Role of central IL-1 in regulating peripheral IGF-I during endotoxemia and sepsis

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Lang, Charles H., Jie Fan, Margaret M. Wojnar, Thomas C. Vary, and Robert Cooney. Role of central IL-1 in regulating peripheral IGF-I during endotoxemia and sepsis. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R956–R962, 1998.—Inflammatory cytokines may mediate the host response to infection via central nervous system, endocrine, and/or paracrine/autocrine signaling mechanisms. Previous studies have shown that intravenous administration of interleukin (IL)-1β alters the concentration of the anabolic hormone insulin-like growth factor (IGF)-I in plasma and various tissues. The purpose of the present study was to determine 1) whether the intracerebroventricular injection of IL-1β can influence peripheral IGF-I levels in control animals and 2) whether the central administration of a IL-1 receptor antagonist (IL-1ra) can prevent the changes in peripheral IGF-I induced by endotoxin [lipopolysaccharide (LPS)] or sepsis produced by cecal ligation and puncture. In the first experiment, injection of IL-1β (100 ng/rat) decreased IGF-I levels in plasma, liver, and gastrocnemius muscle 28–36% by 1.5 h in conscious fasted rats. IGF-I levels remained reduced at 3 h, but returned to baseline by 6 h. IGF-I content was not altered in soleus, kidney, spleen, intestine, or whole brain after IL-1β. In the second series of experiments, LPS injected intravenously decreased IGF-I levels in plasma, liver, and gastrocnemius at 1.5 h, and levels were even further reduced at 3 and 6 h in these tissues (59, 57, and 48%, respectively). Moreover, the IGF-I content was also decreased in soleus (30–35%) and increased in kidney (2- to 3-fold) after LPS. In the third experiment, changes in IGF-I levels in plasma and tissues, similar to those seen in LPS-treated rats, were detected 24 h after induction of peritonitis. Intracerebroventricular infusion of IL-1ra did not alter any of the changes in IGF-I produced by either LPS or sepsis, although it did attenuate the concomitant changes in growth hormone levels. These data suggest that, although central IL-1β is capable of modulating peripheral IGF-I levels, central administration of IL-1ra was unable to modulate the changes in peripheral IGF-I in blood and tissues produced by either endotoxemia or peritonitis.

interleukin-1; interleukin-1 receptor antagonist; insulin-like growth factor I; endotoxin; growth hormone; insulin; corticosterone; intracerebroventricular injection; rats

INSULIN-LIKE GROWTH FACTOR (IGF)-I is a critical anabolic hormone that can function as a classical endocrine hormone or as a paracrine/autocrine mediator of metabolism. The concentration of IGF-I in the blood and selected target tissues is diminished during a variety of stress/inflammatory conditions (7, 8, 15, 27). Cytokines have been implicated in mediating the host response to inflammation. In this regard, the intravenous administration of interleukin (IL)-1β mimics the changes produced in the IGF system produced during endotoxemia (9). In addition, IL-1β attenuates both basal and growth hormone-stimulated IGF-I synthesis and secretion in isolated hepatocytes (28). It has been suggested that reduced IGF-I levels are responsible for part of the muscle wasting characteristic of the septic response. Studies have shown that systemic administration of a specific IL-1 receptor antagonist (IL-1ra) prevents the sepsis-induced decrease in IGF-I content in muscle, and this response was associated with an enhanced rate of protein synthesis in this tissue (15). Collectively, these results provide evidence that circulating IL-1β is an important mediator of IGF-I during at least one type of catabolic illness.

Inflammatory cytokines may mediate the host response to infection via the central nervous system (CNS) as well as through endocrine or paracrine/autocrine signaling mechanisms. Cytokines present in the systemic circulation can gain access to the CNS through areas in brain lacking the blood-brain barrier and via specific saturable transport mechanisms (1). Furthermore, systemic administration of bacteria or bacterial cell wall products also increases the mRNA content and protein concentration of various proinflammatory cytokines within the CNS (10, 21, 24). However, the relative importance of central cytokine production in mediating the metabolic sequelae to infection remains largely unknown. Of the cytokines produced centrally, IL-1 appears to be responsible for many of the behavioral and metabolic responses to bacterial challenge. It is well established that intracerebral administration of IL-1α or IL-1β produces a metabolic response similar to that seen during infection (11, 16, 23). Moreover, the local coadministration of IL-1ra can abrogate some, but not all, aspects of the host response (13, 14, 23). Although previous studies have shown that intravenous administration of IL-1β is capable of altering the concentration of IGF-I in plasma and various tissues, the relative importance of IL-1 within the CNS in regulating peripheral IGF-I levels has not been defined. Hence, the purpose of the present study was to determine 1) whether central administration of IL-1β into naive rats alters peripheral IGF-I levels and 2) whether the intracerebroventricular infusion of IL-1ra, which inhibits endogenous IL-1 activity within the brain, prevents the changes in IGF-I produced acutely by endotoxin [lipopolysaccharide (LPS)] or chronically by sepsis.

