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Temporal Profiles of Interleukin-1β, Interleukin-6, and Tumor Necrosis Factor-α in the Plasma and Hypothalamic Paraventricular Nucleus after Intravenous or Intraperitoneal Administration of Lipopolysaccharide in the Rat: Estimation by Push-Pull Perfusion

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Abstract. Lipopolysaccharide (LPS) is known to stimulate the synthesis and secretion of various proinflammatory cytokines in both the peripheral immune cells and the brain. Yet, the relative contribution of peripheral and central cytokines to the LPS-induced activation of the hypothalamo-pituitary-adrenal axis is still poorly understood. In this study, utilizing the push-pull perfusion technique of the rat brain, we attempted to characterize in detail the temporal profiles of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α after intravenous (iv) or intraperitoneal (ip) administration of LPS in both the general circulation and the hypothalamic paraventricular nucleus (PVN), which is the primary source of corticotropin releasing hormone (CRH). Temporal changes in plasma adrenocorticotropic hormone (ACTH) and CRH levels in the PVN were also monitored. We collected blood and perfusates every 30 min from 11 : 00 to 17 : 00 h. At 12 : 00 h, 1.0 or 2.5 mg/kg body weight of LPS was given via an iv or ip route, respectively. Peak ACTH response occurred 30 min after iv LPS and 1.5 h after ip LPS. Of the three cytokines measured in the plasma, TNF-α showed the fastest rise in synchrony with peak ACTH secretion after both iv and ip LPS. Although plasma IL-6 also showed a robust rise, its peak level occurred later than the ACTH peak. Elevation of plasma IL-1β was the smallest among the three cytokines. CRH levels in the PVN reached their peaks 1 and 2.5 h after the ACTH peak following ip and iv LPS, respectively. Irrespective of the route of LPS administration, IL-6 and TNF-α levels in the PVN showed significant rises 1–2 h after the ACTH peak, but IL-1β in the PVN did not significantly change during the entire period of observation. The results of the present study suggest that circulating TNF-α may play the most important role in triggering the early, peak phase of ACTH secretion after both iv and ip LPS. Although it is possible that brain TNF-α, IL-6, and circulating IL-6, may be involved in the later, protracted phase of ACTH secretion induced by LPS, IL-1β in both the brain and peripheral circulation seems to play the smallest role in ACTH secretion. This is the first study to characterize the LPS-induced temporal changes in IL-1β, IL-6, and TNF-α in both plasma and PVN simultaneously in conscious, freely moving rats.

Key words: Lipopolysaccharide, Interleukin-1β, Interleukin-6, Tumor necrosis factor-α, Push-pull perfusion

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IT is known that lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, stimulates the release of various kinds of bioactive mediators including cytokines, prostaglandins, platelet-activating factor, oxygen free-radicals, and other mediators [1]. Among these mediators, such cytokines as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α are now established as potent activators of the hypothalamo-pituitary-adrenal (HPA) axis [2, 3]. Thus, it is no wonder that such cytokines produced by activated monocytes and macrophages in the periphery are considered as

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mediating the LPS effects on the HPA activity. However, it is also known that the brain, pituitary, and adrenal gland are all capable of synthesizing and secreting various cytokines in response to LPS stimulation [2, 3], which raises the possibility that cytokines produced by such tissues may regulate the HPA activity in a paracrine manner.

