Role of circulating endotoxin and interleukin-6 in the ACTH and corticosterone response to intraperitoneal LPS

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Lenczowski, M. J. P., A.-M. Van Dam, S. Poole, J. W. Larrick, and F. J. H. Tilders. Role of circulating endotoxin and interleukin-6 in the ACTH and corticosterone response to intraperitoneal LPS. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1870–R1877, 1997.—Peripheral administration of lipopolysaccharide (LPS) may activate the hypothalamus-pituitary-adrenal (HPA) axis by way of both neural and humoral mechanisms. We have investigated whether biologically active endotoxin appears in the general circulation after intraperitoneal administration of LPS (5 or 100 μg/kg) to rats and whether this is a prerequisite for activation of this HPA axis. Within 15 min, endotoxin appeared in the general circulation, whereas elevations of plasma adrenocorticotropic hormone (ACTH), corticosterone, and interleukin (IL)-6 concentrations were not detected until 90 min after LPS injection. At this time, a marked interindividual variation was observed in plasma concentrations of endotoxin, ACTH, corticosterone, and IL-6. Elevated levels of plasma endotoxin were associated with elevated levels of ACTH, corticosterone, and IL-6. Intravenous administration of the LPS antagonist cationic antimicrobial protein 18 (5 mg/kg), which did not affect cytokine production in the peritoneal cavity, markedly reduced plasma ACTH, corticosterone, and IL-6 levels after 5 μg/kg LPS. Our results suggest that circulating endotoxin is required for the activation of the HPA axis. They also favor a role for circulating IL-6 in this response.

interleukin-1β; adrenocorticotropic hormone; cationic antimicrobial protein

IN ANIMALS AND HUMANS, peripheral administration of gram-negative bacteria-derived lipopolysaccharides (LPS) induces an array of brain-mediated responses, including fever (14), sickness behavior (13), hyperalgesia (17), and activation of the hypothalamus-pituitary-adrenal (HPA) axis (3, 25). Proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, which are produced by cells of the immune system and other cells in response to LPS (7, 8), are considered to mediate these nonspecific symptoms of illness (26, 31, 32).

However, the signaling pathways by which LPS or proinflammatory cytokines affect the brain to induce these symptoms are still poorly understood. After intraperitoneal administration of LPS, primary vagal afferents have been described as crucial in the transduction of immune signals to the brain. Thus subdiaphragmatic vagotomy blocked the induction of sickness behavior (5) and the induction of hyperalgesia (39). In addition, vagotomy reduced the LPS-induced expression of Fos in hypothalamic corticotropin-releasing hormone neurons, as well as the concomitant plasma adrenocorticotropic hormone (ACTH) response (10). Subdiaphragmatic vagotomy also inhibited the elevation of plasma ACTH concentrations after intraperitoneal administration of the proinflammatory cytokine IL-1β (12), as well as IL-1β-induced hyperalgesia (40), fever (38), and sickness behavior (4). These results have led to the hypothesis that intraperitoneal administration of LPS stimulates the production of IL-1β in the peritoneal compartment, which, subsequently, stimulates primary sensory fibers of the vagal nerve.

After intravenous administration of LPS, humoral signaling pathways have been proposed to play a crucial role in the induction of nonspecific symptoms of illness. LPS or LPS-induced proinflammatory cytokines may act as humoral signals at the brain either by active transport across the blood-brain barrier, as demonstrated for IL-1α and IL-6 (1, 2), or by passing the barrier at the circumventricular organs (28). In addition, circulating endotoxin or IL-1β may affect brain functions indirectly by acting at cerebral endothelial cells. Indeed, after intravenous administration of LPS, immunoreactive prostaglandin E2 (PGE2), a major mediator of nonspecific symptoms of illness (18, 36), has been detected in the microvasculature of the brain (33). Furthermore, mRNA encoding for type I IL-1 receptors has been found to be associated with brain vasculature (9). Accordingly, with respect to PGE2 production, functional type I IL-1 receptors have been demonstrated on rat brain endothelial cells in vitro (34).

Taken together, the above observations support the view that the route of LPS administration determines the predominant signaling pathways involved in the induction of brain-mediated symptoms of illness. However, subdiaphragmatic vagotomy, which effectively blocked the plasma ACTH response to a low dose of intraperitoneally administered LPS, was only partially effective after a high dose of LPS administered by the same route (10). Therefore, it was speculated that low doses of intraperitoneally administered LPS signal the brain primarily by vagal afferents, whereas at high doses LPS might also activate humoral signaling pathways. Because it has been reported that endotoxin concentrations in rat arterial plasma increased gradually in time after the induction of peritonitis, despite a high endotoxin clearance capacity of the rat liver (22, 23), we postulate that endotoxin, even at low doses,
may reach the general circulation after intraperitoneal administration to activate vagal afferents.

