Vagal and splanchnic afferents are not necessary for the anorexia produced by peripheral IL-1β, LPS, and MDP

M. H. PORTER,1 B. J. HRUPKA,1 W. LANGHANS,1 AND G. J. SCHWARTZ2

1Institute for Animal Sciences, Physiology and Animal Husbandry, Swiss Federal Institute of Technology, 8092 Zurich, Switzerland; and 2Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Porter, M. H., B. J. Hrupka, W. Langhans, and G. J. Schwartz. Vagal and splanchnic afferents are not necessary for the anorexia produced by peripheral IL-1β, LPS, and MDP. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R384–R389, 1998.—We investigated the extrinsic gut neural mediation of the suppression of food intake in male Sprague-Dawley rats induced by peripheral intraperitoneal administration of 2 µg/kg interleukin-1β (IL-1β), 100 µg/kg bacterial lipopolysaccharide (LPS), and 2 mg/kg muramyl dipeptide (MDP). Food intake during the first 3 and 6 h of the dark cycle was measured in rats with subdiaphragmatic vagal deafferentation (n = 9), celiac superior mesenteric ganglionectomy (n = 9), combined vagotomy and ganglionectomy (n = 9), and sham deafferentation (n = 9). IL-1β, LPS, and MDP suppressed food intake at 3 and 6 h in all surgical groups. The results demonstrate that neither vagal nor nonvagal afferent nerves from the upper gut are necessary for the feeding-suppressive effects of intraperitoneal IL-1β, LPS, or MDP in the rat and suggest that peripheral administration of immunomodulators produces anorexia via a humoral pathway.

interleukin-1β; lipopolysaccharide; muramyl dipeptide; food intake; brain-gut communication; cytokine; bacterial products

A reduction of food intake is a prominent component of the host's acute phase response to bacterial infection (10). The bacterial products lipopolysaccharide (LPS) and muramyl dipeptide (MDP) trigger the acute phase response and may be involved in the accompanying hypophagia, because peripheral administration of both LPS and MDP reduces food intake (16). In addition, these bacterial products promote the synthesis and release of the immunomodulatory cytokine interleukin (IL)-1β, which also suppresses feeding (16).

Although the neural and/or humoral mediation of the feeding-suppressive effects of peripheral administration of these compounds is unclear, a role for subdiaphragmatic vagal afferent fibers in the hypophagia produced by bacterial products and cytokines has been suggested from studies evaluating the effects of surgical transection of the vagus nerve. For example, subdiaphragmatic vagotomy, which nonselectively transects all gut vagal sensory and motoneurons, attenuated the ability of intraperitoneally injected LPS and IL-1β to suppress instrumental lever pressing for food in mice (2). Subdiaphragmatic vagotomy has also been shown to inhibit a variety of other effects of peripheral LPS and IL-1β, such as LPS-stimulated fever (33), LPS-induced central c-fos expression (32), IL-1β-induced depression in social exploration (1), and IL-1β-induced hyperalgesia (35). However, more recent work from our group demonstrated that selective subdiaphragmatic vagal rootlet deafferentation, eliminating primarily vagal sensory traffic between the gut and the brain, failed to attenuate the suppression of solid food intake induced by peripherally administered LPS or IL-1β (29).

The sensory component of the subdiaphragmatic vagus nerve is not the only extrinsic neural pathway that could mediate the feeding suppression produced by peripheral administration of these agents. The sympathetic celiac-superior mesenteric ganglion complex is the main neuroanatomic pathway through which splanchnic, nonvagal visceral afferents flow from the upper gastrointestinal tract to the central nervous system (CNS). Systemic administration of IL-1β and LPS alters the efferent outflow in renal, splenic, and adrenal sympathetic nerves in rats (14, 20), suggesting that these compounds may also activate splanchnic visceral afferents that signal CNS sites controlling feeding. Thus nonvagal, splanchnic afferents may mediate the hypophagic effects of bacterial products and IL-1β.

The present study used subdiaphragmatic vagal deafferentation, nonvagal visceral deafferentation by celiac-superior mesenteric ganglionectomy, and a combination of these two surgical procedures to further elucidate the roles of subdiaphragmatic vagal and nonvagal visceral afferents in the suppression of food intake produced by peripherally administered IL-1β, LPS, and MDP.

