Interleukin-1β-converting enzyme-deficient mice resist central but not systemic endotoxin-induced anorexia

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Burgess, William, Gilles Gheusi, Jianhua Yao, Rodney W. Johnson, Robert Dantzer, and Keith W. Kelley. Interleukin-1β-converting enzyme-deficient mice resist central but not systemic endotoxin-induced anorexia. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1829–R1833, 1998.—Interleukin-1β (IL-1β) is a proinflammatory cytokine that is synthesized as an inactive 31-kDa precursor molecule (6, 32). IL-1β-converting enzyme (ICE), also known as caspase 1, generates and stimulates the release of the active 17-kDa form of IL-1β by cleaving the precursor protein at two specific aspartic acid residues (11, 25). IL-1β is an important mediator of the inflammatory response induced by lipopolysaccharide (LPS), the active component of the cell wall of gram-negative bacteria. LPS administered centrally or peripherally increases IL-1β mRNA and protein in both peripheral tissues and brain (15, 16). Intracerebroventricular or intraperitoneal injections of either LPS or IL-1β induce behavioral symptoms associated with inflammation, such as decreased food intake and reductions in social exploration and food-motivated behavior (2, 5, 17, 19, 28, 30). Additionally, studies using the IL-1 receptor antagonist (IL-1ra), an endogenous inhibitor of IL-1β activity, have established that intracerebroventricular IL-1ra inhibits deficits in food-motivated behavior induced by intraperitoneal and intracerebroventricular injections of IL-1β (21, 22, 29). These findings suggest that the sickness-inducing effects of IL-1β are centrally mediated.

Although IL-1β has been the most studied, several other LPS-induced proinflammatory cytokines can lead to sickness behavior, including IL-1α, TNF-α, and IL-8 (4, 7, 8, 10, 28). These cytokines and their receptors have been identified not only in peripheral tissues but also in the brain, and it has been demonstrated that they can elicit sickness by functioning via central mechanisms as well (3, 5, 13, 16, 20, 24). There is not only a great deal of redundancy in the functions of these cytokines, but they have been found to act synergistically as well (4, 31, 33). It has therefore been difficult to fully evaluate the contribution of central versus peripheral IL-1β in the sickness response.

In the present report, we have addressed this issue by using ICE-deficient (ICE −/−) mice that are incapable of generating ICE and do not produce active IL-1β (26, 27). Both wild-type (wt) and ICE −/− mice were injected intracerebroventricularly and intraperitoneally with either LPS or IL-1β, followed by measurement of both food consumption and food-motivated behavior. ICE −/− and wt mice responded similarly to intraperitoneal injections of LPS, but ICE −/− mice were much less sensitive to LPS when given intracerebroventricularly. This was not caused by an insensitivity to IL-1β, because intracerebroventricular injections of IL-1β caused a similar reduction in food intake and food-motivated behavior in wt and ICE −/− mice. These data establish that the central effects of LPS on food intake are mediated through IL-1β, whereas other cytokines can compensate for the loss of IL-1β in response to peripheral LPS.

MATERIALS AND METHODS

Male 12- to 16-wk-old ICE −/− mice and their age-matched wt inbred controls (kindly supplied by Dr. Tara Seshadri from BASF Research Corporation) were used in all experiments (26, 27). For the food consumption experiment, animals were housed individually in wire mesh metabolic cages with ad libitum access to water and food. Mice were maintained at 30°C with a 12:12-h light/dark cycle (lights on at 0700). For the food-motivated behavior experiment, mice were housed individually in polypropylene cages with ad libitum access to food.
water. Mice were maintained at 90% of their free-feeding body weight. The ambient temperature was maintained at 22 ± 1°C.

For intracerebroventricular cannula placement, mice were anesthetized with an intraperitoneal injection of ketamine (87 mg/kg) and xylazine (13 mg/kg). The head was oriented in a Kopf stereotaxic instrument so that the plane formed by the parietal and frontal bones was parallel to the instrument tabletop. A 26-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was inserted in the lateral cerebral ventricle using the following coordinates: anterior-posterior: −0.6 mm, lateral: 1.6 mm to the bregma, horizontal: −2.0 mm to the dura mater. Cannulas were secured with two stainless steel screws and cranioplast cement. All surgeries were done under aseptic conditions, and mice recovered for 1 wk before experiments were performed. All procedures were approved by the Laboratory Animal Care Advisory Committee at the University of Illinois and the French National Care Advisory Committee.

Injections into the lateral ventricle were administered with a 28-gauge cannula attached to a syringe pump using a 250-µl Hamilton syringe as a reservoir (Hamilton, Reno, NV). This cannula was inserted into the guide cannula. Injections of LPS (serotype 0127:B8, Sigma Chemical, St. Louis, MO) and recombinant mouse IL-1β (PharMingen, San Diego, CA) in sterile PBS or PBS alone (control) were used in the intracerebroventricular experiments. LPS serotype 0127:B8 was chosen based on previous results that demonstrated that this serotype produces consistent and reproducible reductions in food-motivated behavior, social exploration, and overall activity of mice (2, 5, 24). A total volume of 2 µl was injected at a rate of 1 µl every 12 s. At the end of each intracerebroventricular experiment, cannula placement was verified by injecting Evan’s blue dye into the cannula followed by brain dissection. Both intracerebroventricular and intraperitoneal injections were given just before the dark phase. All experiments were conducted on a minimum of four mice per treatment.