In the present study, we tested whether intraperitoneally administered LPS can reach the general circulation and, if so, whether circulating endotoxin plays a role in the activation of the HPA axis and the appearance of the proinflammatory cytokines IL-1β and IL-6 in the blood. We determined plasma endotoxin concentrations, as well as plasma ACTH, corticosterone (Cort), and IL-6 concentrations, at various time intervals after intraperitoneal administration of LPS. We also studied the potential role of systemic bioactive endotoxin in the activation of the HPA axis and the induction of IL-1β and IL-6 production by inhibiting the actions of circulating endotoxin. For this, we used a 32-amino acid fragment of the LPS antagonist rabbit cationic antimicrobial protein 18 (CAP18).

MATERIALS AND METHODS

Animals

Male Wistar rats (Harlan CPB, Zeist, The Netherlands) were housed two per cage under controlled temperature (20–22°C) and light-dark conditions (lights on 7 AM, lights off 7 PM) for at least 1 wk before the experiments. Food and water were available ad libitum. At the time of the experiments the animals had body weights of 200–240 g. To reduce nonspecific activation of the HPA axis, rats were habituated to experimental procedures twice daily for 3 successive days before the experiments. The experimental protocol had been approved by the Institutional Committee for Animal Health and Care.

Materials

Lipopolysaccharide (LPS; Escherichia coli 055:B5, Westphal, 1 international unit (IU) equals 100 pg; Difco, Detroit, MI) was reconstituted in sterile pyrogen-free saline and stored at −20°C. Appropriate dilutions were prepared immediately before administration. LPS was injected intraperitoneally in a volume of 500 µl per rat.

A 32-amino acid fragment of the 18 kDa LPS antagonist rabbit cationic antimicrobial protein [CAP18(106–137)], produced by Larrick et al. (16) and designated CAP18 throughout this paper, was dissolved in sterile pyrogen-free saline and injected intravenously (500 µl) into the lateral tail vein. This fragment has been shown to inhibit LPS-induced release of cytokines and nitric oxide from macrophages in vitro, as well as to protect mice from LPS lethality (16).

Blood Collection

Animals were killed by decapitation and trunk blood was collected in ice-cold glass tubes that had been made endotoxin-free by heat treatment (200°C for 5 h). This procedure had also been applied to the glass funnels used for collecting the blood. The tubes contained dextran-poor sodium (Fragmin, 9 IU/ml of blood, Pharmacia, Uppsala, Sweden) as anticoagulant (0.6 IU endotoxin/1,000 IU heparin). Blood samples were centrifuged (2,000 g, 10 min, 4°C), and aliquots of plasma were stored at −20°C until assayed.

Peritoneal Lavage

Within 5 min after rats had been killed, 2 ml of cell culture medium (RPMI 1640, GIBCO BRL Life Technologies, Breda, The Netherlands) was injected intraperitoneally, after which the abdomen was gently massaged. Peritoneal lavage fluid was then collected in ice-cold tubes and centrifuged immediately to separate cells (2,000 g, 10 min, 4°C). Peritoneal lavage fluid was stored at −20°C until analyzed.

Assays

All disposables used during the analysis of plasma samples in which endotoxin concentrations were measured were pyrogen-free.

Endotoxin assay. Endotoxin concentrations in rat plasma were measured using a kinetic chromogenic Limulus amebocyte lysate (LAL) assay (Pyrochrome, Associates of Cape Cod, Woods Hole, MA), according to the supplier’s instructions. The First International Standard for Endotoxin (84/650, WHO) was used as a reference, and endotoxin concentrations are expressed as international units per milliliters. Before testing, samples were diluted 1:1,000 or 1:10,000 with endotoxin-free water to reduce interference of plasma components in the LAL assay. Accordingly, detection limits were 10 or 100 IU/ml plasma, respectively.