METHODS

Male Sprague-Dawley rats (Biological Research Laboratories) initially weighing 220–250 g were used in the experiment. They were individually housed in a temperature-controlled (22 ± 2°C) colony room, in stainless steel drawer cages with wire bottoms. The rats were maintained on an artificial 12:12-h light-dark cycle with the lights on from 2200 to 1000. Water was available ad libitum. Rats were deprived of food and water for 16 h before surgery. They were anesthetized by intraperitoneal injection (1.00 ml/kg body wt) with a mixture of 80 mg/ml ketamine (Ketasol-100, Graub), 20 mg/ml xylazine (Rompun, Bayer), and 0.05 mg/ml acepromazine (Sedaline, Chassot and Cie). Supplemental injections of this mixture were administered as necessary to maintain a surgical level of anesthesia. Body temperature was monitored and maintained at 36–37°C throughout all surgical procedures with an electric heating pad.

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Animals were ranked by weight and divided into four surgical groups, distributing equivalent body weights in each group as closely as possible before the four surgeries: subdiaphragmatic vagal deafferentation (SDA, \( n = 9 \)), celiac superior mesenteric ganglionectomy (CGX, \( n = 9 \)), combined vagotomy and ganglionectomy (COM, \( n = 9 \)), and sham deafferentation (Sham, \( n = 9 \)). The vagal deafferentation procedures were performed according to the procedure of Norgren and Smith (24). The deafferentation procedure consisted of a left vagal rootlet transection at the brain stem level and a contralateral subdiaphragmatic vagotomy of the dorsal trunk. The left vagal rootlet transection cut all of the vagal afferents arising from the common hepatic, accessory celiac, and ventral gastric branches as they entered the brain stem, leaving all of the vagal efferent fibers in these branches intact. This is possible in the rat because the vagus diverges into discrete sensory and motor bundles as it enters the caudal brain stem. The dorsal vagal subdiaphragmatic vagotomy was performed according to the procedure of Smith et al. (30). This unilateral vagotomy disconnected all subdiaphragmatic vagal afferents and efferents from the dorsal vagal trunk, which supplies the dorsal gastric and celiac branches. For the CGX, the stomach and spleen were exposed and gently retracted and held in place with warm saline-soaked gauze. The descending aorta was visualized, and the celiac-superior mesenteric ganglion complex was isolated (9) with fine forceps, severed from the splanchic nerve trunks with fine scissors, and then completely surgically excised. The adjacent celiac and superior mesenteric arteries were not damaged during the procedure. Finally, during the sham surgeries, the left vagus, as it penetrates the posterior foramen of the skull, was exposed but left untouched, and a midline laparotomy and exposure of the dorsal subdiaphragmatic trunk were made, leaving the trunk intact.

Solid food intake measurements. Behavioral testing began 1 week after the completion of the surgical procedures. Three studies were performed. In each study, all rats received a single intraperitoneal injection of drug or corresponding vehicle solution between 20 and 5 min before lights out at 1000. The order of drug and vehicle treatments was counterbalanced between the 2 test days for each surgical group, in which five rats of each surgical group received the drug on the first test day and the other four rats of each group received the drug treatment on the second test day. Each test day was separated by at least 1 intervening day, during which injections of drug or vehicle were not made. In the first study, rats received a single intraperitoneal injection of 2 µg/kg recombined human IL-1β (R & D Systems) (dissolved in sterile physiological saline containing 2.0 µg BSA/10 µl) or BSA-saline vehicle delivered in 0.1 ml/100 g rat. In the second study, rats received a single intraperitoneal injection of 100 µg/kg LPS (from Escherichia coli serotype 0111:B4, Sigma (St. Louis, MO) L-2630, dissolved in sterile saline) or saline vehicle injection delivered in 0.1 ml/100 g rat. In the third study, rats received a single intraperitoneal injection of 2 mg/kg MDP (adjuvant peptide, Sigma A-9519, dissolved in sterile saline) or a saline vehicle injection delivered in 0.1 ml/100 g rat. The doses of IL-1β, LPS, and MDP were selected on the basis of previous studies comparing the effectiveness of these compounds to suppress food intake in rats (16, 17). At the beginning of each test, just before lights out, food containers with ground rat chow diet (Nafag) were placed in the cages. Food intake was measured by manually weighing (±0.1 g) the feeding cups at 3 and 6 h after the onset of the dark cycle at 1000. Spillage was collected on paper spread beneath the cages and was also measured at 3- and 6-h time points.

