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Effects of lipopolysaccharide on neurokinin A content and release in the hypothalamic–pituitary axis

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Abstract

The administration of bacterial lipopolysaccharide (LPS) markedly affects pituitary secretion, and its effects are probably mediated by cytokines produced by immune cells or by the hypothalamo-pituitary axis itself. Since neurokinin A (NKA) plays a role in inflammatory responses and is involved in the control of prolactin secretion, we examined the in vivo effect of LPS on the concentration of NKA in hypothalamus and pituitary (assessed by RIA) and serum prolactin levels in male rats. One hour after the intraperitoneal administration of LPS (250 μg/rat), NKA content was decreased in the posterior pituitary but not in the hypothalamus or anterior pituitary. Three hours after injection, LPS decreased NKA concentration in the hypothalamus and anterior and posterior pituitary. In all the conditions tested, LPS significantly decreased serum prolactin. We also examined the in vitro effects of LPS (10 μg/ml), interleukin-6 (IL-6, 10 ng/ml) and tumor necrosis factor alpha (TNF-α, 50 ng/ml) on hypothalamic NKA release. Interleukin-6 increased NKA release without modifying hypothalamic NKA concentration, whereas neither LPS nor TNF-α affected them. Our results suggest that IL-6 may be involved in the increase of hypothalamic NKA release induced by LPS. NKA could participate in neuroendocrine responses to endotoxin challenge.

Keywords: Tachykinins; Interleukin-6; TNF-α; Hypothalamus; Prolactin

1. Introduction

Neurokinin A (NKA) is a member of the tachykinin peptide family that also includes substance P (SP), neurokinin B (NKB), neuropeptide K (NPK) and neuropeptide gamma (NPγ). Tachykinins participate in diverse physiological functions when they bind to three subtypes of specific receptors: NK-1 (SP-preferring), NK-2 (NKA-preferring) and NK-3 (NKB-preferring), members of the G protein-linked receptor family [1,2].

SP and NKA are contained in hypothalamic neurons and nerve fibers and secretory cells of posterior and anterior pituitary lobes, suggesting that these peptides may have a physiological role in the control of pituitary function. In fact, a body of evidence indicates that these tachykinins regulate the hypothalamo-pituitary–adrenal (HPA) axis and reproductive functions by actions exerted at the three levels of the hypothalamo-pituitary–gonadal (HPG) axis [3,4]. In addition, we have previously shown that both SP and NKA are involved in the control of prolactin secretion [5,6].

Tachykinins not only function as neurotransmitters in the central and peripheral nervous system, but also participate as mediators in inflammation and immune responses [7,8]. Recently, it has been reported that SP and NKA directly influence the host response to viral infection [9]. Some effects of SP on inflammation are related to its ability to induce oxygen reactive species and nitric oxide generation [10]. In addition, SP induce cytokine synthesis either by activation of transcription factors, such as NF-κB, or independent of NF-κB by activation of mitogen-activated protein kinases [11,12]. Endotoxin-induced cytokine synthesis was reported to be attenuated by inhibiting SP release or by blocking of NK-1 receptors [13].

The peripheral administration of bacterial lipopolysaccharide (LPS) stimulates peritoneal macrophages which quickly synthesize and release large amounts of several cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [14]. Peripherally produced cytokines as well as those locally synthesized in brain and pituitary contribute to provoke neuroendocrine

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responses to endotoxin. Shared biological activities of IL-1, IL-6 and TNF-α are activation of the HPA axis and suppression of the HPG axis [15,16]. However, contradictory results in serum prolactin levels have been found after administering LPS or cytokines in different experimental models in rodents [17–19]. We have reported that both peripheral LPS and central TNF-α administration exert inhibitory effects on prolactin secretion by stimulating dopaminergic activity in the hypothalamo-pituitary axis [20].