Hormone assays. Plasma ACTH concentrations were determined by radioimmunoassay (RIA) as previously described (21). Synthetic rat ACTH(1–39) (Peninsula Laboratories, Belmont, CA) was used as a standard, and human ACTH(1–39) (Peninsula) was used for labeling. Specific rabbit antiserum, directed to the mid-section of ACTH (15), was kindly provided by Dr. G. B. Makara (Institute for Experimental Medicine, Budapest, Hungary). The sensitivity of the RIA was 10 pg/ml plasma. Intra- and interassay variations were 5% and 8%, respectively.

Plasma Cort concentrations were determined, using a commercially available RIA (ImmuChem Double Antibody CORT kit, ICN Biomedicals). The sensitivity of the RIA was 1.0 ng/ml plasma. Intra- and interassay variations were 7 and 8%, respectively.

Cytokine assays. IL-6 bioactivity in plasma and peritoneal lavage fluid was measured using the IL-6-sensitive B9 cell line as described elsewhere (11). Using human recombinant IL-6 (lot 1449–10–01, CLB, Amsterdam, The Netherlands) as a standard, half-maximal stimulation of B9 cell proliferation was defined as one unit of IL-6 bioactivity. The sensitivity of the assay was 12.5 U/ml plasma. Intra- and interassay variations were 5% and 6%, respectively.

Rat IL-1β concentrations in plasma and peritoneal lavage fluid were measured using an enzyme-linked immunosorbent assay based on a protocol previously described (27). For this assay, 96-well microtiter plates (Nunc-Immuno Plate MaxiSorp, Roskilde, Denmark) were coated with immunoaffinity-purified sheep anti-rat IL-1β antibodies (NIBSC, 2 µg/ml) in 100 µl coating buffer (0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8.1 mM Na₂HPO₄; pH 7.2) for 16 h at 4°C. The plates were then washed three times with 200 µl dilution buffer (0.5 M NaCl, 2.5 mM NaH₂PO₄, 7.4 mM Na₂HPO₄, 0.1 % vol/vol Tween 20; pH 7.2). Samples and standards (100 µl) were added and incubated in duplicate for 16 h at 4°C. Standards were diluted in normal rat plasma or RPMI 1640, depending on the sample to be measured. Appropriate standard curves were included on each plate. Subsequently, the plates were washed three times with dilution buffer and incubated for 1 h at room temperature with biotinylated immunoaffinity-purified sheep anti-rat IL-1β antibodies (NIBSC) in 100 µl dilution buffer, containing 1% normal goat serum (DAKO A/S, Glostrup, Denmark). After the plates had been washed three times with dilution buffer, streptavidin, conjugated to poly-horseradish-peroxidase (Poly-HRP-Strepta-E⁺, 20 ng/ml, CLB, Amsterdam, The Netherlands) was added in 100 µl dilution buffer and wells were incubated for 30 min at room tempera-
tured. *P, presented as median values. Significance was defined at the level of nonparametrically by the Mann-Whitney test and repre-

The reaction was stopped with 150 μl 1.0 M H2SO4, and optical densities were determined at 495 nm using a microplate reader (ICN Biomedicals, Zoetermeer, The Netherlands). The sensitivity of the assay was 1.5 pg/ml plasma. The intra-assay variation was 6%.

Statistical Analysis

Data were analyzed by two-way analysis of variance (2-way ANOVA), followed by a t-test for independent samples when values were normally distributed. If necessary, data were log transformed before analysis. IL-1β data could not be transformed to meet criteria of ANOVA and were, therefore, tested nonparametrically by the Mann-Whitney U test and represented as median values. Significance was defined at the level of P ≤ 0.05. The statistical evaluation was carried out using the SPSS statistical program (release 6.1) for Windows.

RESULTS

Plasma Endotoxin, ACTH, Cort, and IL-6 Concentrations After Intraperitoneal Administration of LPS

To determine alterations in plasma endotoxin, ACTH, Cort, and IL-6 concentrations after intraperitoneal administration of LPS, 30 male Wistar rats were divided into five groups (n = 6). Animals from four groups were injected with LPS (100 μg/kg body wt ip) and decapitated 5, 15, 30, or 90 min thereafter. Control animals (0 min) did not receive LPS. As shown in Fig. 1, endotoxin appeared in the general circulation after 15 min and reached an average plasma concentration of 1,480 IU/ml after 90 min. This equals ~150 ng/ml plasma, demonstrating that the amount of endotoxin in the general circulation represented ~10% of the injected dose of LPS. In all groups, one or more animals had plasma endotoxin concentrations below the detection limit of the LAL assay (100 IU/ml plasma). Plasma endotoxin concentrations from control animals (0 min) were all below this detection limit.

