Endogenous brain IL-1 mediates LPS-induced anorexia and hypothalamic cytokine expression

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Laye´, Sophie, Gilles Gheusi, Sandrine Cremona, Chantal Combe, Keith Kelley, Robert Dantzer, and Patricia Parnet. Endogenous brain IL-1 mediates LPS-induced anorexia and hypothalamic cytokine expression. Am J Physiol Regulatory Integrative Comp Physiol 279: R93–R98, 2000.—The present study was designed to determine the role of endogenous brain interleukin (IL)-1 in the anorexic response to lipopolysaccharide (LPS). Intraperitoneal administration of LPS (5–10 µg/mouse) induced a dramatic, but transient, decrease in food intake, associated with an enhanced expression of proinflammatory cytokine mRNA (IL-1β, IL-6, and tumor necrosis factor-α) in the hypothalamus. This dose of LPS also increased plasma levels of IL-1β. Intracerebroventricular pretreatment with IL-1 receptor antagonist (4 µg/mouse) attenuated LPS-induced depression of food intake and totally blocked the LPS-induced enhanced expression of proinflammatory cytokine mRNA measured in the hypothalamus 1 h after treatment. In contrast, LPS-induced increases in plasma levels of IL-1β were not altered. These findings indicate that endogenous brain IL-1 plays a pivotal role in the development of the hypothalamic cytokine response to a systemic inflammatory stimulus.

hypothalamus; lipopolysaccharide; interleukin-1; receptor antagonist; food intake

PROINFLAMMATORY CYTOKINES mediate the local and systemic components of the acute-phase response. Interleukin (IL)-1 is one of the key cytokines for the development of the centrally mediated signs of sickness, including depression of food intake and food-motivated behavior (8, 28). Peripheral and central administration of recombinant IL-1β decreases food intake in rats and mice (21, 26). This effect is abrogated by pretreatment with the IL-1 receptor antagonist (IL-1ra) (19, 25, 27). IL-1β is synthesized in the hypothalamus, together with other proinflammatory cytokines including tumor necrosis factor-α (TNF-α) and IL-6, in response to peripheral inflammatory stimuli (11, 23). Proinflammatory cytokines act in a network fashion at the periphery, meaning that each cytokine induces its own synthesis and the synthesis of other cytokines that potentiate or oppose its effects. The possibility that a similar network exists in the brain has been suggested by time-course studies of the expression of transcripts of proinflammatory cytokines in the brain in response to peripheral administration of the cytokine inducer lipopolysaccharide (LPS). Intraperitoneal administration of a behaviorally active dose of LPS increases the expression of TNF-α and IL-1β mRNA in the brain within 1 h after the treatment, and 3 and 6 h later IL-6 and IL-1ra mRNA are expressed (11, 23).

To determine whether endogenous brain IL-1 plays a pivotal role in LPS-induced behavioral depression, we measured food intake and proinflammatory cytokine mRNA expression in the hypothalamus of mice that were treated by a peripheral injection of LPS and a central injection of IL-1ra. To ensure that centrally injected IL-1ra blocked exclusively brain IL-1β, we measured circulating levels of IL-1β.

We confirmed that LPS induced a decrease in food intake that was accompanied by an increase in proinflammatory cytokine synthesis in the hypothalamus. LPS-induced anorexia was partially blocked by central administration of IL-1ra. LPS-induced cytokine mRNA expression in the hypothalamus was fully blocked by IL-1ra, whereas increased plasma levels of IL-1β were not affected.

