Cafeteria feeding induces interleukin-1β mRNA expression in rat liver and brain

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Hansen, Michael K., Ping Taishi, Zutang Chen, and James M. Krueger. Cafeteria feeding induces interleukin-1β mRNA expression in rat liver and brain. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1734–R1739, 1998.—Food intake affects gut-immune function and can provide a strong intestinal antigen challenge resulting in activation of host defense mechanisms in the digestive system. Previously, we showed that feeding rats a cafeteria diet increases non-rapid eye movement sleep by a subdiaphragmatic mechanism. Food intake and sleep regulation and the immune system share the regulatory molecule interleukin-1β (IL-1β). Thus this study examined the effects of a cafeteria diet on IL-1β mRNA and IL-1 receptor accessory protein (IL-1RAP) mRNA expression in rat liver and brain. Rats were fed normal rat chow or a palatable diet consisting of bread, chocolate, and shortbread cookies (cafeteria diet). After 3 days, midway between the light period of the light-dark cycle, rats were killed by decapitation. Feeding rats a cafeteria diet resulted in increased IL-1β mRNA expression in the liver and hypothalamus compared with rats fed only the normal rat chow. In addition, cafeteria feeding decreased IL-1RAP mRNA levels in the liver and brain stem. These results indicate that feeding has direct effects on cytokine production and together with other data suggest that the increased sleep that accompanies increased feeding may be the result of increased brain IL-1β. These results further suggest that cytokine-to-brain communication may be important in normal physiological conditions, such as feeding, as well as being important during inflammatory responses.

Interleukin-1 receptor accessory protein; cytokine; reverse transcription-polymerase chain reaction; sleep; vagus nerve

INTERLEUKIN-1β (IL-1β) is a proinflammatory cytokine involved in the regulation of several physiological central nervous system processes (e.g., sleep and appetite regulation) and plays a role in neural-immune responses to tissue damage and infection (reviewed in Ref. 25). Feeding has direct effects on gut-immune function and can provide a strong intestinal antigen challenge and thereby activate digestive system host defense mechanisms (16). For example, compared with other organs of the body, the intestine is routinely exposed to an enormous number of antigenic macromolecules. These macromolecules are derived from many sources, including ingested food, resident bacteria, and invading viruses. Furthermore, the gut is now recognized as a cytokine-producing organ (10, 15), and substantial amounts of IL-1 are produced in the normal intestine (39). However, evidence that IL-1β is produced following meal intake has not been thoroughly investigated in rats.

Cytokines, such as IL-1β and tumor necrosis factor-α (TNF-α), are known to inhibit meal intake whether administered systemically or centrally (31). The anorexic effects of cytokines are most pronounced during pathological conditions, such as during infection, and this effect appears to be mediated by brain IL-1β (32). Thus, considering the acute antigen challenge of food intake, proinflammatory cytokines may also serve a role in signaling central nervous system satiety during normal physiological conditions. In fact, we have previously shown that feeding rats a cafeteria diet increases non-rapid eye movement sleep by a subdiaphragmatic mechanism (17). Furthermore, IL-1β plays a key role in physiological sleep regulation (25), and there is now considerable evidence indicating that the vagus nerve is involved in transmitting cytokine signals to the brain (reviewed in Refs. 3 and 37). IL-1β increases vagal afferent activity (26, 29), and IL-1 receptors are found on paraganglia in the hepatic vagus (13). Thus the aim of the present study was to determine whether cafeteria diet feeding results in increased IL-1β mRNA expression in rat liver and brain. The current experiments also sought to determine variations in IL-1 receptor accessory protein (IL-1RAP) mRNA levels in response to a cafeteria diet. The IL-1RAP is necessary for IL-1 binding and signal transduction (14, 38). Current results suggest that these mechanisms occur during normal physiological processes such as feeding.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 300–400 g were used in this study. The animals were housed individually and maintained on a 12:12-h light-dark cycle (lights on at 0600) and at 25 ± 1°C ambient temperature in an American Association for the Accreditation of Laboratory Animal Care-accredited animal facility. Rats were acclimated to these conditions for at least 10 days before the experiments began, with food and water continuously available. Experimental design was approved by the Institutional Animal Care and Use Committee.