MATERIALS AND METHODS

Experimental protocols. Male Sprague-Dawley rats (325–350 g; Taconic Farms, Germantown, NY) were housed at a constant temperature, exposed to a 12:12-h light-dark cycle,
and maintained on standard rodent chow and water ad libitum for 1 wk. Subsequently, stereotaxic surgery was performed to implant a cannula unilaterally into the lateral ventricle of the brain, as previously described in detail (14, 16). After surgery, animals were placed in individual cages and allowed to recover for 7 days. All animals used in these experiments had regained presurgical body weight and had normal food and water consumption for at least 3 days before the experiment. On the day before starting the experiment, animals were fasted overnight but were provided water ad libitum. All experiments were started the following morning between 0800 and 0900.

In the first series of experiments, rats received a nonlethal intracerebroventricular injection of recombinant human IL-1β (100 ng/rat; Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute) or an equal volume (5 µl) of sterile artificial cerebrospinal fluid (aCSF). Another group of animals was injected intravenously with the same dose of IL-1β to determine whether any of the observed effects of the intracerebroventricular injection of IL-1β resulted from the translocation of the centrally administered cytokine into the peripheral circulation. A small number of animals (n = 4) were injected centrally with IL-1β that was inactivated by heating at 100°C for 60 min. Animals were killed by decapitation at 1.5, 3, or 6 h after injection. These time points and the dose of IL-1β used were chosen based on previous investigations conducted by our laboratory using intracerebroventricular and intravenous administration of IL-1 demonstrating an activation of the hypothalamic-pituitary-adrenal axis and modulation of the growth hormone-IGF axis (9, 16).

In the second series of experiments, rats received an intracerebroventricular infusion of IL-1ra (2 mg/kg 2 mg·kg⁻¹·h⁻¹; Amgen, Boulder, CO) or the same volume (5 µl + 5 µl/h) of sterile aCSF. The primed, constant infusion of IL-1ra was started 30 min before the intravenous injection of Escherichia coli LPS (100 µg/100 g body wt; 026:B6; Difco, Detroit, MI) and continued throughout the remainder of the experimental protocol. Rats were lightly restrained by wrapping them in a towel for no longer than 1–2 min and LPS was injected into a tail vein, after which animals were returned to their individual cages. Animals injected intracerebroventricularly with IL-1ra and with saline intravenously (i.e., drug control) were not used in this study because previous studies have shown that IL-1ra itself does not alter the levels of IGF-I or IGF binding proteins in control animals (15). Animals were then killed at selected intervals.

In the third experimental series, peritonitis was produced by cecal ligation and a single puncture (18-gauge needle) (CLP), as described by Wicherman et al. (26). A laparotomy with intestinal handling was performed on control animals. Three groups of animals were used: sham control + aCSF (intracerebroventricular), septic + aCSF (intracerebroventricular), and septic + IL-1ra (intracerebroventricular). All animals were fasted, and fluid resuscitation consisted of 30 ml/kg of 0.9% saline administered subcutaneously. As in the second series of experiments, there was no group of control animals that received IL-1ra. The infusion of IL-1ra was started immediately after CLP and continued for the next 24 h, at which time animals were killed. During this experimental period, the morality of these groups was ~0% (0 of 7), 22% (2 of 9), and 12% (1 of 8). All experiments were approved by the Institutional Animal Care and Use Committee at the Pennsylvania State College of Medicine and adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

In all experiments, blood was collected at the time of death in chilled heparinized tubes. Blood was centrifuged (13,000 g for 2 min, 4°C) and plasma was collected for determination of total IGF-I. Growth hormone, insulin, and corticosterone, known hormonal mediators of IGF-I, were also measured in plasma. Selected tissues (liver, gastrocnemius, soleus, kidney, spleen, intestine, and whole brain) were excised, dissected free of connective tissue, and frozen in liquid nitrogen. Plasma and tissue samples were stored at −70°C until analyzed.

