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Tumor Necrosis Factor-α Mediates Endotoxin Induced Suppression of Gonadotropin-Releasing Hormone Pulse Generator Activity in the Rat

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Abstract. Bacterial endotoxin lipopolysaccharide (LPS) is known to suppress gonadotropin secretion and this effect is assumed to be mediated by cytokines. In the present study, we examined whether LPS affected hypothalamic electrical activity associated with LH pulses, and whether tumor necrosis factor-α (TNF-α), a major cytokine induced by LPS, was involved in this process. Ovariectomized rats were fitted with chronically implanted electrode arrays in the mediobasal hypothalamus, and multiunit activity (MUA) was recorded under conscious, unrestrained conditions. Blood samples were withdrawn every 6 min through an indwelling atrial catheter for determining serum LH concentrations. Intravenous (iv) injection of LPS (1 μg) suppressed characteristic increases (volleys) in MUA associated with LH pulses throughout the experimental period up to 5 h. This suppressive effect of LPS on MUA volleys was significantly attenuated by simultaneous intracerebroventricular (icv) injection of the antibody (50 ng) to TNF-α through an indwelling cannula in the lateral ventricle. These changes in MUA were faithfully reflected in the LH secretory pattern. Further, either iv (0.4–2 μg) or icv (20–250 ng) injection of TNF-α suppressed the frequency of MUA volleys and associated LH pulses in a dose-dependent manner. These results suggest that LPS leads to the suppression of gonadotropin-releasing hormone pulse generator activity through a mechanism involving TNF-α.

Key words: GnRH pulse generator, Multiunit activity, LH, Lipopolysaccharide, Tumor necrosis factor-α

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REPRODUCTIVE function is known to be suppressed under various stressful conditions such as strenuous exercise, malnutrition, surgical trauma, and infectious diseases [1, 2]. As a model of infections, bacterial endotoxin lipopolysaccharide (LPS) has been used for years to study the acute phase responses and shown to suppress LH secretion [3, 4]. The action of LPS on the endocrine system is considered to be mediated by cytokines produced by activated macrophages and glial cells. In fact it has been reported that interleukin-1 (IL-1) administered intracerebroventricularly suppresses pulsatile LH secretion [5–7].

Among cytokines, tumor necrosis factor-α (TNF-α) is detected in extremely large amounts in peripheral circulation and cerebrospinal fluid, when animals are loaded with LPS [8–10]. Interestingly, C3H/HeJ mice, being unable to produce TNF-α because of a genetic lesion, are far more susceptible to infections than normal mice [11]. Further, TNF-α was shown to be a potent secretagogue of ACTH [12, 13]. TNF-α is therefore regarded as one of the most important cytokines responsible for the coordination of host defense mechanisms and also for the bi-directional communication between the immune and endocrine systems. The question therefore arises

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how TNF-α can suppress reproductive function as an endogenous mediator of infectious stress.

To assess the reproductive activity, we have established the multiunit activity (MUA) recording system for monitoring electrical activity of the hypothalamic GnRH pulse generator that initiates a cascade of neuroendocrine events necessary for expressing reproductive function [14]. In the present study, we investigated whether LPS affected GnRH pulse generator activity and whether TNF-α was involved in mediating LPS actions.

Materials and Methods

Animals

Adult female Wistar-Imamichi rats were housed at a constant temperature (23–24 °C) with controlled lighting (lights on; 0500–1900 h) and given free access to food and water. All the rats were ovariectomized under ether anesthesia at the age of 7–8 weeks (180–200 g body weight).