Experimental protocol. Rats (n = 6/group) were fed either normal rat chow or a palatable diet consisting of bread, chocolate, and shortbread cookies (cafeteria diet) as previously described (17). The diet was offered daily at dark onset (1800), and both groups of rats were allowed ad libitum access to their respective diet. Between 1730 and 1800, the cages were cleaned, fresh food and water were given, and body weights were measured. After 3 days, midway between the light period of the light-dark cycle (1200), rats were killed by decapitation. The liver and brain were quickly removed and snap-frozen in liquid nitrogen and stored at −80°C until RNA extraction.

RNA extraction. Total cellular RNA was isolated as previously described (19). Liver and brain samples from each rat were homogenized and processed individually. The integrity of the RNA was checked by denaturing agarose gel electrophoresis and ethidium bromide staining. The total amount was measured by spectrophotometry at an absorbance of 260 nm.
Internal standard cRNAs. The preparation of the internal standard cRNAs has been previously reported in detail (19). Briefly, plasmids containing a mutated fragment of the rat IL-1β gene and rat IL-1RAP gene were generated by PCR cloning. The IL-1β mutant plasmid contained a 217-bp deletion of the coding region, and the IL-1RAP mutant plasmid contained a 66-bp deletion within the coding region. The internal standards for RT-PCR were generated by in vitro transcription of the mutated plasmids, and DNA template was removed by extensive DNase I digestion. The mutant cRNAs were used as internal controls for RT-PCR. Because of the exponential nature of PCR, small differences in either RT or PCR efficiencies may result in large errors, which make quantitation of the wild-type mRNA difficult.

In the current experiment, mutant cRNA is added before the RT reaction, which controls for differences in RT efficiencies. In addition, the same pair of primers amplifies both wild-type and mutant cDNA, thereby allowing normalization of differences in PCR amplification efficiency among the samples. Finally, because the wild-type and mutant RNAs are different sizes, they can be separated by gel electrophoresis and quantified by the ratio of densitometric measurements of the RT-PCR products visualized on ethidium bromide-stained gels.

RT-PCR

First-strand cDNA was synthesized by random priming using 2 µg (liver samples) and 2.5 µg (brain samples) total RNA, internal standard RNAs, 50 ng DNA random hexanucleotides, and 200 units of Superscript II RTase H-RT (GIBCO BRL, Gaithersburg, MD) as previously described (19). Aliquots (4 µl for brain samples, 1 µl for liver samples) of the RT reaction were amplified by PCR. The primers for IL-1β were 5'-GACCTGTTCCTTGAGGCTGAC-3' (sense) and 5'-TCCATCTTCTCTTGGGTATTGTGTT-3' (antisense), which amplify a 578-bp product corresponding to wild-type IL-1β and a 361-bp product corresponding to the mutant IL-1β. The primers for the IL-1RAP were 5'-CAGCAGCTACTGCGACCAAGTTGGC-3' (sense) and 5'-AGGGTGACTTTCTTGATGCTGAA-3' (antisense), which amplify a 616-bp product corresponding to wild-type IL-1RAP and a 550-bp product corresponding to the mutant IL-1RAP. For the brain and liver samples, respectively, cDNA for IL-1β was amplified for 33 and 34 cycles, whereas cDNA for IL-1RAP was amplified for 26 and 27 cycles. These cycle numbers were chosen based on a previous study (19) determining the linear range of amplification for each respective molecule. In each PCR, denaturation was at 95°C for 45 s, annealing was at 60°C for 45 s, and extension was at 72°C for 2 min (for the final cycle, extension was 7 min). Furthermore, for each cDNA, PCR was performed in duplicate. DNA sequencing, performed at the University of Tennessee Molecular Resource Center, was used to confirm sequence specificity.

After amplification, aliquots of the PCR products (10 µl for IL-1β, 5 µl for IL-1RAP) were electrophoresed on agarose gels as previously described (19). The gels were stained with ethidium bromide and photographed under ultraviolet light using a charge-coupled device camera. Band densities were obtained by densitometric measurements of the RT-PCR products using public domain software National Institutes of Health Image 1.54 for 1-D gels according to the protocol provided. The amount of IL-1β and IL-1RAP mRNA was expressed as a ratio of densitometric measurements derived from the target message and the internal standard.

