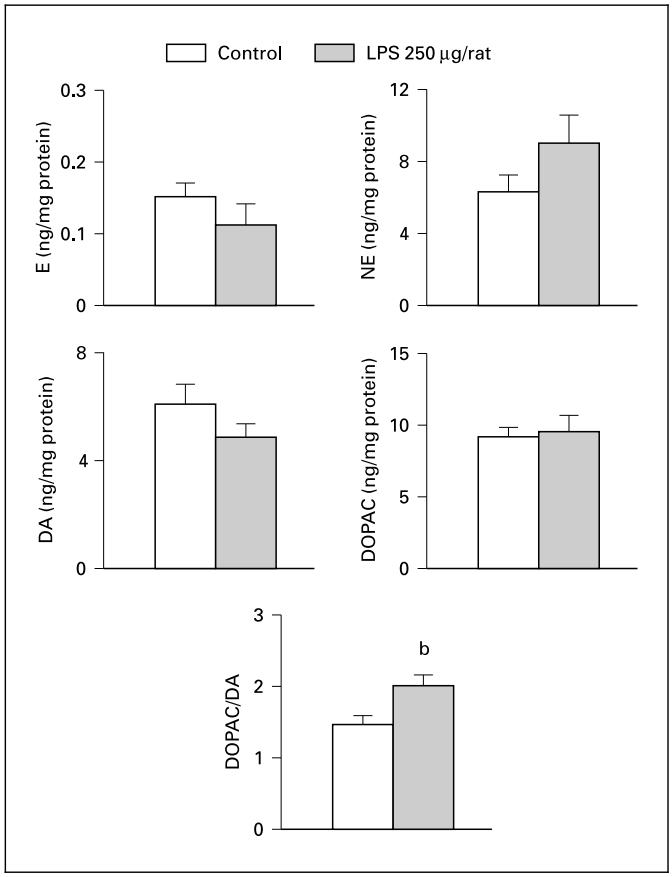

Fig. 5. Effect of LPS administration (i.p., 250 μg/rat, 3 h) on epinephrine (E), NE, DA and DOPAC concentrations and DOPAC/DA ratio in the posterior pituitary. Values represent means ± SEM of 6–8 determinations per group. Data were evaluated by Student's t test. [b] $p < 0.01$ vs. control.

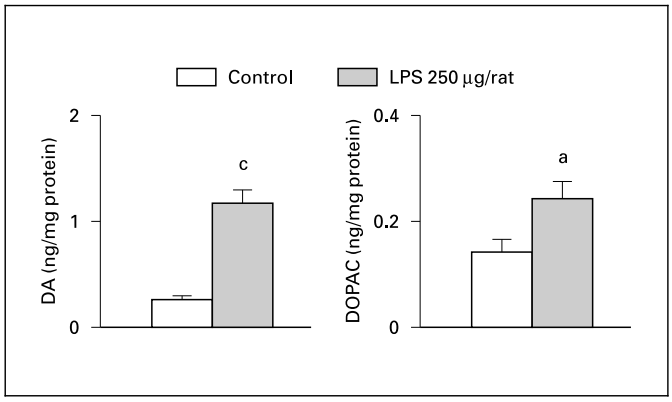

Fig. 6. Effect of LPS administration (i.p., 250 μg/rat, 3 h) on DA and DOPAC concentrations in the anterior pituitary. Values represent means ± SEM of 6–8 determinations per group. Data were evaluated by Student's t test. [a] $p < 0.05$, [c] $p < 0.001$, vs. control.

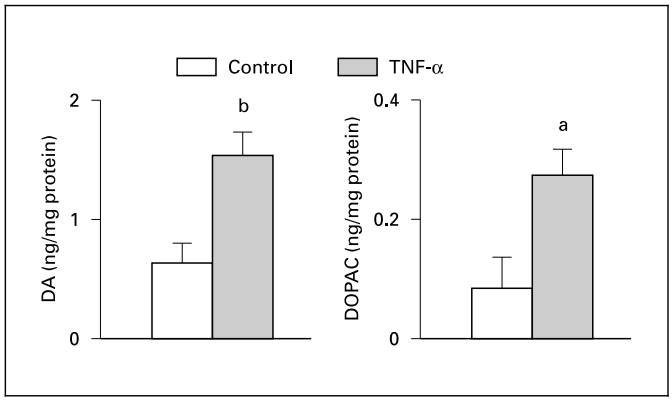

Fig. 7. Effect of TNF-α administration (i.c.v., 100 ng/rat, 1 h) on DA and DOPAC concentrations in the anterior pituitary. Values represent means ± SEM of 8 determinations per group. Data were evaluated by Student's t test. [a] $p < 0.05$, [b] $p < 0.01$, vs. control.