Electrode implantation

More than 2 weeks after ovariectomy, the rats were fitted with chronically implanted electrode arrays under sodium pentobarbital anesthesia (30 mg/kg) as previously described [14]. Briefly, the electrode assembly consisted of four 75 μm Teflon-insulated platinum (90%)-iridium (10%)-wires incased in a stainless steel guide tube (650 μm in diameter). The impedance of each electrode measured at 1 kHz was 50–100 kΩ. According to the stereotaxic atlas of the rat brain by Albe-Fessard *et al.* [15], electrodes were implanted unilaterally into the mediobasal hypothalamus (MBH). Each animal also had a stainless steel cannula (23 G) implanted in the lateral ventricle. The electrodes and cannula were fixed to the skull with anchor screws and dental cement. For intravenous injection and blood sampling, a silastic catheter was inserted into a jugular vein to reach the right atrium.

MUA recording

After a recovery period of a few days, MUA was recorded from freely moving rats. The electrodes were connected to a buffer amplifier where signals were passed through to a biophysical amplifier (AVB-21, Nihon Kohden, Tokyo, Japan) with low and high cutoff frequencies of 500 Hz and 3 kHz, respectively, and amplified signals were displayed on an oscilloscope (VC-11, Nihon Kohden). In the present study, only the rats that exhibited characteristic increases (volleys) in MUA (approximately 30% of the rats prepared) were subjected to the experiments. Neural spikes were discriminated by their amplitude, and the number of spikes was counted with a pulse counter (MET-1100, Nihon Kohden). Outputs were recorded as a histogram with a personal computer. During MUA recording, a blood sample (120 μl) was withdrawn through an indwelling jugular catheter at 6 min intervals, and an equal volume of heparinized saline (10 IU/ml) was replaced after each bleeding. In the present experiment, the duration of blood sampling was shorter than that of MUA recording due to the limitation of the sampling volume.

Drug injection

After a pretreatment period of 1–2 h, a single injection of LPS (*Escherichia coli* 055: B5, Sigma, St. Louis, MO, 1 μg/rat) was given intravenously (iv). Anti-murine TNF-α monoclonal antibody (MM-350C, Endogen, Boston, MA, 50 ng/rat) was simultaneously injected intracerebroventricularly (icv) into some of the rats. Using different rats, a bolus iv or icv injection of TNF-α (recombinant human TNF-α, R&D System, Minneapolis, MN) was given at doses of 20, 50 and 250 ng/rat or 0.4, 1 and 2 μg/rat, respectively. Each dose of drugs was dissolved in 10 μl (for iv injection) or 5 μl (for icv injection) of sterile-filtered physiological saline solution containing 0.1% bovine serum albumin. Equal doses of vehicle were injected as controls. After each treatment, MUA recording continued for an additional 3–5 h.

LH determinations

Serum concentrations of LH were measured by double antibody radioimmunoassay with materials supplied by the NIDDK as reported previously [14]. The reference standard for LH assay was NIDDK-

rLH-RP-3. The intra- and interassay coefficients of variation for LH assays, which were calculated from 5 replicate determinations for the pool of rat serum containing 2.7 ng/ml, were 13.8% and 11.3%, respectively. All samples from each animal were measured in a single assay.

Statistical analysis

The data were statistically analyzed by analysis of variance, followed by Duncan's multiple range test. Differences were considered significant at $P<0.05$.

Results

Effect of LPS (iv) alone or in combination with anti-TNF-α antibody (icv) on hypothalamic MUA and serum LH

As shown in Fig. 1 (upper panel), iv injection of LPS (1 μg) suppressed MUA volleys and associated LH pulses which were visually detected. Effects of LPS on the frequency of MUA volleys and serum LH levels are summarized in the upper panels of Figs. 2 and 3, respectively. LPS did not affect the volley frequency or serum LH levels during the first 1 h-period, but significantly decreased them throughout the rest of the experimental period.

The antibody to TNF-α (50 ng) given in the lateral ventricle much attenuated the inhibitory effect of LPS on the expression of MUA volleys and LH pulses (Fig. 1, lower panel). Although both volley frequency and serum LH levels remained suppressed for 2–4 h after the antibody treatments (Figs. 2 and 3, lower panels), the former returned to the pretreatment level by 5 h after the treatments. The antibody alone did not affect volley frequency (data not shown).