In this respect, it is worth noting that Kakucska *et al.* [4] have suggested a crucial role of central IL-1 in mediating the corticotropin-releasing hormone (CRH)-adrenocorticotropin (ACTH) response to peripheral administration of LPS in the rat. However, the 8-hour time interval at which they examined HPA activity after LPS appeared to be about 6–7 h later than the usual time of peak ACTH response expected following systemic LPS treatment [2, 3]. In addition, most of the previous studies examining IL-1 messenger ribonucleic acid (mRNA) or its protein levels within the brain after peripheral administration of LPS have reported a long time course (6–10 h) of endogenous IL-1 production [5–11]. Thus, it is possible that the above-mentioned conclusion of Kakucska *et al.* [4] may not be true with an early, peak phase of ACTH secretion after peripheral administration of LPS. In support of this possibility, we recently reported that intracerebroventricular administration of IL-1 receptor antagonist (ra), a member of the IL-1 family antagonizing the biological activity of IL-1, did not significantly affect the plasma ACTH response to intravenous (iv) injection of LPS in the rat [12]. Several previous studies have reported the changes that take place in the bioactivity of IL-6 and TNF-α mRNA levels in the hypothalamus after LPS stimulation [7, 9, 13–16]. However, none of these quantitative studies of IL-1β, IL-6, and TNF-α in the brain have assessed the effects of endotoxin modulation upon HPA activity. There are several previous studies which examined the effects of inhibition of cytokine action on the HPA response to endotoxin in rodents. With respect to IL-1, an early study of Rivier *et al.* [17] reported that pretreatment of anti-IL-1 receptor antisera markedly suppressed ACTH response to endotoxin. This is in agreement with a later study of Schotanus *et al.* [18] that systemic treatment of IL-1ra abolished ACTH response induced by endotoxin. In contrast to these two reports, the above-mentioned study of Kakucska *et al.* [4] reported that an iv infusion of IL-1ra had no effect on the HPA response to endotoxin. In turn, Perlstein *et al.* [19] reported that while inhibition of either IL-1, IL-6, or TNF-α suppressed the HPA response to a large dose of endotoxin, only anti-IL-6 antisera were completely effective in inhibiting the HPA response to a low dose, suggesting an obligatory role of IL-6 in moderate stimuli. However, subsequent studies of Ebisui *et al.* [36] and Turnbull and Rivier [38] employed anti- TNF-α antisera and concluded that TNF-α in the periphery may be the most important cytokine mediating an early component of ACTH response after systemic LPS treatment. Taken as a whole, the relative contribution of peripheral and central cytokines to the LPS-induced activation of the HPA axis is still far from being clarified.

In turn, it has been reported that temporal profiles of peripheral cytokines after LPS stimulation may differ depending on its route of administration, *i.e.* iv *vs.* intraperitoneal (ip) [20]. In addition, recent reports have suggested that intraabdominal vagus nerves may play an intermediary role in the HPA activation after ip administration of LPS [21, 22] or IL-1β [23].

Based on all the relevant findings reported to date, our objective in the present study was two-fold. First, in order to have a better understanding of the relative contribution of peripheral and central cytokines to the LPS stimulation of ACTH secretion, we attempted to characterize in detail the temporal profiles of IL-1β, IL-6, and TNF-α after systemic LPS treatment in both the general circulation and the hypothalamic paraventricular nucleus (PVN). Temporal changes in plasma ACTH and CRH levels in the PVN were also monitored. To accomplish this, we employed the push-pull perfusion (PPP) technique of the rat brain as in our previous reports [24–29]. Second, we compared the effects of iv versus ip injections of LPS to determine if the route of administration causes any fundamental differences in response of the three cytokines in either the peripheral circulation or the PVN. We chose the PVN as the site to determine cytokine levels, because that nucleus is where the CRH neuronal cell bodies are located, and we [27] and other investigators [30, 31] have previously reported that the PVN may be a potential site within the hypothalamus where brain-derived IL-1 activates the HPA axis.

Materials and Methods

Animals and PPP protocol

Adult male rats (350–400 g) of the Wistar-Imamichi strain were used. They were housed in an air conditioned room with controlled lighting (light 08 : 00–20 : 00 h), and were given free access to laboratory chow and tap water.

Animals were anesthetized with sodium pentobarbital [40 mg/kg body weight (BW), ip], placed on a stereotaxic instrument, and guide cannulae for PPP were lowered into the brain. As described in our previous reports [27–29], PPP cannulae (outer diameter, 0.7 mm) were constructed in our laboratory according to a design similar to that described by Levine and Ramirez [32]. The guide cannulae with a removable inner stylette were stereotaxically implanted towards the PVN. Implantation coordinates for the PVN, taken from the atlas of Pellegrino *et al.* [33], were 0.6 mm anterior to, and 0.5 mm lateral to the bregma, and 7.7 mm ventral from the dura. The device was fixed onto the skull with anchor screws and dental cement. Immediately after implantation, the animals were injected intramuscularly with 20,000 U/kg BW of benzyl penicillin potassium (Meiji Seika, Ltd., Tokyo, Japan). They were given a minimum recovery period of 14 days until experimentation. Two days prior to PPP, the animals were implanted with a jugular vein catheter filled with heparin solution under light ether anesthesia.