Statistical analysis. All data are expressed as means ± SE. Data from the three brain regions were analyzed by two-way ANOVA for repeated measures. The first factor was the treatment (normal diet vs. cafeteria diet) and the second factor was the region (hypothalamus, hippocampus, and brain stem). If data failed on normality, two-way repeated-measures ANOVA on ranks was applied. When appropriate, post hoc analysis was done using the Student-Newman-Keuls (SNK) multiple-comparison test. Data for the liver were analyzed separately using Student's t-tests. In all tests, an α-level of P < 0.05 was taken as an indication of statistical significance.

RESULTS

Controls. The sequences of the IL-1β and IL-1RAP PCR products, as well as their respective internal controls, corresponded to the appropriate mRNA, as determined by DNA sequence analysis. Furthermore, each had the expected electrophoretic mobility (e.g., Fig. 1). Two additional controls were included in the PCR experiments to rule out possible genomic DNA contamination and general DNA contamination. In the first control, rat genomic DNA was amplified with appropriate sense and antisense primers. It was found that either no product or a larger product was amplified, indicating that the primers either spanned exons or covered introns. This control was necessary because the genomic structures of rat IL-1β and rat IL-1RAP are unknown. The second control was carried out by PCR amplification in the absence of RT to rule out possible DNA contamination; no bands were observed.

Body weight gain. Confirming previous reports (8, 17), cafeteria diet feeding resulted in a significant increase in weight gain (t0 = 6.85, P < 0.0001). The mean weight gain in cafeteria diet-fed rats compared with the rats fed only the normal diet was 13.0 ± 1.25 and 3.0 ± 0.47 g, respectively.

IL-1β mRNA. An example of the RT-PCR-amplified IL-1β mRNA and corresponding internal standard cRNA for the liver is shown in Fig. 1. The averaged values for IL-1β mRNA in the liver, brain stem, hippocampus, and hypothalamus are shown in Fig. 2. Cafeteria feeding significantly increased IL-1β mRNA levels in the liver (t0 = 4.15, P < 0.002) compared with rats fed only normal rat chow. For the brain, ANOVA indicated a significant treatment and region interaction [F(2,20) = 4.2, P = 0.0299]. Post hoc analysis revealed a significant increase in IL-1β mRNA in the
hypothesis of the cafeteria diet-fed rats [SNK test: q(3,20) = 4.358, P < 0.05] compared with the normal diet-fed rats. No significant differences in IL-1β mRNA levels were found in the hippocampus or brain stem between the normal diet- and cafeteria diet-fed rats.

IL-1RAP mRNA. An example of the RT-PCR-amplified IL-1RAP mRNA and corresponding internal standard cRNA for the liver is shown in Fig. 1. The averaged values for IL-1RAP mRNA in the liver, brain stem, hippocampus, and hypothalamus are shown in Fig. 3. IL-1RAP mRNA was highly expressed in the rat liver and in all brain regions examined. Cafeteria feeding significantly decreased IL-1RAP mRNA levels in the liver (t10 = 2.35, P < 0.05) compared with rats fed only normal rat chow. For the brain, ANOVA indicated a significant treatment and region interaction [F(2,20) = 5.65, P = 0.0113]. Post hoc analysis revealed a significant decrease in IL-1RAP mRNA in the brain stem of the cafeteria diet-fed rats [SNK test: q(3,20) = 5.1653, P < 0.01] compared with the normal diet-fed rats. No significant differences in IL-1RAP mRNA levels were found in the hypothalamus and hippocampus between the cafeteria diet- and normal diet-fed rats.