Since NKA plays a role in inflammatory processes and is involved in the regulation of pituitary hormone secretion, the aim of this study was to explore whether NKA could be involved in the neuroendocrine response to endotoxin. Therefore, we investigated the effect of LPS administration on NKA-immunoreactivity in the hypothalamus and anterior and posterior lobes of the pituitary and serum prolactin levels. In addition, we examined the in vitro effect of LPS, IL-6 and TNF-α on NKA release from hypothalamic explants.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–250 g were used. The animals were fed lab chow and water ad libitum and kept in 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.

2.2. Drugs

All materials, including bacterial lipopolysaccharide (*Escherichia coli* serotype 0111:B8), were purchased from Sigma (St. Louis, MO, USA), except recombinant hTNF-α and hIL-6 (Promega, Madison, WI, USA), anti-NKA serum (Peninsula Laboratories, Belmont, CA, USA) and NKA-[^{125}I] (NEN™ Life Science Products, Boston, MA, USA).

2.3. Experimental protocols

2.3.1. In vivo experiments

Endotoxic shock was induced by a single intraperitoneal (i.p.) injection of LPS dissolved in pyrogen-free isotonic saline at a dose of 250 μg/rat, and rats were sacrificed 1 or 3 h later. Doses and times were chosen on the basis of previous experiments conducted in our laboratory [32] and/or known in the literature to effectively induce the corresponding endotoxic shock. Control animals were injected with vehicle alone. All animals were sacrificed by decapitation and trunk blood was collected for prolactin measurement. Serum was separated by centrifugation. After sacrifice, the anterior and posterior pituitary gland and 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, then homogenized in 1 N acetic acid. The tissue homogenates were heated at 100 °C for 10 min and centrifuged at 14500 rpm for 20 min. The supernatants were stored at −70 °C until determination of NKA. Protein concentration in tissue homogenates was determined by the method of Lowry et al., using bovine serum albumin as standard.

2.3.2. Incubation of hypothalamic fragments

For NKA release, one hypothalamic fragment was preincubated for 15 min in a Dubnoff shaker (60 cycles per 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 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.18 mM NaH_2PO_4, 1.18 mM $MgSO_4$, 24.88 mM $NaHCO_3$, pH 7.4) containing 10 mM glucose, 10 mM HEPES, 1 mM ascorbic acid, 0.1 mM bacitracin and 0.1% bovine serum albumin. Then, the medium was replaced with fresh KRB containing the substances to be tested and the tissues were incubated for 60 min. At the end of the incubation period, media were acidified with 1 N acetic acid and heated at 100 °C for 10 min and tissues treated as described above. Media and tissue supernatants were quickly frozen on dry ice.

2.4. NKA radioimmunoassay

Incubation media and tissue supernatants were neutralized with 1 N OHNa and diluted in PBS buffer (50 mM NaCl, 81 mM Na_2HPO_4, 19 mM NaH_2PO_4), pH 7.4 containing 0.1% bovine serum albumin and 0.1% Triton X-100. The samples were incubated with rabbit anti-NKA serum for 24 h at 4 °C. Then, NKA-[^{125}I] was added as tracer and incubated for 24 h at 4 °C. The reaction was stopped by the addition of sheep anti-rabbit IgG serum diluted 1:15 in PBS–Triton X-100 buffer with 1% normal rabbit serum for 2 h. After the addition of cold 6% PEG, the samples were centrifuged at 3000 rpm for 20 min. The radioactivity was quantified in a gamma counter. All samples from animals tested within each specific experimental paradigm were measured in the same RIA to avoid interassay variability. The intraassay coefficient of variation was lower than 5%, and assay sensitivity was 7.8 pg/tube.

2.5. Prolactin determination

Prolactin was measured by a double antibody radioimmunoassay utilizing the RP3 reference preparation and anti-rPRL-S-9 serum provided by the National Hormone and Pituitary Program (Torrance, CA, USA). The intrassay

coefficients of variation was lower than 9% and the assay sensitivity was 20 pg/tube.