All the following experiments were done under conscious, freely moving condition. At about 07 : 00 h on the day of PPP, the inner stylette was removed and replaced with the inner cannula perfusion assembly. Immediately thereafter, we started infusing artificial cerebrospinal fluid, of which constituents were the same as in our previous studies [24–29], through the push cannula, and collected perfusates from the pull cannula at a flow rate of 15 μl/min. Subsequently, the jugular vein catheter was exteriorized to allow for sequential blood sampling. The dead space of the pull system (from the tip of the guide cannula to the farthest end of the pull tubing) was adjusted to 225 μl (corresponding to a 15-min period of perfusion) so that each blood sample could be drawn just in the middle of collecting a timely matched perfusate. After an equilibration period of 3.5–4 h, perfusion fractions (450 μl) and blood samples (500 μl) were collected every 30 min over a total period of 360 min (11 : 00–17 : 00 h). To prevent the loss of circulating plasma volume, 500 μl of 0.9% NaCl was injected iv immediately after each blood collection. At 12 : 00 h, 1.0 or 2.5 mg/kg BW of LPS (Escherichia coli, serotype O127: B8, Sigma Chemical Company, St. Louis, Mo., USA) was given via an iv or ip route, respectively. Just prior to use, LPS was dissolved in 0.9% NaCl at the concentration of 1.0 mg/ml. Two subsets of animals were injected with the vehicle only through an iv or ip route, and served as control groups. The perfusates were immediately frozen on dry ice, lyophilized, and stored at -70°C until assayed for CRH, IL-1β, IL-6, and TNF-α. The blood was collected in EDTA-2Na (2.5 mg/ml)-containing tubes, centrifuged, and the plasma was stored at -20°C until assayed for ACTH and the three cytokines (IL-1β, IL-6, TNF-α). Within 30 min after the experiment was over, the animals were killed by decapitation, and their brains were removed and stored at -20°C for histological checking.

Assays

The lyophilized perfusates were reconstituted with 450 μl of a buffer (0.1% BSA, 81 mM Na_2HPO_4, 19 mM NaH_2PO_4, 50 mM NaCl, 0.1% NaN_3, 0.1% Triton X-100, pH 7.4), and subjected to RIA of CRH, and enzyme-linked immunoadsorbent assays (ELISA) of rat IL-1β, IL-6, and TNF-α. CRH-RIA was carried out using the above-mentioned buffer as an assay buffer, and antisera and iodinated peptide, which were purchased from Peninsula Laboratories (Belmont, CA, USA) or New England Nuclear (Boston, MA, USA), respectively. Sensitivity of this assay was 10 pg/ml. ELISAs of the three cytokines in the plasma and perfusates were performed using kits produced by BioSource International (Camarillo, CA, USA). Sensitivities of these assays were 3.0, 10, or 2.0 pg/ml for the plasma IL-1β, IL-6, or TNF-α, respectively. Similarly, their sensitivities in the perfusates were 1.4, 5.0, or 0.5 pg/well, respectively. CRH and the three cytokines were also measured in reconstituted lyophilizates from blank perfusates (5 samples per rat) containing 450 μl of the pure artificial cerebrospinal fluid, and its mean value was subtracted from the levels in all the actual per-

fusates from each animal. Plasma ACTH levels were measured with a commercial immunoradiometric assay kit produced by Mitsubishikagaku Biochemicals (Tokyo, Japan), and its sensitivity was 5 pg/ml. In all the above assays, both the intra- and interassay coefficients of variation were less than 10%.