Effect of TNF-α Administration on Plasma Prolactin and Catecholamine Levels

Plasma prolactin levels were significantly lower 1 h after the intracerebroventricular administration of TNF-α (100 ng/rat) when compared with control animals injected with the same volume of PBS (control: 4.20 ± 0.30 ng/ml; TNF-α: 1.78 ± 0.10, n = 8, $p < 0.01$). Central TNF-α administration did not modify plasma catecholamine levels (data not shown).

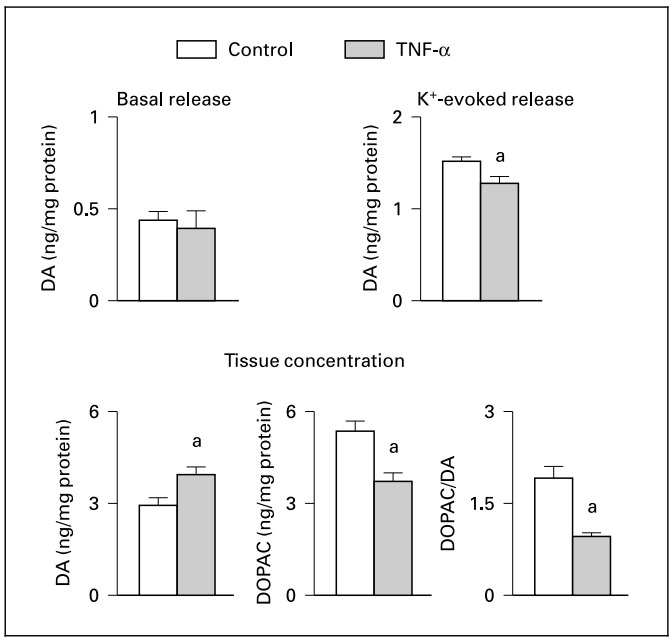

Fig. 8. Effect of TNF-α (50 ng/ml) on basal and K^+-evoked DA release from hypothalamic explants and DA and DOPAC tissue concentration. Values represent means ± SEM of 6–7 determinations per group. Data were evaluated by Student's t test. [a] $p < 0.05$ vs. control.

Effect of TNF-α Administration on Catecholamine Concentrations in Hypothalamic Explants and Pituitaries

The concentrations of catecholamines in hypothalamic fragments or posterior pituitary glands were not significantly modified when measured 1 h after intracerebroventricular administration of TNF-α (100 ng/rat). However, TNF-α decreased DOPAC concentrations in the posterior pituitary, without significantly affecting the DOPAC/DA ratio (table 1). On the contrary, TNF-α significantly increased DA and DOPAC concentrations in the anterior pituitary (fig. 7).

In vitro Effect of TNF-α on Catecholamine Release from Hypothalamic Explants and Posterior Pituitary Glands

The presence of TNF-α in the incubation medium reduced K^+-evoked DA release from hypothalamic explants although it did not affect its basal release. TNF-α increased the DA content in the remaining tissue whereas it decreased DOPAC concentration and the DOPAC/DA ratio (fig. 8). TNF-α neither significantly modified hypothalamic epinephrine nor NE release nor its tissue concentration (data not shown).

Conversely, TNF-α significantly increased basal and K^+-evoked DA release from posterior pituitaries incubated in vitro (fig. 9). Although TNF-α did not affect DA concentrations in this tissue, it significantly increased DOPAC concentrations and the DOPAC/DA ratio. Also, TNF-α stimulated basal and K^+-evoked epinephrine release and basal NE release (table 2).

Table 1. Effect of TNF-α administration (i.c.v., 100 ng/rat, 1 h) on epinephrine (E), NE, DA and DOPAC concentrations (ng/mg protein) and DOPAC/DA ratio in the hypothalamus and posterior pituitary

	Hypothalamic explants		Posterior pituitary	
	control	TNF-α	control	TNF-α
E	0.30 ± 0.04	0.37 ± 0.03	0.23 ± 0.05	0.23 ± 0.02
NE	63.68 ± 2.34	63.22 ± 1.58	6.39 ± 0.59	7.52 ± 0.98
DA	15.34 ± 0.27	15.32 ± 0.74	4.40 ± 1.13	4.23 ± 1.28
DOPAC	3.07 ± 0.14	3.12 ± 0.25	6.12 ± 0.78	4.26 ± 0.50*
DOPAC/DA	0.38 ± 0.02	0.42 ± 0.04	1.39 ± 0.27	1.00 ± 0.32

Values represent means ± SEM of 7–8 determinations per group. Data were evaluated by Student's t test. * $p < 0.05$.