Analytic procedures and statistics. For measurement of IGF-I, plasma was extracted with the use of a modified acid-ethanol cryoprecipitation procedure, as described by our laboratory (8, 15). Tissues were homogenized in acid and extracted using Sep-Pak (C₁₈). This method removes >99% of the IGF binding proteins. The eluate was evaporated, and the dried sample was reconstituted with phosphate buffer for IGF-I determination. IGF-I in plasma and tissues was determined by radioimmunoassay (RIA). Recombinant human [Thr⁵⁹]IGF-I (UBI, Lake Placid, NY) was used for iodination and standards, as previously described (8, 15). The mean effective dose of this assay is 0.03–0.08 ng/tube. Interassay and intra-assay coefficients of variation are 10 and 7%, respectively.

The concentrations of insulin and corticosterone (Diagnostic Products; Los Angeles, CA) and growth hormone (Amersham, Arlington Heights, IL) were determined on each plasma sample by RIA. Experimental values are presented as means ± SE. The number of rats per group is indicated in the legends to Figs. 1 and 6 and Table 1. Data were analyzed by analysis of variance and then, where appropriate, by Student-Newman-Keuls to determine treatment effect. Statistical significance was set at P < 0.05.

RESULTS

Intracerebroventricular administration of IL-1β. The plasma concentration of IGF-I was decreased 36% by 1.5 h after intracerebroventricular injection of IL-1β, compared with time-matched control values (Fig. 1, top). Levels remained decreased (39%) at 3 h, but had returned to control values by 6 h. A similar temporal pattern was observed for the IGF-I content in liver, with a 30–40% decrease occurring at 1.5 and 3 h and normal levels at 6 h (Fig. 1, bottom). In gastrocnemius (fast-twitch muscle), IL-1β decreased IGF-I by 28% at 1.5 h and by 53% at 3 h (Fig. 2, top). Although gastrocnemius IGF-I was decreased at 6 h, this change was not statistically significant. In contrast, the intracerebroventricular administration of IL-1β did not significantly alter IGF-I content of soleus (slow-twitch muscle) at the time points examined (Fig. 2, bottom). The IGF-I content of kidney (Fig. 3), spleen, intestine (jejunum), and whole brain was also not altered by IL-1β, compared with values from control animals (data not shown). No changes in plasma or tissue IGF-I were detected in rats injected intravenously with the same dose of IL-1β (Figs. 1–3) or after the intracerebroventricular injection of heat-inactivated IL-1β (data not shown).

Growth hormone concentrations were decreased by >50% at all three time points examined following intracerebroventricular injection of IL-1β (Fig. 4, left). Corticosterone levels were elevated 5- to 10-fold, com-
pared with time-matched control values, at all time points (Fig. 5, top). The intravenous injection of IL-1β resulted in a transient increase in corticosterone (2-fold), which was present at 1.5 h but had returned to control values by 3 h. In contrast, insulin levels were only increased at 1.5 h (45%; Fig. 5, bottom). However, this hyperinsulinemia was only transient and insulin levels were comparable to basal values at 3 and 6 h.

Influence of intracerebroventricular administration of IL-1ra on peripheral response to LPS. The intravenous injection of LPS decreased the concentration of IGF-I in plasma (29%) and liver (22%) at 1.5 h, compared with control values (Fig. 1). The magnitude of this decrease was greater at 3 h (60 and 45%, respectively), and levels remained similarly reduced at 6 h. LPS also decreased the IGF-I content in both the gastrocnemius and soleus (30–50%; Fig. 2). The decrease was apparent at both 3 and 6 h, and was of greater magnitude in the gastrocnemius compared with the soleus. Intracerebroventricular infusion of
IL-1ra did not alter these LPS-induced changes in plasma, liver, or muscle IGF-I. In contrast to other tissues, LPS increased the IGF-I content in kidney by twofold at 1.5 h and by more than threefold at 3 and 6 h (Fig. 3). However, this LPS-induced increase in renal IGF-I was also not altered by intracerebroventricular infusion of IL-1ra. IGF-I levels in the intestine, spleen, and brain were not altered by LPS and/or IL-1ra infusion (data not shown).