Functional vagotomy verification. Previous studies have demonstrated that left vagal afferent rootlet transection combined with dorsal vagal subdiaphragmatic transection blocks the suppression of feeding produced by low (1–6 µg/kg) but not higher (8–16 µg/kg) intraperitoneal doses of the brain-gut peptide cholecystokinin (CCK) (23, 30). To obtain a functional verification of the vagal deafferentation procedures in the present study, consumption of the ground rat chow diet was measured in the animals of each group 30 min after lights out at 1000. The rats received a single intraperitoneal injection of 4 µg/kg CCK or saline vehicle solution delivered in 0.1 ml/100 g rat body wt between 10 and 5 min before lights out at 1000. The order of the drug and vehicle treatments was counterbalanced between the 2 test days for each group as previously described. Again, each test day was separated by at least 1 intervening day, during which injections of drug or vehicle were not made. Water was available ad libitum at all times.

Histological vagotomy verification. All rats were killed between 6–7 wk post surgery for histological verification of vagotomy to minimize the possibility of functional regeneration of the vagus nerves (T. L. Powley, personal communication). To verify the subdiaphragmatic vagal deafferentations in the SDA and COM rats, and also in the SDA-CGX animals, we used a fluorescent tracer strategy (27). Briefly, immediately after the completion of all behavioral food intake tests, each animal received two intraperitoneal injections (0.5 ml) of fluorogold (Fluorochrome) solution (2 mg/ml of saline). Three days after the fluorogold injections, rats were anesthetized with a mixture of ketamine and xylazine and perfused with three solutions into the left ventricle. The first solution consisted of 250 ml of PBS with 0.2 ml of heparin (1,000 U/ml) delivered over a period of 15 min. The second solution contained 500 ml of 4% paraformaldehyde dissolved in PBS, followed by 200 ml of 20% sucrose in 4% paraformaldehyde.

For SDA and COM rats, the fixed brain was exposed and examined in situ under microscopic observation (×20) to verify that the vagal afferent rootlets were 1) cut on the left side and 2) intact on the right side as they entered the medulla. The brain and brain stem were then removed and cryoprotected in 20% sucrose in PBS. The medulla was blocked and sectioned sagittally at 50 µm on a sliding microtome. Sections for the fluorogold analyses were thaw mounted on slides, air dried, dehydrated by clearing in alcohols and xylene, and placed under a coverslip with DPX (Aldrich, Milwaukee, WI). Fluorogold label in the brain stem was examined with a Zeiss (Thornwood, NY) epifluorescence microscope. Because fluorogold is taken up and transported retrogradely in intact neurons but not in neurons with transected axons, a successful dorsal trunk subdiaphragmatic vagotomy was confirmed by 1) the absence of label in the right dorsal motor vagal nucleus, where the cut dorsal subdiaphragmatic vagal trunk would project, and 2) the presence of label in the left dorsal motor vagal nucleus, where the intact efferents of the ventral vagal subdiaphragmatic trunk project (27). Additional confirmation of the dorsal subdiaphragmatic vagotomy was also made by microscopic inspection and localization of the silk sutures around the cauterized dorsal vagal nerve trunks, with no visible fibers in between the proximal and distal stumps. With use of these verification procedures, all SDA and COM rats were determined to have successful, complete vagal deafferentations.

Verification of splanchnic nerve section and celiac-superior mesenteric ganglionectomy was performed postsurgically by examining the descending aorta at the region between the branch points between the celiac and superior mesenteric arteries. The complete absence of any neural and lymphatic...
tissue surrounding these vessels indicated a successful transection in all cases. This verification procedure is consistent with the neuroanatomic pathway taken by splanchnic afferents en route to the upper gut (9).

Data analyses. Differences between group means were analyzed using separate two-way repeated-measures ANOVA at each of the time points, followed by a Student-Newman-Keuls test when appropriate. P values < 0.05 were considered significant.

RESULTS

IL-1β. IL-1β (2 µg/kg) significantly inhibited food intake in every surgical group at 3 and 6 h [3 h overall F(3,32) = 43.6, P < 0.001; 6 h overall F(3,32) = 79.47, P < 0.001] (Fig. 1). There was no significant interaction between surgical group and drug effect at either 3- or 6-h time points [3 h F(3,32) = 0.378, P > 0.75; 6 h F(3,32) = 1.961, P > 0.15]. At each time point, food intake after saline vehicle administration did not differ significantly across surgical groups [3 h F(3,57) = 2.265, P > 0.05, post hoc t-test after significant ANOVA]. However, IL-1β administration resulted in a greater suppression of food intake in CGX rats (P < 0.01) compared with the SDA and Sham rats at 6 h (Fig. 1).