Histology

Using a microtome cryostat, serial coronal sections of the brain, 50-μm thick, were cut, stained with thionin, and subjected to histological examinations of the PPP cannula placement. The PVN perfusion was considered successful when the tip of the push cannula was found anywhere in the whole PVN region. Special care was not taken as to whether the principal site of perfusion was the parvocellular or magnocellular region of the PVN, because data obtained were very similar as long as the cannula placement was somewhere in the PVN region. Fig. 1 shows representative section of the perfused brain.

Statistical Analyses

Results were expressed as the mean$\pm$SE. One-way or two-way ANOVA followed by Scheffe's post hoc test was used for statistical analyses. The level of significance was set at $P<0.05$.

Results

Effects of iv administration of LPS on the plasma levels of ACTH, IL-1β, IL-6, and TNF-α, and the PVN levels of CRH and the three cytokines

Fig. 2 shows the temporal profiles of ACTH, IL-1β, IL-6, and TNF-α in the plasma, and CRH and the three cytokines in the PVN before and after the iv administration of LPS or vehicle only. In the control group, the plasma levels of ACTH and the three cytokines did not show a significant change during the entire period of observation. Even in the control group, both IL-6 [16$\pm$10 (lowest time point value)$\sim$31$\pm$19 pg/ml (highest time point value)] and TNF-α [48$\pm$4 (lowest)$\sim$85$\pm$12 (highest)] were measurable in most plasma samples, whereas the plasma IL-1β was nearly undetectable ($<$3.0 pg/ml). CRH and cytokine levels in the PVN of the control group were detectable in most or a small proportion of the perfusates, respectively. LPS induced a significantly higher secretion of ACTH than that of the control, with its peak level occurring 30 min after injection. Although LPS significantly elevated all three of the cytokines in plasma, differences were observed in their temporal secretory patterns. The plasma concentration of IL-1β reached a significantly higher level than the control and reached a peak, 1.5 h after LPS. Similarly, plasma IL-6 started to significantly increase 1 h after LPS administration and reached its peak in 1.5 h. Differing from these two cytokines, the plasma TNF-α showed a significant

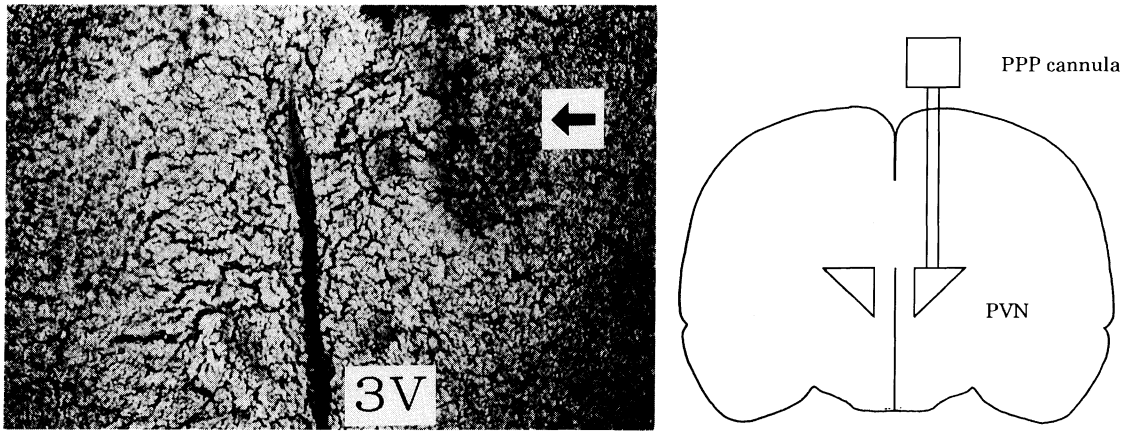

Fig. 1. A representative section of the perfused brain. Arrow indicates the localization of the tip of PPP cannula. 3V, third ventricle. PVN, paraventricular nucleus.