Intravenous injection of LPS decreased growth hormone levels by ~75% at 1.5 h, and levels remained significantly reduced for the remainder of the experimental protocol (Fig. 4, right). The intracerebroventricular infusion of IL-1ra completely prevented the LPS-induced decrease in growth hormone at all time points. Intravenous injection of LPS also elevated plasma corticosterone between 5- and 10-fold, but did not alter plasma insulin, compared with time-matched values from control animals (Fig. 4). Intracerebroventricular administration of IL-1ra, however, did not significantly influence the circulating levels of corticosterone or insulin in LPS-treated rats.

Influence of IL-1ra on CLP-induced changes. Peritonitis induced by CLP decreased IGF-I in plasma (36%), liver (44%), and gastrocnemius (46%), but increased IGF-I content in kidney (2.5-fold) (Fig. 6). No changes in IGF-I levels in soleus, spleen, intestine, or brain were observed at this time point after CLP (data not shown). The overnight intracerebroventricular infusion of IL-1ra did not influence the IGF-I levels in blood or tissues in response to CLP.

Growth hormone levels were elevated twofold 24 h after CLP, compared with nonseptic rats (Table 1). The intracerebroventricular infusion of IL-1ra blunted this sepsis-induced increase in growth hormone, with plasma levels being intermediate between septic and control rats. Corticosterone concentrations were also elevated in septic rats (~2-fold), compared with time-matched values from nonseptic animals (Table 1). However, in contrast to the effect on growth hormone, intracerebroventricular infusion of IL-1ra did not significantly alter corticosterone levels in septic rats. Plasma insulin concentrations were not altered by CLP either in the absence or presence of IL-1ra (Table 1).

DISCUSSION

We have previously established that the systemic administration of a nonlethal dose of IL-1β results in diminished IGF-I in plasma, liver, gastrocnemius, soleus, and whole brain, as well as increased IGF-I content in kidney. The possibility exists that the alterations in IGF-I in peripheral tissues and brain are mediated centrally rather than peripherally by IL-1β. Therefore, we examined the ability of IL-1β administered intracerebrally to mimic changes in IGF-I content observed following intravenous injection of IL-1β. The first series of experiments demonstrated that central...
administration of a nonlethal dose of IL-1β is capable of decreasing the IGF-I concentration in blood, liver, and gastrocnemius. However, the magnitude of the reduction in IGF-I concentration following central IL-1β is less pronounced than following intravenous administration. Moreover, central IL-1β injection did not modulate IGF-I in soleus, whole brain, or kidney. Systemic infusion of IL-1β at a dose comparable to that injected centrally did not alter IGF-I in any of the tissues examined. Thus the changes induced by central administration of IL-1β are probably not mediated by translocation of IL-1 from the brain to the periphery. However, because complete dose-response curves were not generated for either intracerebroventricular or intravenous IL-1β, we cannot definitively conclude whether this differential response occurred from differences in the route of cytokine administration or was simply due to a difference in the severity of the two insults. It was also possible that the peripheral IGF-I response might differ in animals in which central IL-1β is infused over a more extended period of time.

Although the first series of experiments clearly demonstrated central IL-1β was capable of regulating IGF-I, these data provide little insight into whether endogenous elevations of this cytokine within the brain are responsible for the changes in IGF-I observed following inflammation and infection. To address this question, animals were pretreated with IL-1ra, which is a naturally occurring receptor antagonist. IL-1ra binds competitively to IL-1 receptors but has no detectable agonist activity. As such, IL-1ra blocks many of the effects of IL-1β at a dose comparable to that injected centrally.