2.6. Statistics

The results were expressed as means ± S.E.M. Significance of the differences between means was determined by Student's *t*-test. Differences were considered significant when $p < 0.05$. All experiments were performed at least twice. Figures represent results of individual experiments.

3. Results

3.1. Effect of LPS administration on NKA content

One hour after intraperitoneal administration of LPS (250 μg/rat), the content of NKA in the hypothalamic fragments or anterior pituitary gland were not significantly modified.

Table 1
Effect of LPS and TNF-α on NKA release from hypothalamic fragments and tissue concentration

	NKA release (ng/mg protein)	NKA tissue content (ng/mg protein)
Control	0.251 ± 0.043 (6)	8.420 ± 0.480 (8)
LPS (10 μg/ml)	0.315 ± 0.054 (8)	8.420 ± 0.701 (8)
Control	0.329 ± 0.107 (6)	5.980 ± 0.380 (6)
TNF-α (50 ng/ml)	0.225 ± 0.108 (6)	5.800 ± 0.300 (6)

The hypothalamic fragments were incubated with LPS or TNF-α for 60 min. Media and tissues were processed for determination of NKA concentration by RIA. Protein concentration was determined in tissue homogenates. Values represent means ± S.E.M. The numbers of determinations are indicated between brackets. Data were evaluated by Student's *t*-test.

However, LPS significantly ($p < 0.05$) decreased NKA content in posterior pituitary (Fig. 1A). NKA content was significantly ($p < 0.05$) decreased in hypothalamus and anterior and posterior pituitary lobes 3 h after LPS administration (Fig. 1B).

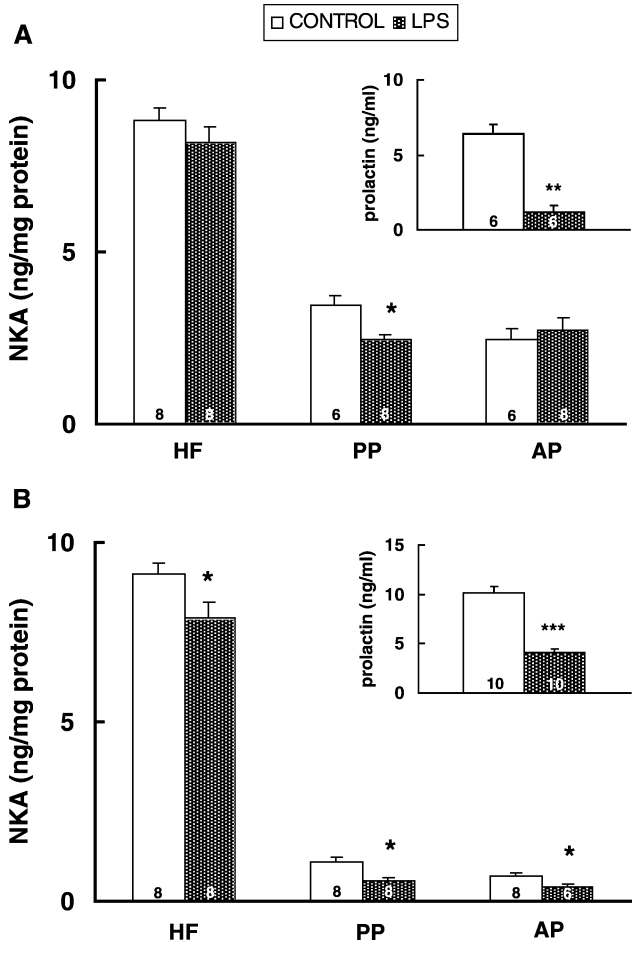

Fig. 1. Effect of LPS administration (i.p., 250 μg/rat) on NKA immunoreactivity in hypothalamic fragments (HF), posterior pituitary (PP), anterior pituitary (AP) and serum prolactin levels (insets) 1 h (A) and 3 h (B) postinjection. Values represent means ± S.E.M. The number of rats is indicated inside each column. Data were evaluated by Student's *t*-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control).