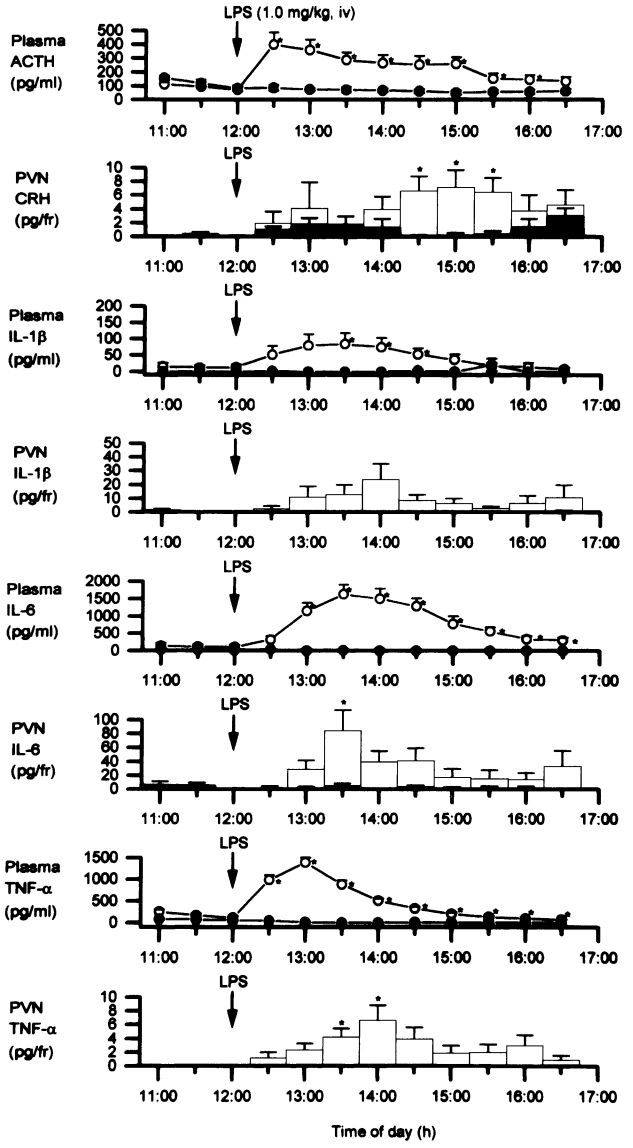

Fig. 2. Effects of iv administration of LPS (1.0 mg/kg) or vehicle only on the plasma levels of ACTH, IL-1β, IL-6, and TNF-α, and the PVN levels of CRH and the three cytokines [expressed as per perfusate fraction (fr)]. The number of rats examined was 6–7 per group. In this and next figures, (1) open circles and columns indicate the data of the LPS group, and closed circles and columns the control group, (2) the described time of day for perfusate collection is shifted 15 min ahead of the actual time of perfusion, because the dead space of the pull system (225 μl) corresponded to a 15-min period of perfusion (flow rate, 15 μl/min), (3) the asterisks indicate statistically significant differences (at least $P<0.05$) *vs.* control values, and (4) where standard errors are not shown, they were smaller than the symbols.

increase as early as 30 min after LPS, and reached its peak 1 h postinjection. Among the three cytokines in the plasma, there were also differences in the magnitude of elevation after LPS. The magnitude of increase (expressed as -fold) from the basal (at 12 : 00 h) to peak values was 6.5 for IL-1β (from 13.1 ± 9.1 to 84.8 ± 33.3 pg/ml), 15.1 for IL-6 (from 108 ± 54 to $1{,}626\pm276$ pg/ml), and 11.6 for TNF-α (from 119 ± 29 to $1{,}386\pm110$ pg/ml). CRH levels in the PVN were significantly stimulated by LPS over