Table 1. Plasma hormone concentrations in septic and nonseptic rats

<table>
<thead>
<tr>
<th>Condition</th>
<th>Growth Hormone, ng/ml</th>
<th>Corticosterone, ng/ml</th>
<th>Insulin, µU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonseptic</td>
<td>21 ± 3*</td>
<td>152 ± 21*</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>Septic</td>
<td>44 ± 6†</td>
<td>360 ± 17†</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>Septic+IL-1ra</td>
<td>30 ± 5†</td>
<td>394 ± 27†</td>
<td>21 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 in each group. IL-1ra, interleukin-1 receptor antagonist. Groups with different symbols are significantly different (P < 0.05) from each other.
Although glucocorticoids and insulin are important modulators of the IGF system, the most critical regulator of IGF-I levels is growth hormone. Clearly, changes in IGF-I are directly proportional to changes in growth hormone levels in many conditions (12). After intracerebroventricular injection of IL-1β, growth hormone levels were decreased within 1.5 h and remained reduced for at least 6 h. Although smaller intracerebroventricular doses of IL-1β have been shown to increase growth hormone in rats (19), our results are consistent with other studies demonstrating a decrease in basal and spontaneous growth hormone secretion with relatively higher doses (19, 25). Although the decrease in growth hormone at 1.5 and 3 h after intracerebroventricular IL-1β could contribute to the concomitant drop in IGF-I at these times, this fall is inconsistent with the normal IGF-I levels in plasma and tissue observed in these animals at 6 h. The intravenous injection of LPS also resulted in a large reduction in growth hormone levels for 6 h (20) and was associated with a reduction in IGF-I. Although the intracerebroventricular infusion of IL-1ra prevented the LPS-induced decrease in growth hormone, the IGF-I concentration remained decreased. IL-1ra also abrogated the increase in growth hormone observed in rats after CLP but failed to attenuate the reduction in IGF-I. Collectively, these data suggest that factors other than circulating levels of growth hormone are important in regulating tissue and plasma levels of IGF-I during endotoxemia and sepsis. The ability of centrally administered IL-1ra to blunt the LPS-induced increase in growth hormone secretion has been previously described (20).

In conclusion, these data demonstrate that central IL-1β is capable of modulating peripheral IGF-I levels. However, intracerebral infusion of IL-1ra, which inhibits endogenous IL-1 activity within the paraventricular region of the brain, did not modulate the changes in peripheral IGF-I observed during either acute endotoxemia or following CLP.

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Inflammatory responses attributed to IL-1 (4). Because of its relatively short half-life, IL-1ra was infused continuously in these experiments to maintain an effective dose. The ability of centrally administered IL-1ra to regulate peripheral IGF-I was studied in two models of "sepsis." In the first model, rats were challenged with an intravenous injection of E. coli LPS to determine whether IL-1ra could prevent the acute (i.e., within several hours) changes in IGF-I previously described (8). The second model, that produced by CLP, was used to determine whether IL-1ra was able to regulate changes in IGF-I produced during a more chronic hypermetabolic septic state. Despite these two relatively different animal models, central infusion of IL-1ra did not modulate peripheral IGF-I changes induced by either LPS or CLP. Thus we were unable to demonstrate a significant regulatory role for central IL-1 on peripheral IGF-I in experimental sepsis. We cannot, however, exclude the possibility that there are areas of the brain distant from the ventricular system in which the concentration of IL-1ra was too low to be efficacious. Furthermore, these data do not rule out the possibility that central IL-1 mediates peripheral IGF-I in other inflammatory conditions or even in other models of infection in which the septic insult persists for days. Although the dose of IL-1ra administered did blunt the sepsis- and LPS-induced alterations in growth hormone, thus indicating efficacy of the antagonist, we cannot eliminate the possibility that higher doses of IL-1ra might also be capable of modulating peripheral IGF-I levels.

Previous studies have shown that glucocorticoids are important mediators of the IGF system. An elevation in glucocorticoids has been shown to decrease IGF-I levels (17). In addition, the intravenous injection of IL-1β dramatically elevated circulating levels of corticosterone in rats (9). Although pretreatment of rats with the glucocorticoid antagonist RU-486 completely prevented the reduction in blood and hepatic IGF-I, it did not abrogate the decrease in muscle IGF-I or increase in renal IGF-I produced by intravenous IL-1β (9). The intracerebroventricular administration of IL-1β also elevated corticosterone levels (16, 22); however, the intracerebroventricular infusion of IL-1ra failed to attenuate the LPS- or sepsis-induced increase in corticosterone (5, 18). Therefore, the presence of high levels of glucocorticoids may be responsible, at least in part, for some of the decline in IGF-I observed in blood and liver in all three groups of experimental animals.

Insulin is an important positive regulator of IGF-I synthesis and secretion by the liver. In primary rat hepatocytes, insulin produces a dose-dependent stimulation of IGF-I secretion (3), whereas IGF-I levels are generally reduced in insulin-dependent diabetes mellitus with insulinopenia (2). The intracerebroventricular injection of IL-1β transiently increased plasma insulin concentrations. Hence, the general reduction in IGF-I in blood and tissues induced by IL-1β (as well as that produced by LPS and CLP) in the present study cannot be explained by the presence of insulinopenia.

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