MATERIALS AND METHODS

Animals and materials. Seven-week-old male mice of the CD-1 (ICR) BR strain (Charles River), weighing 30–35 g at the start of the experiment, were housed in polypropylene cages in a room that was maintained at 23 ± 1°C. Lights were turned on from 8 PM to 8 AM, and food and water were available ad libitum except for food intake experiments. LPS (Escherichia coli, serotype 0127:B8) was obtained from Sigma Chemical (St. Louis, MO). This serotype was used because it had been demonstrated to reliably increase body temperature and metabolic rate in the rat, despite the fact that the rat is relatively insensitive to endotoxin (15). Recombinant mouse IL-1β and sheep antibodies to mouse IL-1β were obtained from National Institute for Biological Standards and Control (Potters Bar, UK). IL-1ra was obtained from Synergen (Boul-

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Twenty microliters of cDNA products were separated according to size. The amount of radioactivity incorporated was quantified with a PhosphorImager (Molecular Dynamics). To compare the expression of cytokine mRNAs in the different experimental groups, results were expressed as the ratio of cytokines per insert to β2-microglobulin per insert × 100.

RESULTS

Baseline food intake did not differ between treatments (P > 0.05; Fig. 1). LPS significantly depressed food intake for the duration of measurement (P < 0.001). Intracerebroventricular administration of IL-1ra significantly attenuated the depression in food intake induced by LPS at 4, 6, 8, and 10 h after treatment (P < 0.05). Mice treated with IL-1ra intracerebroventricularly and LPS intraperitoneally ate significantly less than mice treated with saline intracerebro-
ventricularly and intraperitoneally when food intake was measured 8 and 10 h after treatment (P < 0.05). These results show that intracerebroventricular administration of IL-1ra attenuated the decrease in food intake induced by LPS.

To assess the role of IL-1 in the induction of the expression of proinflammatory cytokines in the hypothalamus in response to LPS, mice were pretreated intracerebroventricularly with IL-1ra or saline just before they were injected intraperitoneally with LPS (10 µg/mouse) or saline. The effect of the different treatments under study was assessed by semiquantitative RT-PCR with 10 pg of insert per sample. Figure 2 illustrates a typical RT-PCR gel showing a weak basal expression of IL-1β, IL-6, and TNF-α mRNAs and a marked induction of the expression of cytokine mRNAs in the hypothalamus in response to LPS. pMus amplification was the same in all experimental groups. After intracerebroventricular IL-1ra treatment, LPS-induced cytokine mRNA expression was attenuated (Fig. 2). In all cases, the expression of β2-microglobulin, a housekeeping gene, was not affected by the treatments. Figure 3 illustrates in a quantitative manner the levels of IL-1β, IL-6, and TNF-α mRNA expressed as percentage of β2-microglobulin mRNA. A one-way ANOVA revealed a significant effect of the treatments on the expression of the different cytokines under study: IL-1β (P < 0.05), IL-6 (P < 0.01), and TNF-α (P < 0.01). When administered alone, LPS significantly induced IL-1β (P < 0.05), IL-6 (P < 0.05), and TNF-α (P < 0.01) mRNA expression in the hypothalamus of mice compared with saline injection. IL-1ra injected intracerebroventricularly abrogated LPS-induced IL-1β, IL-6 mRNA, and TNF-α mRNA expression in the hypothalamus, as evidenced by the comparison of the intraperitoneal LPS-intracerebroventricular saline and intraperitoneal LPS-intracerebroventricular IL-1ra groups (IL-1β (P < 0.05), IL-6 (P < 0.01), and TNF-α (P < 0.01)).

To ensure that the effect of IL-1ra was specific to the brain, IL-1β levels were measured in the plasma of IL-1ra-treated and nontreated mice. IL-1β levels were very low in the plasma of saline-treated mice (~8 pg/ml). LPS treatment dramatically increased plasma levels of IL-1β (saline ip-saline icv vs. LPS ip-saline icv, P < 0.01; saline ip-IL-1ra icv vs. LPS ip-IL-1ra icv, P < 0.001), and this increase was not significantly altered by intracerebral administration of IL-1ra (Fig. 4).

**DISCUSSION**

The findings of the present study show that blockade of brain IL-1 receptor activation by intracerebroven-
IL-1ra in the brain of LPS-treated rats was detected by other authors (4, 31). The sensitivity of the technique used for measuring IL-1β is likely to be critical, since the levels of IL-1β that occur in the brain in response to peripheral LPS treatment are very low.