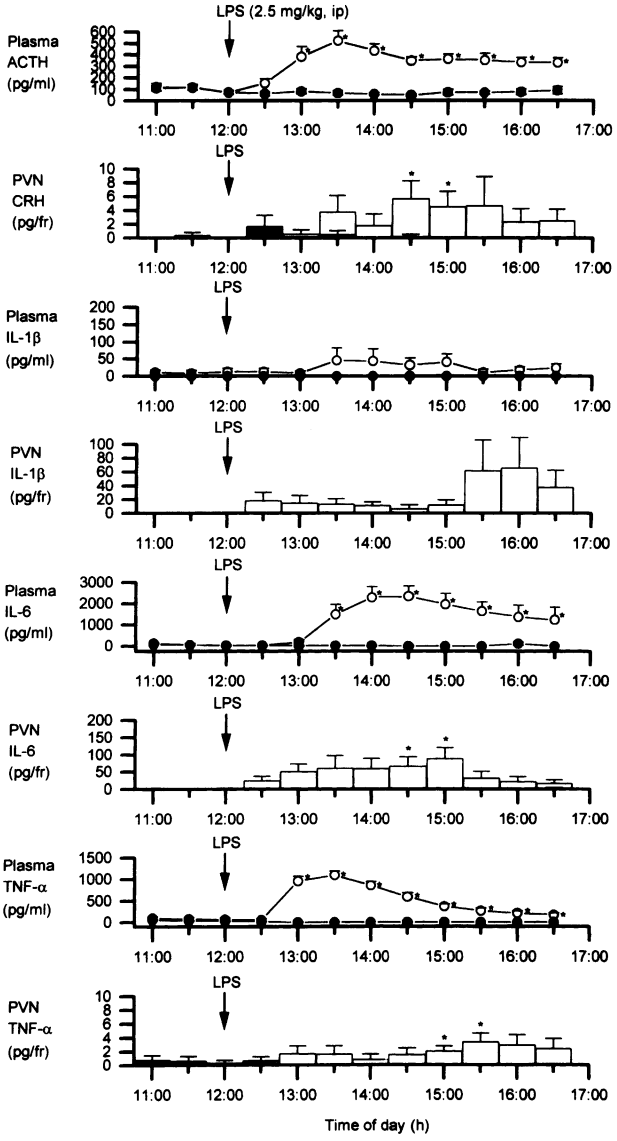

Fig. 3. Effects of ip administration of LPS (2.5 mg/kg) or vehicle only on the plasma levels of ACTH, IL-1β, IL-6, and TNF-α, and the PVN levels of CRH and the three cytokines. The number of rats examined was 6 in both groups.

the control values during the period between 14 : 15 and 15 : 45, and its peak level was observed 3 h after LPS (2.5 h after peak ACTH response). Although IL-1β levels in the PVN showed a relatively wide variation of data, they were not significantly stimulated by LPS over those of the control group, whereas both IL-6 and TNF-α in the PVN were significantly elevated by LPS with their peak levels occurring 1.5 or 2 h after LPS (1 or 1.5 h after peak ACTH response), respectively.

Effects of ip administration of LPS on the plasma levels of ACTH, IL-1β, IL-6, and TNF-α, and the PVN levels of CRH and the three cytokines

Fig. 3 shows the temporal profiles of all the above-mentioned parameters before and after the ip administration of LPS or vehicle only. As in the case of iv injection of vehicle only, the ip vehicle did not significantly affect the values of each parameter during the entire observation period. Differing from the secretory pattern of ACTH after iv LPS, ip LPS caused a more protracted secretion of the hormone with peak ACTH response 1.5 h postinjection. Differences were also observed in the plasma-cytokines profiles after iv *vs* ip LPS. Although the peak ACTH levels attained were statistically indistinguishable between iv (397 ± 88 pg/ml) and ip LPS (520 ± 86 pg/ml), the plasma IL-1β response to ip LPS was not statistically significant compared to the value after vehicle alone. Of the remaining two cytokines in the plasma, only TNF-α showed a significant rise as early as 1 h after ip LPS, and reached a peak 1.5 h postadministration. The peak value ($1{,}099 \pm 88$ pg/ml) was statistically the same as after iv LPS. It is notable that the temporal profile of plasma TNF-α after ip LPS was well synchronized with the ACTH response. After the TNF-α response, plasma IL-6 started to increase 1.5 h after ip LPS and reached a peak 2.5 h postadministration. Even so, similarly to TNF-α, the peak level of IL-6 ($2{,}326 \pm 510$ pg/ml) after ip LPS was statistically indistinguishable from that after iv LPS. CRH levels in the PVN were significantly stimulated by LPS over the control values during the period between 14 : 15 and 15 : 15, and its peak level was observed 2.5 h after LPS (1 h after the peak ACTH response). Results of cytokine measurement in PVN perfusates after ip LPS were very similar to those after iv LPS. IL-1β levels in the PVN were not significantly stimulated by ip LPS compared to the values of the control group. By contrast, both IL-6 and TNF-α in the PVN were significantly stimulated by ip LPS with their peak levels occurring 3 or 3.5 h postadministration (1.5 or 2 h after the peak ACTH response), respectively.