In rats killed 5, 15, and 30 min after LPS administration, plasma concentrations of ACTH, Cort, and IL-6 were not different from control values (Fig. 2). However, markedly elevated plasma ACTH, Cort, and IL-6 concentrations were observed 90 min after intraperitoneal LPS injection. Thus the appearance of endotoxin in the general circulation preceded the elevation of plasma ACTH, Cort, and IL-6 concentrations. At 90 min, a large interindividual variation preceded the elevation of plasma endotoxin and in plasma ACTH, Cort, and IL-6 concentrations. At 90 min, a large interindividual variation preceded the elevation of plasma endotoxin and in plasma ACTH, Cort, and IL-6 concentrations. As shown in Fig. 3, three of six rats (triangles) had plasma ACTH (Fig. 3A), Cort (Fig. 3B), and IL-6 (Fig. 3C) concentrations similar to those observed in control animals. These “nonresponding” animals all had plasma endotoxin concentrations below the detection limit of the LAL assay. In contrast, “responding” animals with elevated plasma ACTH, Cort, and IL-6 levels all had detectable endotoxin concentrations in the general circulation. These results suggest a correlation between plasma endotoxin concentrations and plasma ACTH, Cort, or IL-6 concentrations. However, because endotoxin concentrations in “nonresponding” animals were undetectable as a result of the detection limit of the LAL assay, correlation coefficients could not be computed. Therefore, we will discuss the data on the basis of an “association” between the appearance of detectable amounts of endotoxin in the circulation and elevated plasma ACTH, Cort, and IL-6 concentrations.
Effect of CAP18 on LPS-Induced Activation of the HPA Axis and Cytokine Production

To investigate whether the presence of biologically active endotoxin in the general circulation is a prerequisite for the appearance of proinflammatory cytokines in the blood and/or for the activation of the HPA axis, we studied the effect of blockade of the actions of circulating endotoxin by intravenous administration of the LPS antagonist CAP18. For this, 48 rats were divided into six groups (n = 8). Animals from three groups were pretreated with CAP18 (5 mg/kg body wt iv), whereas the other animals received sterile pyrogen-free saline (iv). Four minutes later, animals were injected with 5 or 100 µg/kg body wt ip LPS or with sterile pyrogen-free saline. Ninety minutes later, rats were killed by decapitation, and trunk blood, as well as peritoneal lavage fluid, was collected.

Plasma endotoxin concentrations. In CAP18-pretreated rats, plasma endotoxin concentrations could not be determined because of interference of CAP18 in the LAL assay. In vehicle-pretreated rats, intraperitoneal administration of 5 and 100 µg/kg LPS resulted in mean endotoxin concentrations of 120 ± 31 and 5,427 ± 1,614 IU/ml plasma, respectively. Again, at 100 µg/kg LPS, an association between the appearance of endotoxin in the general circulation and elevated plasma ACTH, Cort, IL-6, and IL-1β concentrations was observed (Fig. 3, circles). A similar association was observed at the lower dose of LPS. Seven rats with plasma endotoxin concentrations above those observed in control animals had increased plasma ACTH, Cort, and IL-6 concentrations, whereas the one rat with a control level of plasma endotoxin also had control levels of these plasma variables (data not shown). Moreover, at both concentrations of LPS, a complete association existed between the appearance of IL-6 in plasma and the appearance of elevated plasma ACTH and Cort concentrations.

Plasma ACTH and Cort concentrations. Intraperitoneal LPS administration (5 or 100 µg/kg) significantly increased plasma ACTH and Cort concentrations (2-way ANOVA, P < 0.001; Fig. 4, A and B). CAP18 pretreatment markedly reduced the ACTH response to 5 µg/kg LPS (P < 0.01), but did not attenuate the ACTH response to 100 µg/kg. Similarly, CAP18 reduced plasma Cort concentrations (P = 0.05) in response to the lower dose of LPS, but not in response to the higher dose.

Plasma IL-1β and IL-6 concentrations. Ninety minutes after intraperitoneal administration, the low dose of LPS (5 µg/kg) did not affect plasma IL-1β concentrations (Fig. 4C). After injection of the higher dose of LPS (100 µg/kg), median plasma IL-1β concentrations increased from 1.5 (saline-treated animals) to 52 pg/ml (LPS-treated animals). As illustrated, CAP18 markedly reduced plasma IL-1β concentrations induced by this dose of LPS (Mann-Whitney U test, P = 0.05, corrected for ties).