**DISCUSSION**

In the present study, feeding rats an assortment of palatable, energy-rich foods increased IL-1β mRNA levels in the liver and hypothalamus 3 days after presentation of the cafeteria diet. This time was chosen because, in a separate study (17), this period corresponded to peak sleep periods induced by the cafeteria diet. Nevertheless, it is possible that this time frame did not coincide with peak values of IL-1β mRNA expression reached at other times after the onset of the cafeteria diet. Furthermore, we cannot rule out the possibility that IL-1β mRNA was increased or decreased in the brain stem and/or hippocampus at times not examined in this experiment. Regardless of such considerations, the increase in hypothalamic IL-1β mRNA induced by the cafeteria diet was relatively small compared with the increase in hypothalamic IL-1β mRNA induced by intraperitoneal injections of IL-1β that we previously reported (19). It is likely that the smaller changes reported here reflect physiological rather than pathological processes.

The finding of increased IL-1β mRNA levels in the liver of cafeteria diet-fed rats suggests that the cafeteria diet has direct effects on gut-immune function. This is consistent with a recent study that found increased numbers of neutrophils and platelets and decreased lymphocyte counts following meal intake in humans (16). In that study, they did not examine liver or brain production of cytokines; however, they failed to find changes in plasma cytokine production after one meal. This failure could reflect the short half-life of IL-1β in blood; random blood sampling may miss the peaks in activity. Furthermore, circulating concentrations of cytokines likely do not reflect their tissue concentrations. For example, even in pathological conditions many central nervous system manifestations of the acute phase response occur in the absence of measurable circulating cytokines (24). Finally, the current results, e.g., increased cytokines, and the recruitment of immune cells following food intake (16), clearly indicate that meal intake affects the gut immune system.

The normal intestinal immune system is constantly being stimulated by food and bacteria. The stimulatory molecules present in the intestinal lumen that activate and induce subsequent mucosal immunological and inflammatory events include bacterial cell wall products, such as peptidoglycans and lipopolysaccharides (LPS). Consistent with this notion, intestinal mononuclear cells are in a heightened state of activation compared with peripheral blood mononuclear cells (30), and this may be important for their distinct role in mucosal defense. Previously, immunoinflammatory cells...
were thought to express and produce IL-1 only when activated by pathological processes (11), but recent evidence indicates that there is also a constitutive secretion of IL-1β by lamina propria monocytes under nonpathological conditions (33, 39). Furthermore, mucosal inflammation is associated with a dramatic increase in cytokine levels (33, 39), and the gut is now recognized as a cytokine-producing organ. For example, the gut produces cytokines in response to hemorrhagic shock (10), and changes in the gut microbiota modulate the systemic cytokine response to hemorrhagic shock (15). The intestinal immune system also seems to play a role during infection, as it is well appreciated from clinical and epidemiological data that nutritional deficiencies are related to an increased incidence of infection (6). In addition, there is a relatively large literature on the effects of dietary factors on bacterial translocation from the gastrointestinal tract to the mesenteric lymph nodes and other extraintestinal organs, including the liver and spleen, and blood (9). For example, total parenteral nutrition promotes bacterial translocation from the gut (1). Bacterial translocation is also a spontaneous process in normal animals; however, whether cafeteria diet feeding (or increased feeding) results in increased bacterial translocation remains to be determined.

In the periphery, IL-1β is produced by many cell types in response to microbial pathogens and tissue injury (11). Peripheral IL-1β induces many central nervous system-controlled manifestations of the acute-phase response, including anorexia, fever, and excess sleep (11, 25). However, the mechanisms by which peripherally released cytokines signal the brain have not been conclusively identified. Cytokines are relatively large, lipophobic peptides and are not expected to readily cross the blood-brain barrier. Saturable transport systems for several cytokines exist (2); however, it is uncertain whether the amounts shown to enter the brain are sufficient to activate central mechanisms (37). Furthermore, various behavioral and central actions of peripheral IL-1β or LPS are inhibited by vagotomy (3, 37), suggesting a neural route of communication via vagal afferents. For example, subdiaphragmatic vagotomy inhibits systemic IL-1β- or LPS-induced sleep (18, 22), fever (36), and decreased food-motivated behavior (5). IL-1β induces dose-dependent and long-lasting increases in vagal afferent activity (26, 29), and IL-1 receptors are found in liver paraganglia (13). Thus it is likely that IL-1 receptors on these structures could respond to local increases in IL-1β and subsequently send cytokine information to the brain; this mechanism could account for the failure to observe increases in circulating IL-1β during times of neurological manifestations of the acute-phase response.