Discussion

Kakucska *et al.* [4] have suggested that endogenous IL-1 in the brain may play a crucial role in mediating the HPA activation after ip LPS in the rat. However, in our study IL-1β was the only cytokine among the three examined whose level in the PVN did not significantly change after either iv or ip LPS. These results appear to weigh against a significant contribution of brain-derived IL-1β in HPA activation after systemic LPS treatment. The conclusion of Kakucska *et al.* [4] was derived from data of CRH mRNA at the PVN which was determined 8 h after ip LPS, and they did not examine the CRH gene expression at an earlier time point. Moreover, their time point of evaluation (8 h after LPS) appeared to be about 6–7 h later than the usual time of peak-ACTH responses expected after peripheral administration of LPS [2, 3]. Indeed, most of the previous studies examining IL-1 mRNA or its protein levels within the brain after systemic LPS treatment have reported a long time course (6–10 h) of endogenous IL-1 production [5–11]. Based on all these previous reports including our present data, we may conclude that the previously suggested important role of brain-derived IL-1 in modulating the CRH-ACTH response to peripheral administration of LPS [4] may not be true with an early, peak phase of ACTH secretion after either iv or ip LPS. It is to be noted that the increase of plasma IL-1β after iv and ip LPS was the smallest among the three cytokines, and moreover, the cytokine's elevation after ip LPS was statistically insignificant. This appears in agreement with previous reports that the plasma IL-1β response after LPS was small or not constantly detectable [20, 34]. Although Givalois *et al.* [35] have reported that iv LPS significantly stimulated circulating IL-1β levels, the cytokine's response to the smallest dose of LPS they used was considerably lower than those of IL-6 and TNF-α. At any rate, our present data do

not support the previous studies of Rivier *et al.* [17] and Schotanus *et al.* [18] in which a significant role of circulating IL-1 in LPS stimulation of the HPA axis was reported.

In contrast to the circulating IL-1β levels, both IL-6 and TNF-α in the plasma showed robust rises after both iv and ip LPS. In agreement with previous reports [35–37], our study also demonstrated that TNF-α was the first cytokine increased in plasma after both iv and ip LPS, later followed by IL-6. It is worth noting that the temporal profile of plasma TNF-α was well synchronized with that of plasma ACTH after both iv and ip LPS, although the elevation of plasma IL-6 was delayed. This temporal pattern of plasma TNF-α that we observed differs from that reported by Givalois *et al.* [35], since in their study neither IL-1β, IL-6, nor TNF-α had significantly increased in the plasma by the time plasma ACTH had already shown a significant rise. Although this discrepancy may be due to the different methods employed for TNF-α assay (bioassay by Givalois *et al.* [35] *vs.* a specific ELISA by us), it is likely that our data obtained by the specific immunoassay are more reliable. Even though fewer in number than the reports on IL-1β in the brain, several previous studies have examined the mRNA levels or bioactivity of IL-6 and TNF-α in the brain after systemic LPS treatment [7, 9, 13–16]. All these studies generally agreed that both IL-6 and TNF-α or their mRNA levels showed significant rises in the hypothalamus 1–3 h after LPS. These results do not disagree with the temporal profiles of the two cytokines in the PVN that we observed. However, irrespective of the route of LPS injection, the significant rises and peaks of the two cytokines in the PVN occurred later than the peak ACTH responses.