LPS. LPS (100 µg/kg) significantly inhibited food intake in every surgical group at 3 and 6 h [3 h F(3,32) = 60.6, P < 0.001; 6 h overall F(3,32) = 125.2, P < 0.001] (Fig. 2). There was no significant interaction between surgical group and drug effect at either 3- or 6-h time points [3 h F(3,32) = 0.423, P > 0.73; 6 h
At each time point, food intake after saline vehicle administration did not differ significantly across surgical groups [3 h F(3,32) = 0.59, P > 0.6; 6 h F(3,57) = 0.543, P > 0.65; 6 h F(3,57) = 1.37, P > 0.25]. There were also no significant differences between the surgical groups in the reduced food intake induced by LPS at each time point [3 h F(3,57) = 1.3, P > 0.25; 6 h F(3,57) = 0.89, P > 0.45].

MDP. MDP (2 mg/kg) significantly inhibited food intake in every surgical group except SDA [3 h overall F(3,32) = 47.5, P < 0.001] (Fig. 3) and in all surgical groups at 6 h [overall F(3,32) = 125.2, P < 0.001] (Fig. 3). There was no significant interaction between surgical group and drug effect at either 3- or 6-h time points [3 h F(3,32) = 0.855, P > 0.45; 6 h F(3,32) = 0.51, P > 0.6]. At each time point, food intake after saline vehicle administration did not differ significantly across surgical groups [3 h F(3,57) = 0.924, P > 0.4; 6 h F(3,57) = 0.48, P > 0.69].

CCK. During the 30-min feeding trial, CCK (4 µg/kg) significantly reduced food intake in Sham-operated rats but not in SDA or COM rats [overall F(2,24) = 6.64, P < 0.005] (Fig. 4). COM and SDA rats did not differ statistically in their lack of feeding suppression in response to CCK (P < 0.05).

**DISCUSSION**

The present results replicate our previous finding that subdiaphragmatic vagal afferents are not necessary for the feeding-suppressive effects of intraperitoneal IL-1β and LPS and extend those findings to include another bacterial cell wall compound, MDP. Our functional test of complete subdiaphragmatic vagal deafferentation confirmed in both SDA and COM groups that low doses of CCK (4 µg/kg) failed to suppress food intake. Furthermore, these data reveal that neither splanchic nor vagal subdiaphragmatic visceral afferents are necessary to mediate the hypophagia produced by intraperitoneal administration of IL-1β, LPS, or MDP, because combined subdiaphragmatic vagal deafferentation and celiac-superior mesenteric ganglionectomy failed to alter the ability of these compounds to suppress feeding.

The present finding that subdiaphragmatic vagal afferent fibers are not required for the hypophagic effect of intraperitoneal IL-1β, LPS, or MDP is consistent with results from other studies showing that intact vagal afferent fibers are not required to mediate several other effects of peripheral immunomodulator administration. For instance, hepatic branch vagotomy fails to attenuate intravenous IL-1α and intraperitoneal LPS-induced anorexia in rats (17, 19). In guinea pigs, the febrile response to intramuscular injection of MDP is...
not significantly altered by subdiaphragmatic vagotomy (7). Furthermore, systemic treatment with capsaicin fails to reverse LPS- and IL-1β-induced instrumental bar pressing for food in rats and mice (3).

Results from studies involving complete subdiaphragmatic vagotomy, which nonselectively transects all subdiaphragmatic vagal sensory and motor fibers, have demonstrated that the intact vagus contributes to a wide range of the behavioral and neural effects of peripheral IL-1β and LPS administration, including hyperthermia, hyperalgesia, and food-motivated behavior (28, 34). Unlike the present study, previous work evaluating the role of the intact vagus in food intake measured instrumental food-motivated behavior rather than actual food intake, making it difficult to compare results across such experiments. In addition, differences between the ineffectiveness of the SDA preparation in this study and the ability of total subdiaphragmatic vagotomy to attenuate feeding-related responses to LPS and IL-1β in other studies may be due in part to the fact that SDA spares one-half of the vagal efferent supply of the gut, whereas total subdiaphragmatic vagotomy transects all subdiaphragmatic vagal afferents and efferents.