Effect of iv or icv treatment with TNF-α on hypothalamic MUA and serum LH

The effects of increasing doses of TNF-α given either iv or icv on hypothalamic electrical activity and serum LH profiles were monitored. Representative examples of MUA volleys and LH pulses after iv (1 μg) and icv (50 ng) injections of TNF-α are shown in Fig. 4, upper and lower panels,

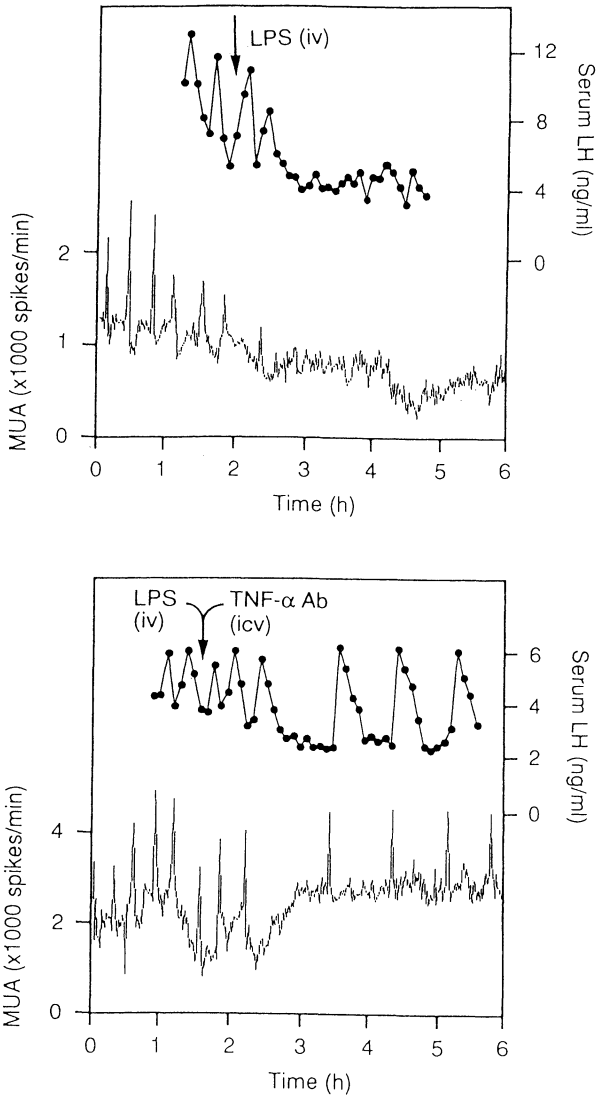

Fig. 1. Effect of iv injection of LPS (1 μg) alone (upper) or in combination with icv injection of anti-TNF-α antibody (TNF-α Ab, 50 ng) (lower) on hypothalamic MUA and serum LH profiles. Arrows indicate the time of injection.

respectively. Figure 5 illustrates a summary of the effects of TNF-α on the frequency of MUA volleys. Although iv injection of 400 ng TNF-α did not affect the frequency of MUA volley, that of 1 or 2 μg significantly decreased it. The suppressive effect of 1 μg TNF-α on volley frequency was discernible during the second 1 h-period after the injection, while 2 μg TNF-α decreased volley frequency during the whole post-treatment period of 3 h. In the case of icv injection, 20 ng TNF-α did not affect