Microbial products and cytokines in the periphery induce cytokine expression in the brain (11, 19, 27). Intraperitoneal injections of IL-1 increase IL-1β mRNA levels in the liver and several brain regions (19); the increase in brain IL-1β mRNA is inhibited by subdiaphragmatic vagotomy. Vagotomy also blocks LPS-induced IL-1β gene expression in brain (27). Furthermore, the central inhibition of IL-1β blocks many of the central effects of peripherally administered microbial products and cytokines, such as increased sleep (34) and fever (23). Thus it is likely that the induction of IL-1β, and possibly other cytokines, in brain is crucial for many of the systemic IL-1β-induced responses. Centrally, IL-1β regulates several gastrointestinal functions, such as gastric acid secretion (35) and intestinal motility (12). Furthermore, IL-1β acts directly in the central nervous system to suppress feeding by inhibiting glucose-sensitive neurons in the lateral hypothalamic area (32). Brain IL-1β also plays a key role in sleep responses to infection and in normal physiological sleep regulation (reviewed in Ref. 25). For example, administration of exogenous IL-1β via intraperitoneal, intravenous, or intracerebroventricular routes results in relatively large increases in sleep, whereas inhibition of endogenous IL-1 reduces spontaneous sleep and sleep rebound after sleep deprivation. In addition, food intake is one of the determining factors of the daily amount of sleep; e.g., an excess of sleep occurs when there is increased feeding (8, 17). The electroencephalogram of food-satiated animals shows a marked increase in the amount of high-voltage, low-frequency activity (20), and refeeding after food deprivation results in increased sleep (4, 21). In contrast, starvation suppresses sleep (21). Finally, subdiaphragmatic vagotomy blocks the increase in sleep that accompanies cafeteria diet feeding (17). Collectively, these data suggest that feeding may play a role in normal, everyday sleep regulation possibly by maintaining IL-1β levels and further suggest that the increase in sleep that accompanies cafeteria diet feeding is mediated, in part, by IL-1β.

IL-1β is one member of a family of molecules currently containing at least nine members (reviewed in Ref. 11). These include three ligands, IL-1α and β and the IL-1 receptor antagonist; two receptors; a soluble receptor; an IL-1 receptor-associated kinase; the IL-1 converting enzyme; and the recently identified IL-1RAP (14, 28). In principle, the up- or downregulation of any one component of the IL-1 family could influence the level of activation of the entire IL-1 system. In the present study, IL-1RAP mRNA was highly expressed in all regions examined; to obtain a discernible signal, cDNA for IL-1RAP was amplified either 26 or 27 cycles, whereas the same cDNA required 33 or 34 cycles of amplification for IL-1β mRNA in the brain and liver samples, respectively. Cafeteria diet feeding decreased IL-1RAP mRNA levels in the liver and brain stem. Again, it is possible that the time at which samples were taken did not coincide with peak values reached, nor can we rule out the possibility that IL-1RAP mRNA was increased or decreased in the hypothalamus and/or hippocampus at times not examined in this experiment. To our knowledge, this is the first demonstration of a physiological event inducing any change in IL-1RAP mRNA expression. The physiological significance of these findings remains unknown; however, it is possible that the downregulation of IL-1RAP mRNA in the liver provides a means to limit the actions of IL-1β.
in the liver. Consistent with this notion, IL-1RAP mRNA levels increase in the liver 1.5 h after systemic lipopolysaccharide (19), whereas 24 h after systemic LPS administration IL-1RAP mRNA levels decrease (28). Furthermore, meal intake is a strong stimulus of lipopolysaccharide (19), whereas 24 h after systemic mRNA levels increase in the liver 1.5 h after systemic

In conclusion, cafeteria feeding resulted in the upregulation of IL-1β mRNA in the liver and hypothalamus and a decrease in IL-1RAP mRNA levels in the liver and brain stem. The systemic immune responses to food intake, as well as the subsequent induction of IL-1β in brain, may contribute to the complex pattern of signals inducing central nervous system-controlled symptoms of satiety.

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