Discussion

Hyperprolactinemia has been observed in many cases of infectious or inflammatory diseases [24, 25]. However, contradictory results in serum prolactin levels have been obtained after LPS or cytokine administration in different experimental models in rodents [22, 26, 27]. Also, cytokines have shown either an increase or a decrease in the in vitro release of prolactin from anterior pituitaries [1, 28–30]. It has been shown recently that T cell-dependent immune responses require an early activation of TRH and prolactin. The ensuing neuroendocrine modifications deeply differ from those occurring in inflammatory or T cell-independent responses such as those produced by LPS [31]. We have shown that the intraperitoneal administration of LPS caused decreases in serum prolactin levels 1 or 3 h after injection. An increase in prolactin 30 min after the intravenous administration of a similar dose of LPS to male Sprague-Dawley rats has been reported previously [26]. In the present experimental conditions, LPS decreased prolactin secretion 1 h after its administration and its effect lasted for at least 3 h. These conflicting responses are likely due to differences in the experimental design between the studies. Different strains of rats were used, the routes of LPS administration differed and different time courses were studied. Furthermore, in one study, the rats were implanted with a jugular catheter 24 h prior to the experiment which could have modified the response due to a priming effect of a previous surgical procedure. Also, it has been reported that the intraperitoneal administration of LPS stimulates peritoneal macrophages which quickly synthesize and release large amounts of several cytokines [32].

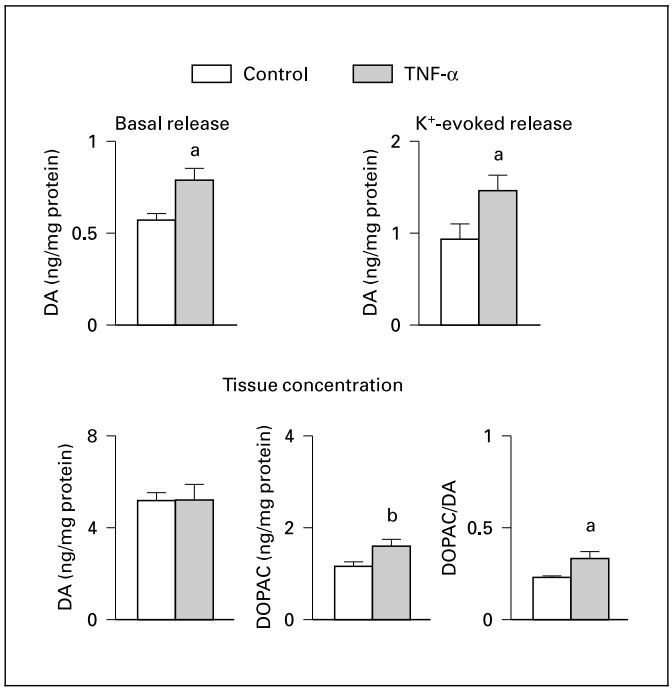

Fig. 9. Effect of TNF-α (50 ng/ml) on basal and K^+-evoked DA release from posterior pituitary and DA and DOPAC tissue concentration. Values represent means ± SEM of 6–7 determinations per group. Data were evaluated by Student's t test. [a] $p < 0.05$, [b] $p < 0.01$, vs. control.

The inhibitory effect of LPS on prolactin secretion was not observed when D_2 dopaminergic receptors were blocked by sulpiride, a dopaminergic antagonist. The lack of effect of LPS in the presence of sulpiride suggests the involvement of dopaminergic activity in the decrease of serum prolactin induced by LPS. In fact, we observed that LPS increased hypothalamic DA and DOPAC concentrations and the DOPAC/DA ratio, considered as a good index of dopaminergic-neuronal activity. These results agree with previous evidence indicating an increased DA concentration in the arcuate nucleus after intraperitoneal administration of LPS [8]. The DOPAC/DA ratio was also enhanced in the posterior pituitary by LPS administration. Our data suggest that by activating TIDA, THDA

Table 2. Effect of TNF-α (50 ng/ml) on basal and K^+-evoked release of epinephrine (E) and NE from the posterior pituitary

	E, ng/mg protein		NE, ng/mg protein	
	control	TNF-α	control	TNF-α
Basal release	0.16 ± 0.01	0.29 ± 0.02***	2.69 ± 0.11	3.25 ± 0.17*
K^+-evoked release	0.10 ± 0.01	0.17 ± 0.01**	2.96 ± 0.29	3.48 ± 0.29

Values represent means ± SEM of 6–7 determinations per group. Data were evaluated by Student's t test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, vs. control.

and/or PHDA neurons, LPS may stimulate the release of DA into both the long and short portal vessels, thus increasing the amount of DA reaching the anterior pituitary. After interacting with its specific receptors on the lactotrophs, DA is internalized and incorporated into the secretory granules [33]. In the lactotrophs, DA can be oxidized to DOPAC by the enzyme monoaminooxidase [34]. The increase in DA and DOPAC concentrations in the anterior pituitary induced by LPS reflects an enhanced supply of DA to the lactotroph.