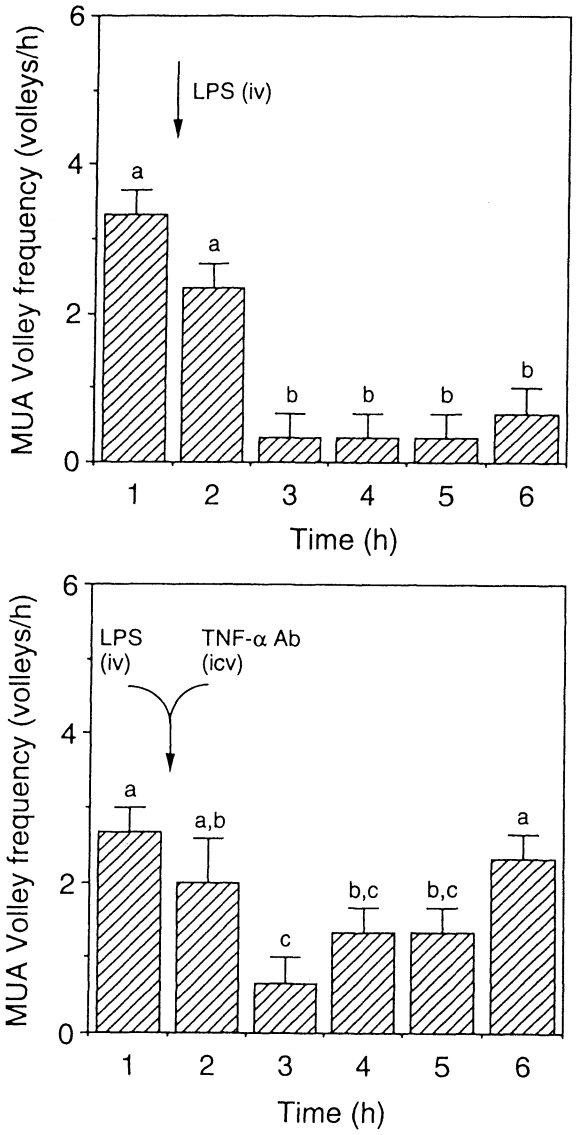

Fig. 2. Summary of the effects of iv injection of LPS (1 μg) alone (upper) or in combination with icv injection of anti-TNF-α antibody (TNF-α Ab, 50 ng) (lower) on MUA volley frequency. Each column and vertical bar represent the mean ± SEM (n=4–5). Values with different letters are significantly different ($P<0.05$) from each other.