As shown in Fig. 4D, LPS significantly elevated plasma IL-6 concentrations (2-way ANOVA, P < 0.001). In vehicle-pretreated animals, mean plasma IL-6 concentrations increased from <12.5 to 1,720 and 8,466 U/ml after intraperitoneal administration of 5 and 100 µg/kg LPS, respectively (P < 0.05). CAP18 significantly reduced plasma IL-6 concentrations induced by 5 µg/kg LPS (P < 0.05), but not by 100 µg/kg LPS.

IL-1β and IL-6 concentrations in peritoneal lavage fluid. To investigate whether intravenous administration of CAP18 affected LPS-induced cytokine concentra-
tions in the peritoneal cavity, levels of IL-1β and IL-6 were measured in cell-free peritoneal lavage fluid. Intraperitoneal administration of 5 and 100 µg/kg LPS to vehicle-pretreated animals induced high levels of both cytokines in the lavage fluid, which were already maximal at the lower dose of LPS (Fig. 5). Concentrations of IL-1β ranged from 19 ± 6 in saline-treated rats to 681 ± 699 and 857 ± 125 pg/ml in 5 and 100 µg/kg LPS-treated rats, respectively, whereas in the same groups of animals IL-6 concentrations ranged from 12.5 to 10,825 ± 3,029 and 17,668 ± 3,156 U/ml. These concentrations were not significantly affected by CAP18, indicating that intravenous administration of this LPS antagonist did not affect LPS-induced cytokine production within the peritoneal cavity.

DISCUSSION

We have shown that intraperitoneally administered LPS reaches the general circulation and that the appearance of endotoxin in the blood is a prerequisite for the appearance of the proinflammatory cytokine IL-6 in the general circulation, as well as for the activation of the HPA axis.

Previously, we reported that plasma ACTH, Cort, and IL-6 concentrations peaked ~2 h after intraperitoneal administration of LPS. In the present study we show that the appearance of endotoxin in the plasma preceded the IL-6 and HPA responses. In addition, 90 min after intraperitoneal injection of both a relatively low (5 µg/kg) and a relatively high dose of LPS (100 µg/kg), our results demonstrate a marked interindividual variation in plasma endotoxin concentrations. This variation could not be explained by a biotechnical failure (unpublished studies involving intraperitoneal injection of blue dextran). Moreover, without exception, all responding and nonresponding animals had elevated levels of IL-1β and IL-6 in peritoneal lavage fluid, which suggests that LPS was appropriately injected. These observations led us to postulate that in most animals bioactive endotoxin reaches the general circulation after intravenous injection.
intraperitoneal injection of LPS, whereas in some animals it does not. The nature of these individual differences remains speculative, although similar observations have previously been published (23). Irrespective of its exact nature, our results show that the appearance of detectable amounts of biologically active endotoxin in the blood coincided with elevated plasma ACTH, Cort, and IL-6 concentrations. Because direct administration of LPS into the general circulation is known to activate the HPA axis and to induce elevated plasma levels of IL-1β and IL-6 (6, 19), we considered the possibility that circulating endotoxin in our experiments may in fact mediate these responses.

Indeed, administration of the LPS antagonist CAP18 into the general circulation significantly reduced plasma ACTH, Cort, and IL-6 concentrations induced by the low dose of LPS (5 µg/kg). Similarly, in mice, systemic anti-LPS antibodies markedly reduced plasma TNF-α, IL-1, and IL-6 levels after intraperitoneal injection of live E. coli bacteria (41). In contrast to its effects on the low dose of LPS, CAP18 did not affect plasma ACTH, Cort, or IL-6 responses to 100 µg/kg ip LPS, possibly because the dose of CAP18 (5 mg/kg) was insufficient to inactivate all circulating endotoxins. It should be noted that 5 µg/kg is already a maximally effective dose of intraperitoneally administered LPS for activation of the HPA axis (29) and that mean plasma endotoxin levels after the high dose of intraperitoneally administered LPS were 50-fold higher than those observed after intraperitoneal administration of the low dose. A limited availability of CAP18 prevented us from testing higher CAP18 doses. Surprisingly, compared with the other variables, the induction of detectable concentrations of IL-1β in the general circulation was less sensitive to intraperitoneal administration of CAP18 than 5 µg/kg LPS, because 5 µg/kg LPS was insufficient to increase plasma IL-1β concentrations. Accordingly, plasma IL-1β concentrations were reduced by CAP18 at the higher dose of LPS, suggesting that inactivation of only a part of the circulating endotoxin was sufficient to diminish the increase in plasma IL-1β concentrations.