The intracerebroventricular injection of TNF-α also significantly lowered plasma prolactin levels whereas it increased DA and DOPAC concentrations in the anterior pituitary suggesting that this cytokine may inhibit prolactin secretion by stimulating dopaminergic activity. Central administration of TNF-α did not modify hypothalamic concentrations of catecholamines or DOPAC. However, TNF-α decreased DOPAC concentration in the posterior pituitary without affecting the DOPAC/DA ratio. It has been demonstrated that the blockade of DA transporters inhibits the reuptake of DA and therefore increases DA concentration in the perivascular space and its diffusion to the portal vessels [21, 35]. The present results suggest that TNF-α may increase DA release from and/or decrease DA reuptake in this tissue. In fact, the in vitro presence of TNF-α significantly stimulated the release of DA from the posterior pituitary. These data suggest that this cytokine may increase DA concentration in the anterior pituitary by affecting the release of DA from dopaminergic neurons projecting to the neurointermediate lobe.

The central production of cytokines has been proposed to be involved in the induction and/or maintenance of neurological manifestations observed during peripheral LPS administration [1–3, 26, 36]. It has been suggested that the ability of peripherally administered LPS to produce an upregulation of brain cytokines such as IL-1 and TNF-α indicates the presence of humoral mechanisms in the periphery that are able to signal the brain and modify the cytokine synthesis within specific brain regions [36]. Also, it was shown that systemically administered LPS induced TNF-α expression in both the anterior and posterior pituitary glands [37]. Since LPS also increased glial fibrillary acidic protein expression in the posterior pituitary, it was suggested that LPS could influence pituitary function by affecting pituicytes, therefore, altering the release of posterior pituitary secretion [37]. It has been shown that the posterior pituitary plays an important role in the regulation of prolactin secretion. In fact, both oxytocin and α-melanocyte-stimulating hormone can stimulate prolactin secretion [21]. Our results suggest that locally synthesized TNF-α could be involved in the decrease in prolactin release as a result of LPS by increasing DA release from the posterior pituitary. Besides, LPS could also decrease prolactin secretion by interacting with specific receptors in the anterior pituitary such as CD14 and Toll-like receptor type 4 [38] and therefore increase TNF-α release [39]. Furthermore, TNF-α has been shown to directly inhibit prolactin release from anterior pituitary cells [40]. Although our results suggest that locally synthesized TNF-α is involved in the stimulatory effect of LPS on dopaminergic activity in the HPA, a direct effect of LPS on dopaminergic neurons or the participation of other cytokines induced by LPS cannot be ruled out and could account for the action of LPS.

Central noradrenergic systems appear to be important for the regulation of the HPA axis, particularly the noradrenergic pathways that innervate the paraventricular nucleus and stimulate corticotropin-releasing hormone and arginine-vasopressin release [2, 41]. Since circulating glucocorticoids inhibit hypothalamic NE release and turnover, NE may be a key neurotransmitter linking the function of stress-responsive systems. Also, some evidence indicates that epinephrine has a stimulatory role on corticotropin-releasing hormone neurons [41, 42]. It also appears that a stimulatory noradrenergic component is involved in the regulation of the stress-induced release of prolactin [43]. However, neither the hypothalamus nor the posterior pituitary demonstrated changes in NE or epinephrine concentrations after LPS or TNF-α administration. On the contrary, TNF-α increased epinephrine and NE release from the posterior pituitary. The role played by these catecholamines released in the posterior pituitary has not been fully elucidated yet. However, the morphological changes observed in pituicytes in response to stimuli that increase the demand of neurohypophysial hormones are mediated, at least in part, by β-adrenergic agonists like epinephrine and NE [44].

Epinephrine is released from the adrenal medulla. Evidence indicates that NE and DA are released from peripheral nonsynaptic sympathetic nerve terminals in lymphoid organs and the zona glomerulosa of the adrenal gland. Circulating and locally released catecholamines are involved in the quick and fine tuning of immune responses, suppressing cellular immunity and boosting humoral immunity [45]. Together with previous evidence, our data indicate that intraperitoneal administration of LPS increases plasma levels of epinephrine, NE and DA. However, circulating catecholamines do not appear to affect ACTH secretion from the pituitary [41]. Since the

concentration of DA in portal blood is approximately 15-fold higher than that in the systemic circulation [46], it is unlikely that peripherally released catecholamines could affect lactotroph secretion.

In conclusion, our observations indicate that DA plays an important role in the inhibition of prolactin release during endotoxemia and suggest that TNF-α synthesized in the brain could be involved in the stimulatory effect of LPS on dopaminergic activity in HPA. The reduction in the secretion of prolactin, considered a proinflammatory factor, could participate in the downregulation of the immune response during the acute phase of endotoxemia.

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