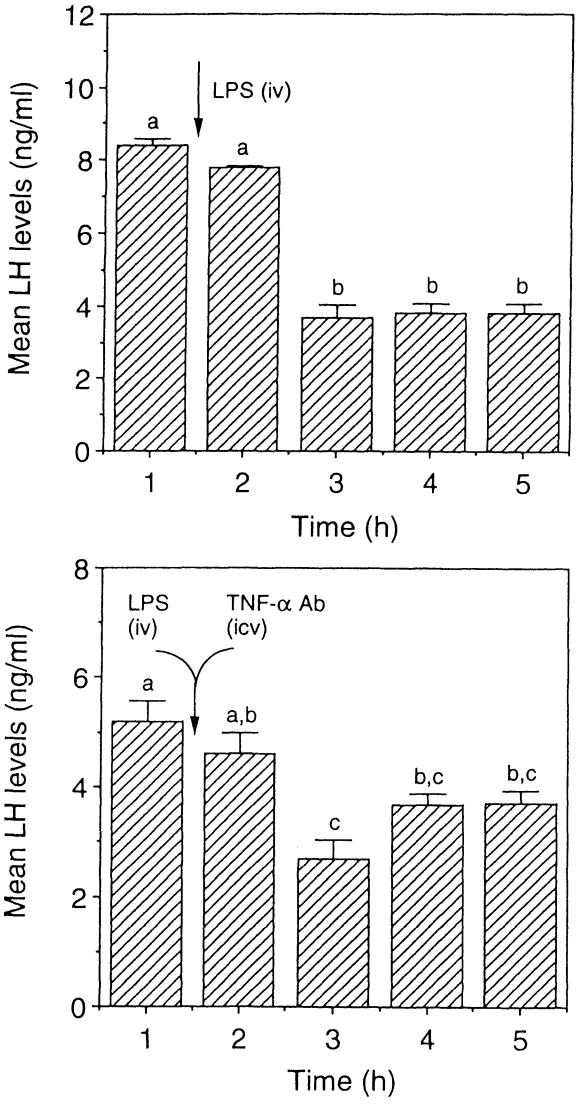

Fig. 3. Summary of the effect of iv injection of LPS (1 μg) alone (upper) or in combination with icv injection of anti-TNF-α antibody (TNF-α Ab, 50 ng) (lower) on serum LH levels. Each column and vertical bar represent the mean ± SEM of individual mean LH during each 1 h-period (n=4). Values with different letters are significantly different ($P<0.05$) from each other.

volley frequency, but 50 ng TNF-α significantly decreased volley frequency during the second to the fourth 1 h-periods after the injection. Following icv injection of 250 ng TNF-α, volley frequency remained suppressed during the whole post-treatment period for 4 h.

Serum LH levels were analyzed in animals given iv (1 μg) or icv (50 ng) injection of TNF-α. As summarized in Fig. 6, either iv or icv injection of TNF-α significantly suppressed serum LH, with a similar time course change as that of MUA volley frequencies described above.

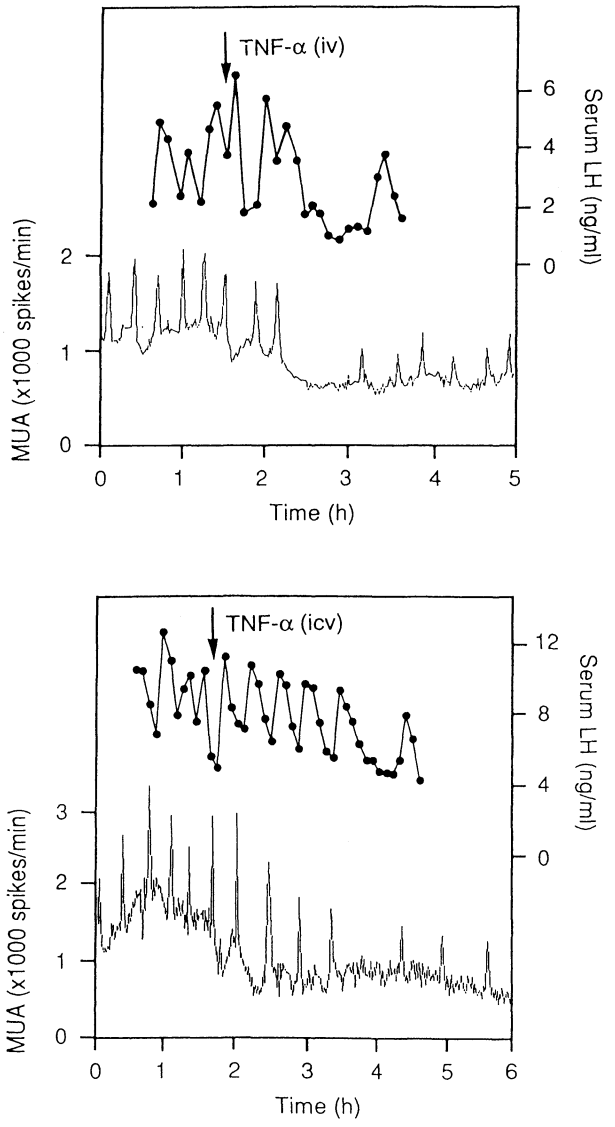

Fig. 4. Effects of iv (1 μg, upper) and icv (50 ng, lower) injection of TNF-α on hypothalamic MUA and serum LH profiles. Arrows indicate the time of injection.