Cumulative food intake was measured by weighing food cups preinjection and then at 2, 4, 8, 12, and 24 h postinjection. Assessment of food-motivated behavior involved training the mice to poke their nose in a modified Skinner box to obtain a food pellet. A photo cell was used to detect a nose poke. An animal would receive a food reward after 20 consecutive nose pokes. Mice were trained for 15 min a day until their response rate had stabilized. Experimental sessions involved a baseline measurement 1 h before treatment followed by retesting at 1, 2, 4, 8, and 24 h postinjection. A testing session involved recording the number of nose pokes in 5 min.

**RESULTS**

Cumulative food intake, as well as food-motivated behavior using operant conditioning techniques, was used to measure the effects of LPS in both wt and ICE−/− mice. As expected, intraperitoneal injections of 125 µg/mouse LPS depressed food intake (Fig. 1, A and B). The same effect was obtained with 10 µg/mouse LPS on food-motivated behavior (Fig. 2, A and B). A three-way ANOVA (treatment × genotype × time) on food consumption revealed a main effect of treatment (P < 0.01), time (P < 0.01), and their interaction (P < 0.01). Post hoc comparisons (least squares means) showed that LPS-treated mice had lower cumulative food intake than controls at 8, 12, and 24 h (P < 0.05). However, wt and ICE−/− mice responded similarly, showing equivalent reductions in cumulative food intake. Nearly identical results were obtained when food-motivated behavior was measured in both strains of mice injected intraperitoneally with LPS (Fig. 2, A and B). A three-way ANOVA (genotype × treatment × time) on percent depression in operant responding revealed a significant main effect of treatment (P < 0.001), time (P < 0.001), and their interaction (P < 0.01). LPS reduced the frequency of operant responding at 1, 2, 4, and 8 h postinjection in wt mice (P < 0.01). As with cumulative food intake, ICE−/− mice responded similarly to wt mice to peripheral injections of LPS (P > 0.05).
In contrast to intraperitoneal administration of LPS, intracerebroventricular administration of 100 ng/mouse LPS had little effect in ICE −/− mice (P > 0.05 at all time points), whereas it still depressed food intake in wt mice (Fig. 1D). Wt mice treated with LPS consumed significantly less food at 4, 8, 12, and 24 h postinjection (P < 0.01) (Fig. 1, C and D). A three-way ANOVA (treatment × genotype × time) revealed main effects for genotype, treatment, and time, as well as a treatment × time × genotype interaction (P < 0.05). Very similar results were obtained when food-motivated behavior was measured as the dependent variable (Fig. 2, C and D). Comparison of the wt and ICE −/− mice using this operant conditioning paradigm showed a significant main effect of genotype (P < 0.01), time (P < 0.001), and treatment × time interaction (P < 0.05). Intracerebroventricular LPS treatment significantly depressed operant responding at 4 h in ICE −/− mice compared with controls (P < 0.05). A similar decrease in operant responding was observed in wt mice at 2 and 4 h after intracerebroventricular LPS treatment (P < 0.05).

We then determined whether wt and ICE −/− mice were equally responsive to central administration of IL-1β (2 ng/mouse). A three-way ANOVA (treatment × genotype × time) revealed a main effect for both treatment and time (P < 0.01). Although ICE −/− and wt mice treated intracerebroventricularly with IL-1β consumed significantly less food than control mice at 4 and 8 h (P < 0.05; Fig. 1E), there were no differences between wt and ICE −/− mice at any time point. Similarly, intracerebroventricular administration of IL-1β reduced food-motivated behavior (P < 0.01), as assessed by operant conditioning techniques (Fig. 2E). A three-way ANOVA (genotype × treatment × time) revealed significant main effects for treatment (P < 0.01), time (P < 0.001), and their interaction (P < 0.001), but did not reveal any difference between wt and ICE −/− mice in the reduction in food-motivated behavior induced by intracerebroventricular administration of IL-1β.