In the present study we used semiquantitative RT-PCR to accurately determine relative amounts of transcripts. The validity of this technique was first checked to confirm the lack of a plateau amplification of the internal standard (pMus3) and cDNA and to ensure that β2-microglobulin mRNA levels remained stable in hypothalamus of mice injected systematically with LPS. Because this was the case, the results were expressed as the ratio of cytokine amplification to β2-microglobulin × 100. As previously demonstrated, intraperitoneal administration of LPS induced expression of cytokine mRNA in the hypothalamus of mice 1 h after treatment (22). The basal expression of proinflammatory cytokines in the hypothalamus was relatively high in the present study compared with our observations in previous studies (22, 23). This difference could be due to a propagation of the cytokine signal induced by implantation of the cannula into the brain (18, 35).

However, this possibility still needs to be checked by a direct comparison of cytokine expression in the brain of implanted and nonimplanted mice. Despite this increase in basal levels of cytokines, exogenous injection of IL-1ra in the lateral ventricle of the brain was able to fully block the LPS-induced expression of IL-1β, TNF-α, and IL-6 mRNAs. Contradictory data have been reported concerning the effect of intracerebroventricular IL-1ra on LPS-induced IL-6 protein (1, 24). In the rat, Luheeshi et al. (24) could not detect any effect of intracerebroventricularly injected IL-1ra on LPS-induced IL-6 in the cerebrospinal fluid, whereas it was blocked in the cat (1). In both studies, IL-6 was measured by ELISA. Inasmuch as more cerebrospinal fluid can be collected from the brain of a cat than from the brain of a rat, it might be easier to pick up significant differences due to IL-1ra in the cat than in the rat.

In terms of mechanisms of action, IL-1ra binds to both types of IL-1 receptors: type I (IL-1RI), which is the active receptor, and type II (IL-1RII), which acts as a decoy target for IL-1 (2, 5). IL-1ra abrogates the effect of IL-1 on its receptors by preventing the formation of the complex IL-1RI–IL-1–IL-1R accessory protein, which is thought to be the transducing receptor complex (14, 34). Although these data have been obtained in peripheral immune and nonimmune cells, there is evidence that brain cells also express IL-1RI, IL-1RII, and IL-1RacP that do not differ from peripheral IL-1 receptor subtypes (10, 12, 17) and that the in vivo effects of IL-1 in the brain are mediated by IL-1RI (6, 30) and its accessory protein (unpublished observation), whereas the brain IL-1RII downregulates the effects of IL-1 (7).

Fig. 4. Plasma concentration of IL-1β (pg/ml) measured by ELISA 1 h after intracerebroventricular injection of saline or IL-1ra followed by intraperitoneal saline (open bars) or LPS (shaded bars). Values are means of 5 independent measures. **P < 0.01; ***P < 0.001 compared with intraperitoneal saline-intracerebroventricular saline.
The importance of hypothalamic IL-1 in the induction of proinflammatory cytokines differs from what occurs at the periphery, where TNF-α is usually considered to play a central role in inducing the release of IL-1 and IL-6 during bacterial infections (9). Accordingly, time-course studies confirmed that, in response to LPS, the release of IL-1 and IL-6 was maximal when the levels of TNF-α were declining (33). The present findings, therefore, reinforce the concept that the brain differs from the periphery in the way the cytokine network is activated (22).

In summary, the results obtained in the present study demonstrate that endogenous hypothalamic IL-1 plays a pivotal role in the organization of the neural components of the host response to infection.

Perspectives

Many data on the expression of proinflammatory cytokines in the brain in response to peripheral inflammatory stimuli have been collected during the last decade. However, because of the redundant properties of these cytokines, little was known about their relative importance in the organization of the brain response to systemic insults. The present findings are the first to demonstrate the key role of brain IL-1 in the organization of the hypothalamic cytokine network. These results are important, since they indicate that IL-1 is certainly a better target molecule for controlling inflammation in the brain than at the periphery.

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REFERENCES

24. Plata-Salaman CR, Oomura Y, and Kai Y. Tumor necrosis factor and interleukin-1β suppression of food intake by direct