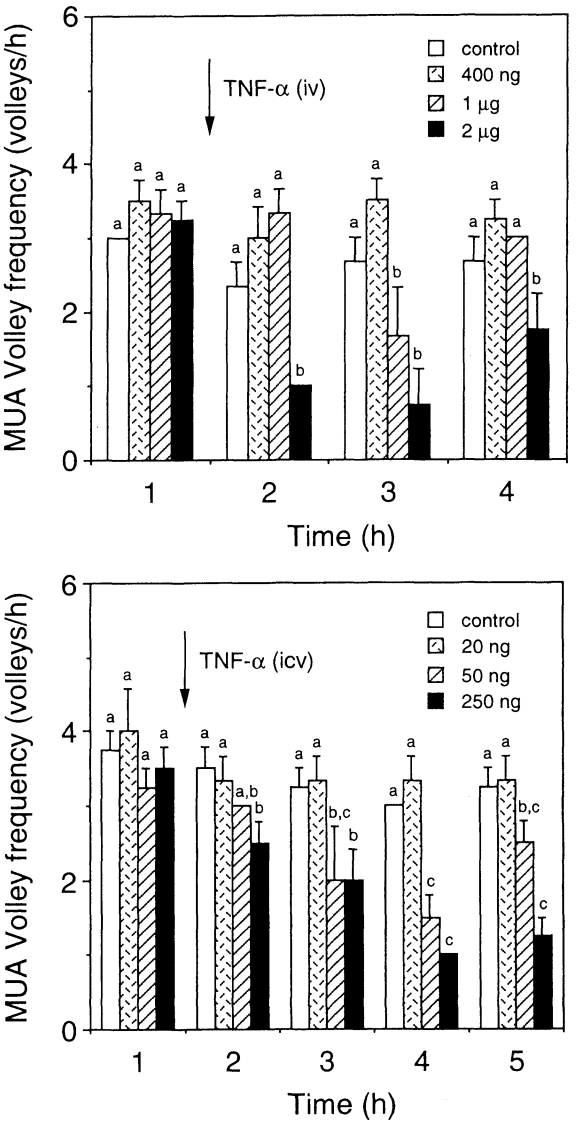

Fig. 5. Summary of the effect of increasing doses of TNF-α (400 ng, 1 and 2 μg for iv, upper; 20, 50 and 250 ng for icv, lower) on MUA volley frequency. The control group received vehicle injection. Each column and vertical bar represent the mean ± SEM (n=4–5). Values with different letters are significantly different ($P<0.05$) from each other.

Discussion

By monitoring MUA volleys, the present study demonstrated that iv injection of LPS suppressed GnRH pulse generator activity and associated LH pulses in the ovariectomized rat. Because the antibody to TNF-α injected icv significantly attenuated this effect of LPS, endogenous TNF-α acting on the central nervous system may at least partially mediate the suppressive effect of LPS on GnRH pulse generator activity. It should be noted, however, that the antibody used did not completely block the responses caused by LPS. Although the possibility of insufficiency in the amount of the antibody is not excluded, factors other than TNF-α, of which release is stimulated by LPS, may also