From all the findings in this study, we interpret our present data as suggesting that circulating TNF-α may play the most important role in triggering the early, peak phase of ACTH secretion after both iv and ip LPS, and that circulating IL-6, and IL-6 and TNF-α in the brain, may be involved in the later, protracted phase of ACTH secretion after LPS. The suggested most important role of circulating TNF-α appears in agreement with the conclusions reached by Ebisui *et al.* [36] and Turnbull and Rivier [38], who both stated that TNF-α in the periphery may be the most important proinflammatory cytokine mediating an early component of ACTH response after systemic LPS treatment. Although our study does not suggest a primary role of IL-6 as reported by Perlstein *et al.* [19], the dose of LPS given may be a crucial factor changing the relative contribution of various cytokines as they suggested. The recent demonstration of the existence of TNF-α receptors in the mouse pituitary [39] and also in AtT-20 cells, mouse pituitary-derived cell lines [40], suggests the possibility that the effect of promptly rising TNF-α in the bloodstream may be exerted at the level of the pituitary. In addition, since we have previously observed that iv administration of TNF-α promptly stimulates the release of CRH from the rat median eminence [26], such a hypothalamic action of TNF-α may also operate in the early phase of ACTH secretion after systemic LPS treatment. Although the receptors for IL-1β and IL-6 have also been demonstrated in the rodent anterior pituitary [41–45], our present data strongly suggest that the direct action of the cytokines on the pituitary may not be important in inducing the early, peak phase of ACTH response to LPS. It has been repeatedly shown that LPS itself is without effect on CRH [46, 47] and arginine vasopressin [47] release from rat hypothalamic fragments *in vitro*. However, mRNAs for IL-1β, IL-6, and TNF-α have all been demonstrated in the anterior pituitary as early as 1-2 h after peripheral administration of LPS [7, 9, 16]. Thus, a possible paracrine effect of such intrapituitary cytokines on ACTH secretion can also not be excluded, even though it has yet to be verified.

Recent reports suggest that intraabdominal vagus nerves may play an important role in mediating peripheral cytokine signaling to the brain. With respect to the HPA axis, Gaykema *et al.* [21] and Kapcala *et al.* [23] reported that subdiaphragmatic vagotomy prevented the plasma ACTH response to ip administration of LPS or IL-1β, respectively. In addition, Laye *et al.* [22] reported that the induction of IL-1β mRNA in the mouse brain after ip LPS was blocked by subdiaphragmatic vagotomy. Bearing these previous observations in mind, our second objective for the present study was to explore whether there exists a fundamental difference in the responses of IL-1β, IL-6, and TNF-α in the periphery and PVN to LPS, depending on its route of administration. The results were that both the magnitudes and temporal profiles (viewing the peak ACTH response as the landmark) of all the three cytokines in the plasma

and PVN, were essentially similar between iv and ip administrations of LPS. Even though our statistical analysis indicated a significant elevation of plasma IL-1β after iv but not ip LPS, the magnitude of the response was very similar between the two treatments. Our findings may, at least in part, be related to our ip dose of LPS, because the degree to which intra-abdominal vagus nerves [21] and also macrophages [48] may contribute to the HPA activation after ip LPS, have been reported to change depending on the dose of endotoxin given. Even so, it is interesting to note that the time of occurrence of peak CRH levels in the PVN, which may presumably represent *de novo* synthesis of the neurohormone following its profuse secretion from the nerve endings, was 1 or 2.5 h behind the peak ACTH levels after ip or iv LPS, respectively. Since the PVN is innervated by the nucleus tractus solitarii, the principal central structure receiving viscerosensory input [49], the possibility can not be excluded that the earlier occurrence of peak CRH levels in the PVN after ip LPS was a reflection of intraabdominal vagal stimulation by LPS.

In summary, in this study we examined for the first time the temporal patterns of IL-1β, IL-6, and TNF-α in the general circulation and the PVN after iv and ip administrations of LPS in the rat. It was suggested that circulating TNF-α may play the most important role in triggering the early, peak phase of ACTH secretion after both iv and ip LPS. Although it is possible that brain TNF-α and IL-6, and circulating IL-6, may mediate the later, protracted phase of ACTH secretion after LPS, our results suggest that the role of IL-1β in both the brain and periphery may be the smallest. In addition, under our present experimental conditions, there were no essential differences in the central and peripheral responses of the three cytokines after iv *vs.* ip administration of LPS.

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