These contrasting vagotomy procedures may produce important differences in the metabolic context in which immunomodulators act to suppress food intake. In the present study, the SDA procedure results in transient weight loss with complete recovery of body weight to sham control levels within 1 wk and subsequent maintenance of normal body weight gain. This is in contrast to the more long-lasting effects of total subdiaphragmatic vagotomy, which are characterized by more severe initial weight loss and a failure to subsequently reach the body weights achieved by sham-operated control rats postsurgically (15). More severe and prolonged body weight loss may reduce the anorectic potential of peripherally administered immunomodulators.

The design of this study reflects the fact that the vagus nerve is not the only afferent gut-brain pathway potentially involved in immunomodulator-induced feeding suppression. Nonvagal, splanchnic afferents might also mediate the hypophagic effects of immunomodulators, secondary to the ability of these compounds to stimulate sympathetic efferent outflow. LPS administration in rats increases the expression of c-fos protein in discrete brain nuclei in regions that mediate sympathetic outflow to sympathetic preganglionic neurons in the spinal cord (31), and systemic administration of IL-1β and LPS alters sympathetic efferent outflow in rats (14, 20, 21). Despite these demonstrations, surgical transection of the celiac-superior mesenteric ganglion complex failed to attenuate IL-1β- and LPS-, and MDP-induced hypophagia, indicating that such changes in sympathetic efferent outflow are not necessary for the hypophagic response to these compounds. The previously reported failure of combined pharmacological α- and β-adrenergic receptor blockade by phenolamine and propranolol to attenuate LPS-induced hypophagia (16) is consistent with this interpretation.

Gut enteric neuronal function is also influenced by cytokine exposure; IL-1β inhibits acetylcholine release and stimulates norepinephrine release in isolated rat small intestinal myenteric plexus (12, 22). However, the present results demonstrate that any potential feeding-suppressive effects of this type of immunomodulator-induced enteric activation do not require either intact vagal or splanchnic afferents.

Finally, IL-1β administration resulted in a greater suppression of food intake in CGX rats compared with SDA and Sham rats at 6 h (Fig. 1). Because celiac-superior mesenteric ganglionectomy not only transects splanchnic gut visceral afferents but also transects gut sympathetic efferents, CGX may eliminate a sympathetic influence that limits the feeding-suppressive effects of IL-1β but is only evident when vagal afferents are present. The nature of this signal remains to be identified.

Perspectives

The inability of selective vagal deafferentation, CGX, or a combination of both surgical procedures to attenuate the hypophagia induced by peripherally administered IL-1β, LPS, and MDP demonstrates that these compounds can act on the CNS to suppress feeding via humoral pathways. Peripherally synthesized cytokines may enter the CNS 1) by crossing the blood-brain barrier (BBB) during infection, when LPS increases BBB permeability (11, 2) through the circumventricular organs that lack a BBB, or 3) by specific transport mechanisms (4). Once available to the CNS, cytokines 1) have potent anorectic effects and 2) may induce the local production of anorectic cytokines in the CNS (26). Intracerebroventricular injection of pathophysiological levels of several cytokines, including IL-1β and tumor necrosis factor (TNF)-α, decreases food intake in rats (25). In addition, humoral access to cytokines or LPS may stimulate endothelial cells at the BBB to produce eicosanoids or other messengers that act on the CNS to suppress ingestion (4). Finally, peripheral immunomodulators may stimulate the production of peripheral humoral signals related to energy balance and food intake. Studies in hamsters (8) and humans (13) have demonstrated that the administration of LPS or TNF and IL-1β increases 1) the expression of mRNA for leptin in adipose tissue and/or 2) plasma levels of leptin, which have been correlated with decreases in food intake (8). Furthermore, IL-1β mediates the ability of peripheral LPS to induce leptin during inflammation (5). However, it has been reported that intraperitoneal LPS causes anorexia in both db/db and db/db mice (8), which lack functional leptin and functional leptin receptors, respectively. Although these data suggest that a functioning leptin signaling pathway is not critical for the feeding-suppressive actions of peripheral LPS, LPS also stimulates increases in plasma cytokines that may act via the other humoral mechanisms described above. Together, these data suggest that progress in understanding the anorexic actions of peripheral immunomodulators will come through sys-
Afferent Nerves and Immunomodulator-Induced Anorexia

R389