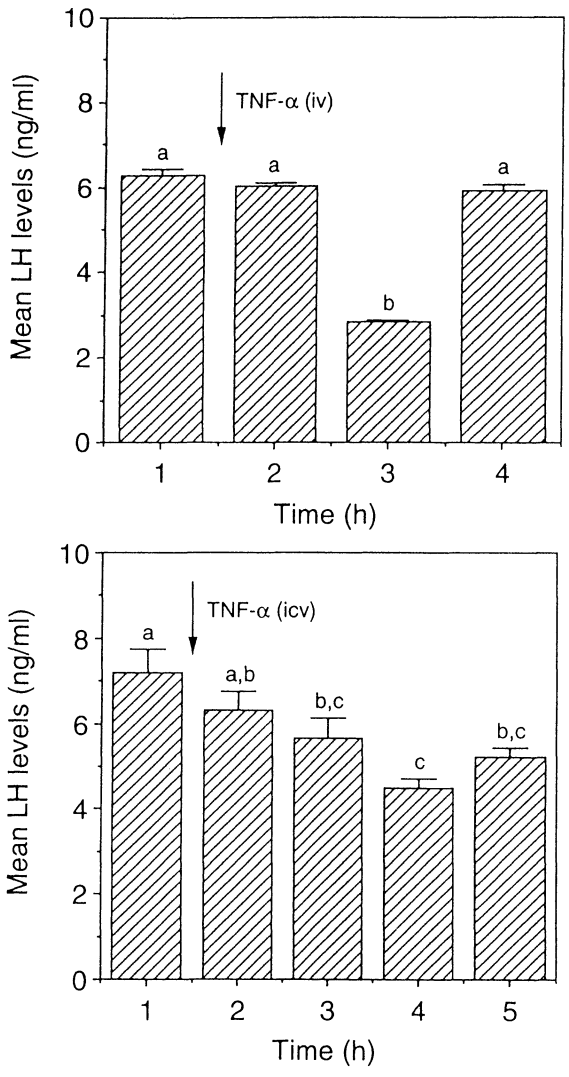

Fig. 6. Summary of the effect of injection of TNF-α (1 μg for iv, upper; 50 ng for icv, lower) on serum LH levels. Each column and vertical bar represent the mean ± SEM for individual mean LH during each 1 h-period (n=4). Values with different letters are significantly different ($P<0.05$) from each other.

participate in mediating the LPS actions.

TNF-α administered alone either iv or icv could decrease MUA volley frequency in a dose-dependent manner. The minimum effective doses in the present study, i.e., 1 μg for iv and 50 ng for icv, are within the range of those reported to cause a moderate increase in plasma ACTH in the rat [12, 13, 16]. ACTH release is enhanced by other cytokines such as IL-1 and IL-6 [12, 17–19], which are known to also inhibit LH secretion in rats [5, 7, 19] and monkeys [6]. Thus the inhibition of the hypothalamo-pituitary-gonadal system, as well as stimulation of the hypothalamo-pituitary-adrenal system, seems to be a common feature of the action of cytokines that occur in the general circulation during the acute-phase response to nonspecific infections.

Although our results suggest a central action of TNF-α in suppressing the pulse generator activity, it is unlikely that TNF-α crosses the blood-brain barrier [20]. How and where peripherally derived TNF-α transmits information to the brain is not well understood, but it has been suggested by several investigators that systemic administration of LPS enhances peripheral as well as central TNF-α synthesis and release [8–10, 21]. Further, many cytokines are known to stimulate production of their own and/or other cytokines in immune cells. For example, production of TNF-α in human monocytes is stimulated by TNF-α itself, IL-1 and interferon-γ (IFN-γ) [22]. On the other hand, TNF-α-like immunoreactivity has been localized in neurons and fibers [23], and specific receptors for TNF-α have been identified in astrocytes and microglia [24, 25]. Taking these observations into account, if TNF-α molecules can enter the brain through the circumventricular organs where the blood-brain barrier is absent, it would presumably evoke a cascade of reactions that stimulate production of the same molecule in the brain.

The suppressive effect of TNF-α and LPS on both MUA volleys and serum LH levels was manifested with a latency of about 1 h in this study. This may imply that another mediator(s) is further involved in the response to TNF-α. In this context, it is pertinent to mention that prostaglandins released from the glial cells appear to play a key role for cytokines to exert their activities on the central nervous system. It has been suggested that they mediate the central actions of cytokines including TNF-α, IL-1, IL-6 and IFN-γ, all of which share similar biological functions such as induction of fever, slow wave sleep, anorexia and ACTH release [26–30]. Experiments are now under way on the assumption that the effect of TNF-α on pulse generator activity may involve prostaglandin-mediated mechanisms.

In conclusion, the present study indicates that TNF-α produced either in the peripheral tissue or

in the brain in response to LPS may lead to a decrease in hypothalamic GnRH pulse generator activity, which would result in suppression of the reproductive function in general. The exact site and mode of actions of TNF-α on the pulse generator remain to be ascertained

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