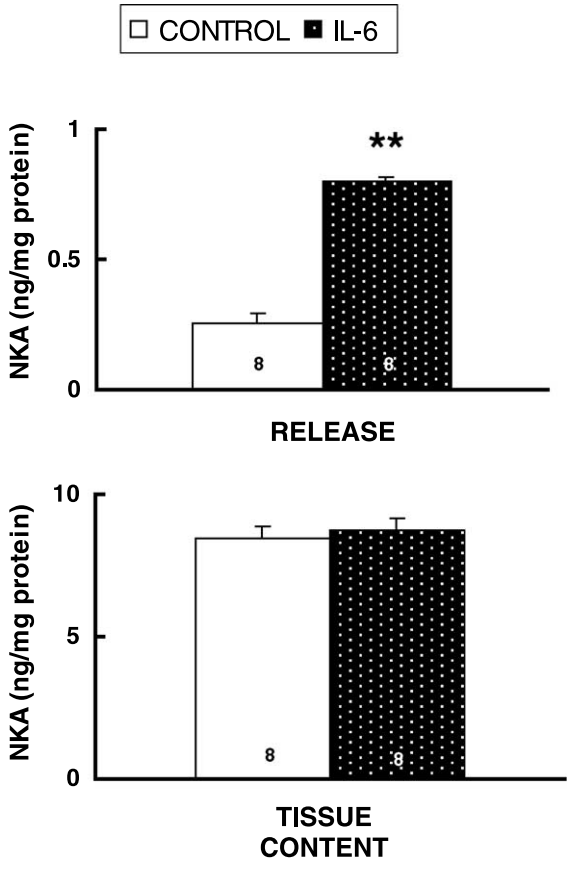

Fig. 2. In vitro effect of IL-6 on hypothalamic NKA release and tissue concentration. The hypothalamic fragments were incubated with IL-6 (10 ng/ml) for 60 min. Media and tissues were processed for determination of NKA concentration by RIA. Protein concentration was determined in tissue homogenates. Values represent means ± S.E.M. The number of hypothalamic fragments is indicated inside each column. Data were evaluated by Student's *t*-test (**$p < 0.01$ vs. control).

Serum prolactin levels were significantly decreased 1 h ($p<0.01$) and 3 h ($p<0.001$) after the intraperitoneal administration of LPS (Fig. 1).

3.2. In vitro effect of LPS, IL-6 and TNF-α on NKA release from hypothalamic fragments

The presence of LPS (10 μg/ml) in the incubation medium did not significantly change NKA release from hypothalamic fragments or tissue concentration (Table 1). IL-6 (10 ng/ml) significantly increased ($p<0.01$) hypothalamic NKA release without modifying its content in tissue (Fig. 2). On the contrary, TNF-α (50 ng/ml) affected neither hypothalamic NKA release nor content (Table 1).

4. Discussion

Our results show that intraperitoneal administration of LPS decreases NKA concentration in the hypothalamus and the pituitary 3 h postinjection. The effect of LPS was also observed in the posterior pituitary 1 h after LPS administration. Evidence indicated that the secretion of proinflammatory cytokines after systemic LPS administration are causally and temporally related, with plasma TNF-α elevated first, then IL-1, and finally IL-6 [15]. Peripherally administered LPS also produces an up-regulation of brain cytokines suggesting that humoral mechanisms in the periphery are able to signal the brain and modify cytokine synthesis within specific brain regions [21]. In addition, systemically administered LPS was shown to induce cytokine expression in both the anterior and posterior pituitary gland [22]. In our experiments, LPS did not appear to exert a direct effect on NKA release so that the action of LPS on tachykinergic neurons could imply cytokine synthesis. In fact, IL-6 increased NKA release from the mediobasal hypothalamus, suggesting that IL-6 may be involved in the decrease of hypothalamic NKA concentration observed after LPS administration. IL-1β has also been shown to increase NKA release from the median eminence and arcuate nucleus of castrated rats [23]. The posterior pituitary expresses both IL-1 and its receptor and their expression in this tissue is strongly up-regulated by LPS [24–26]. Both LPS and IL-1β were shown to stimulate IL-6 synthesis and release in the posterior pituitary [27]. Since IL-1 and IL-6 have been reported to stimulate neurohypophyseal hormone release by hypothalamic explants [28], it is possible that both IL-1 and IL-6 could mediate the changes in NKA concentration induced by LPS administration in hypothalamus and posterior pituitary.