Results from several studies suggest that IL-1β may act as an intermediate in the LPS-induced activation of the HPA axis. For example, intraperitoneal administration of the IL-1 receptor antagonist (IL-1ra) inhibited the ACTH and Cort responses to intraperitoneally administered LPS (29). These results are in line with other data, suggesting that IL-1β in the peritoneal cavity may directly stimulate primary sensory fibers of the vagal nerve to activate the HPA axis (12) as well as to induce other nonspecific symptoms of illness (4, 38, 40). However, our results, showing that nonresponding rats still had high levels of both IL-1β and IL-6 in the peritoneal lavage fluid, challenge the proposed role for peritoneally derived IL-1β (or IL-6) in the activation of the HPA axis after intraperitoneal LPS administration. Our results also do not support a role for circulating IL-1β in the activation of the HPA axis after intraperitoneal LPS administration. First, after administration of a maximally effective dose of LPS (5 µg/kg) for HPA axis activation (29), plasma IL-1β concentrations were <1.5 pg/ml (equivalent to ~0.1 pM) and are unlikely to represent biologically active concentrations. Second, at the higher dose of LPS, CAP18 reduced plasma IL-1β concentrations, whereas plasma ACTH and Cort levels remained unaffected. Third, also at shorter times intervals (30 and 60 min) after intraperitoneal LPS administration (5 µg/kg), IL-1β was not detected in plasma (own unpublished observations), which excludes the possibility that we missed the plasma IL-1β response at 90 min. In line with our results, other investigators also questioned a role for plasma IL-1β in the LPS (ip)-induced activation of the HPA axis (7).

Another proinflammatory cytokine that may be involved in the activation of the HPA axis in response to LPS is IL-6. Intravenous administration of IL-6 markedly stimulated the ACTH and Cort release in rats (20, 35), but its potency (on the basis of weight) was shown to be two- to fivefold lower than that of IL-1β (20; own unpublished results). However, in the present experiments, plasma IL-6 concentrations were ~2–8 ng/ml (1 U of IL-6 equals ~1 pg of IL-6), which was almost 200 times higher than plasma concentrations of IL-1β. Therefore, IL-6 is most likely to play a signaling role in the LPS-induced activation of the HPA axis. Moreover, the LPS dose-response characteristics of plasma IL-6 concentrations (and not those of plasma IL-1β) were similar to those of plasma ACTH and Cort concentrations in that 5 µg/kg LPS induced maximal responses for all three parameters. Furthermore, the reduction in plasma ACTH and Cort concentrations by CAP18 at the lower dose of LPS was accompanied by a reduction in plasma IL-6 concentrations, whereas at the higher dose of LPS, like plasma ACTH and CORT concentrations, plasma IL-6 concentrations remained unaffected by CAP18. These observations, together with the association between the presence of IL-6 in the general circulation and the induction of plasma ACTH and Cort, indicate a possible intermediate role for plasma IL-6 in the activation of the HPA axis.

Both doses of LPS induced high levels of IL-1β and IL-6 in the peritoneal lavage fluid, with plateau levels reached after 5 µg/kg LPS. Therefore, it is unlikely that IL-1β is readily transported from the peritoneal cavity to the general circulation, because IL-1β could not be detected in the plasma after the low dose of LPS. In addition, CAP18 did not affect cytokine levels in the peritoneal cavity, whereas it suppressed plasma concentrations of both IL-1β and IL-6. These data further support the idea that cytokines produced in the peritoneal cavity remain locally in the time frame of our experiment and that cytokines in the blood are primarily derived from endothelial cells or monocytes outside the peritoneal cavity (24, 37).

Taken together, the present results suggest that intraperitoneal administration of a low dose of LPS activates the HPA axis in a manner dependent on the presence of endotoxin in the blood. Moreover, circulating IL-6, rather than circulating IL-1β, seems to play an intermediate role in the activation of this axis. Because results from earlier studies have suggested that low-dose LPS-induced ACTH secretion is mediated...
by the vagal nerve, we postulate that vagal afferents are activated by circulating, rather than by peripherally derived, endotoxins or cytokines, which is supported by recent observations showing that endotoxin in the circulation, or systemically induced pro-inflammatory mediators, may induce nonspecific symptoms of illness by a neural route (30).

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