**DISCUSSION**

Administration of LPS induces a variety of responses in laboratory animals, including profound changes in behavior, anorexia, fever, and body weight loss (1, 2, 5, 18, 28). Here we show that ICE −/− mice may have similar reductions in food consumption and food-motivated behavior as wt mice when injected intraperitoneally with LPS. Because the high dose of LPS that was used in mice submitted to the food intake measurement could have masked a differential sensitivity of these two mouse strains to endotoxin, a lower dose of LPS was used in the food-motivation experiment. Both experiments yielded the same results, indicating that the lack of difference in the sensitivity of wt and ICE −/− mice to the depressing effects of LPS was not a dose-dependent phenomenon. Because ICE −/− mice have been shown to be less sensitive than wt mice to LPS-induced septic shock, the most obvious explanation for their similar response to LPS-induced anorexia is that this phenomenon is mediated by other proinflammatory cytokines than IL-1β. Several studies have demonstrated that animals treated with IL-1β, IL-1α, TNF-α, or IL-8 exhibit similar symptoms of sickness as LPS-treated animals (4, 7, 8, 10, 28, 30, 31). This redundancy has therefore made it difficult to assess which cytokine has the greatest impact on inducing sickness. IL-1β, IL-1α, and TNF-α all induce anorexia, depression of social exploration, and loss of body weight when administered either peripherally or centrally (1, 7, 14, 21, 33). Additionally, these cytokines function synergistically, as demonstrated by the ability of subeffective doses of IL-1α and TNF-α to decrease food intake when administered simultaneously (33). In IL-1β
knockout mice, intraperitoneal injection of LPS decreased food intake and body weight to the same extent as wt mice, and this was accompanied by similar increased serum levels of IL-1α, TNF-α, and IL-6 in both mouse strains (12). In the present experiments, both wt and ICE −/− mice (Figs. 1B and 2B) decreased their food consumption similarly in response to intraperitoneal LPS stimulation. These data suggest that cytokines other than IL-1β are involved in the reduction in food consumption and food-motivated behavior produced by the peripheral administration of LPS. For example, it is possible that IL-1α compensates for the loss of IL-1β, because both ICE −/− and IL-1β knockout mice are able to produce IL-1α on LPS stimulation (12, 26, 27).

In contrast, the present results establish that IL-1β appears to be the cytokine responsible for regulating food motivation in the central nervous system. LPS administered intracerebroventricularly does not reduce food consumption and only partially depresses food-motivated behavior in ICE −/− mice compared with wt mice. This finding implies that the effects of central LPS on food-motivated behavior are mediated primarily by IL-1β and, unlike peripherally, other cytokines are unable to compensate for its absence centrally. This possibility seems likely, because the intracerebroventricular administration of IL-1β produces similar reductions in both food consumption and food-motivated behavior in wt and ICE −/− mice. Previously, we demonstrated that intracerebroventricular administration of the IL-1ra inhibited the reduction in food-motivated behavior when IL-1β was administered intracerebroventricularly but could only partially inhibit the effects of IL-1β administered intraperitoneally (21). LPS administered intracerebroventricularly may not stimulate secretion of sufficient levels of other cytokines to inhibit food consumption. Indeed, IL-1β administered intracerebroventricularly has been found to induce anorexia to a greater extent than either TNF-α or IL-8 alone, but the combination of all three proved to be effective (31). IL-1ra has also been shown to inhibit the behavioral effects of centrally administered TNF-α (4). Compared with controls, ICE −/− mice exhibited a moderate decrease in TNF-α production after LPS treatment both in vivo and in vitro (23, 26, 27). Furthermore, IL-1β knockout mice have reduced amounts of mRNA transcripts for IL-1α and TNF-α in the brain after peripheral stimulation with LPS, which suggests that IL-1β is involved in the communication system that leads to the production of cytokines in the brain (12). These data suggest that IL-1β is critical for intracerebroventricularly administered LPS to be effective in depressing food intake and food-motivated behavior. The present investigations demonstrate that IL-1β plays a primary role in modulating food consumption and food-motivated responses to centrally administered LPS. However, it appears that other cytokines can compensate for the lack of IL-1β in peripheral inflammation.

Perspectives

Cytokines contribute to the sickness induced by a variety of infectious, neoplastic, and autoimmune diseases. A significant clinical problem with many chronic diseases is anorexia and the associated body wasting. The mechanism by which cytokines contribute to the pathogenesis of wasting is not well understood. The brain and several peripheral tissues synthesize cytokines and express their receptors. The possibility exists and our data suggest that the physiological effects of a given cytokine depend on the particular compartment where the cytokine is expressed. For example, we previously demonstrated that IL-1β suppresses sickness and elevates body temperature by a mechanism that uses different receptors (21). The present investigation clearly indicates that, at least for food motivation, a cytokine can produce different physiological responses depending on where it is expressed. These experiments demonstrate that there is clearly a need to investigate the function of particular cytokines within specific physiological compartments. This information will lead to a greater understanding of the anorexia and wasting associated with chronic diseases and will contribute significantly to the ultimate goal of providing successful treatments for these maladies.

The authors thank BASF Bioresearch Corporation and Dr. Tara Seshadri for the generous donation of the ICE-deficient mice. This research was supported by grants to K. W. Kelley from the National Institutes of Health (AG-06246, MH-51569–01A2, and DK-49311) and the Pioneering Research Project in Biotechnology financed by the Japanese Ministry of Agriculture, Forestry and Fisheries. Address for reprint requests: K. W. Kelley, Laboratory of Immunophysiology, Univ. of Illinois, 207 Edward R. Madigan Laboratory, 1201 West Gregory Drive, Urbana, IL 61801.

Received 30 October 1997; accepted in final form 14 March 1998.

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