Some evidence suggests that SP and NKA are not released in significant amounts into the portal vessels and that they may regulate pituitary secretion by modulating the release of hypothalamic neuropeptides and neurotransmitters [4]. Therefore, an increase in hypothalamic NKA neurotransmission induced by cytokines could target neurons in hypothalamic nuclei or their terminals in the median eminence and the posterior pituitary, thus affecting systems involved in the control of anterior pituitary function.

Although serum prolactin levels decreased 1 h after LPS administration, a decrease in hypothalamic NKA concentration was evident only after 3 h. This time lag suggests that the effect of LPS on tachykinergic neurons requires the synthesis, release and action of mediators, such as cytokines. Alternatively, a decrease in NKA content could only be evident after sustained stimulation of peptide release. The increase in hypothalamic NKA release induced by IL-6 may affect the activity of tuberoinfundibular dopaminergic neurons involved in the inhibitory tone that the hypothalamus exerts on prolactin secretion [29]. Dopaminergic neurons in the arcuate nucleus receive synaptic inputs from tachykinin-containing nerve terminals [30]. We have previously shown that SP stimulates dopamine release from hypothalamic fragments [31]. In addition, we have shown that LPS stimulates dopamine turnover in the hypothalamic–pituitary axis suggesting that the increase in dopaminergic activity could mediate the inhibitory effect of LPS on prolactin release [20]. Therefore, NKA could contribute to LPS-induced decrease in serum prolactin levels by stimulating hypothalamic dopamine release. Since NKA decreases oxytocin release from the posterior pituitary [32] this mechanism could also be involved in the inhibition of prolactin secretion during the acute phase of endotoxemia. In fact, IL-6 decreased in vitro oxytocin release from the posterior pituitary (unpublished results).

Tachykinins play an important role in the regulation of the HPA axis, especially in physical or inflammatory stresses. NK-1 agonists, like SP, exert an inhibitory effect on the CRH/ACTH system but stimulate the adrenal cortex, whereas NK-2 agonists like NKA activate both the central and intradrenal levels of the HPA axis [3]. It has been demonstrated that SP does not inhibit the initial response of the HPA axis to restraint stress but reduces the duration of the response to stress suggesting that SP has an important role in the transition between acute and chronic stress [33]. On the contrary, an increase in NKA release induced by cytokines could contribute to activation of the HPA axis during endotoxemia. It has been well established that prolactin increases the immune response [16]. The inhibition of prolactin release together with the activation of glucocorticoid secretion by cytokines [15,17,32] may represent a feedback mechanism that keeps within physiological limits the immune response triggered by acute inflammatory stimuli [16].

Some evidence indicates that NKA, NPK and NPγ have physiological roles as inhibitors of LH secretion, acting at the level of the hypothalamus [4]. Furthermore, NKA was suggested to mediate the inhibitory effect of IL-1β on LH secretion [23,34]. Our results support the notion that NKA could be involved in the inhibitory effect of LPS on reproductive functions.

In conclusion, cytokines synthesized during endotoxemia, in particular IL-6, may increase NKA release from hypothalamic tachykinergic neurons. NKA could participate as mediator of some neuroendocrine responses to an immune challenge such as inhibition of prolactin secretion.

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