Attenuation of lipopolysaccharide anorexia by antagonism of caudal brain stem but not forebrain GLP-1-R

Harvey J. Grill, Jill S. Carmody, L. Amanda Sadacca, Diana L. Williams, and Joel M. Kaplan

Graduate Groups of Psychology and Neuroscience, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Submitted 15 March 2004; accepted in final form 22 June 2004

Grill, Harvey J., Jill S. Carmody, L. Amanda Sadacca, Diana L. Williams, and Joel M. Kaplan. Attenuation of lipopolysaccharide anorexia by antagonism of caudal brain stem but not forebrain GLP-1-R. Am J Physiol Regul Integr Comp Physiol 287: R1190–R1193, 2004. First published July 1, 2004; doi:10.1152/ajpregu.00163.2004.—The central glucagon-like peptide-1 (GLP-1) system has been implicated in the control of feeding behavior. Here we explore GLP-1 mediation of the anorexic response to administration of systemic LPS and address the relative importance of caudal brain stem and forebrain GLP-1 receptor (GLP-1-R) for the mediation of the response. Fourth-intracerebroventricular delivery of the GLP-1-R antagonist exendin-(9–39) (10 μg) did not itself affect food intake in the 24 h after injection but significantly attenuated the otherwise robust (~60%) reduction in food intake obtained after LPS (100 μg/kg) treatment. This result highlights a role for caudal brain stem GLP-1-R in the mediation of LPS anorexia but does not rule out the possibility that forebrain receptors also contribute to the response. Forebrain contribution was addressed by delivery of the GLP-1-R antagonist to the third ventricle with the caudal flow of cerebrospinal fluid blocked by occlusion of the cerebral aqueduct. Exendin-(9–39) delivery thus limited to forebrain did not attenuate the anorexic response to LPS. These data suggest that LPS anorexia is mediated, in part, by release of the native peptide acting on GLP-1-R within the caudal brain stem.

feeding behavior; cachexia; neural systems; nucleus of the solitary tract; glucagon-like peptide-1 receptor

SYSTEMIC INJECTION OF LPS produces a dose-related anorexia (22) widely held to reflect the direct action of circulating and brain perivascular mediators (e.g., cytokines, prostaglandins) on central nervous system circuits (e.g., 2, 9, 12, 13, 21, 25). Researchers have begun to explore the contribution of central neuropeptide systems to LPS anorexia, with attention focused on those peptides implicated more generally in the control of feeding behavior. Positive pharmacological results have been obtained thus far for the melanocortin system (5; see also 4), where peptide receptor antagonism reduced the anorexic potency of LPS treatment. Such contributions of other neuropeptide systems have not yet been reported.

A number of findings taken together suggest glucagon-like peptide-1 (GLP-1) as another potentially important link in the downstream mediation of LPS anorexia. Central GLP-1 treatment itself induces a dose-related inhibition of feeding (14, 20, 23). In addition, stimulation of endogenous GLP-1 activity has been implicated in the mediation of the anorexic effects of LiCl and oxytocin (14, 16, 19). In each case, doses that reduce food intake increase c-Fos expression in GLP-1 neurons, and importantly, the anorexic effects are attenuated or eliminated with central injection of a GLP-1-receptor (GLP-1-R) antagonist (14, 16, 19). c-Fos expression in GLP-1 neurons is also enhanced by systemic LPS treatment (15), although pharmacological evidence supporting a critical contribution of GLP-1 to LPS anorexia has not yet been provided. Here, the GLP-1-mediation hypothesis is explored by intracerebroventricular delivery of exendin-(9–39), an antagonist of the GLP-1-R, alone and in combination with systemic LPS injection.

GLP-1 neurons are situated in the medulla, primarily in the caudal nucleus of the solitary tract (8, 11). They project both to LPS anorexia would be consistent with the GLP-1-R mediation hypothesis and indicate that the peptide’s contribution is transmitted, at least in part, via receptor populations in the caudal brain stem. The possible contribution of specifically forebrain GLP-1-R is evaluated in experiment 2, where the antagonist is delivered through third-intracerebroventricular cannula in rats with the caudal flow of cerebrospinal fluid (CSF) blocked by occlusion of the cerebral aqueduct (17).

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley rats (Charles River) weighing 350–450 g at the time of surgery were housed individually in hanging stainless cages under a 12:12-h light-dark cycle. Pelleted food (Purina 5001) and water were available ad libitum.

Surgery

Rats were anesthetized with ketamine (90 mg/kg), xylazine (2.7 mg/kg), and acepromazine (0.64 mg/kg) delivered intramuscularly. Rats in experiment 1 received a fourth-intracerebroventricular guide cannula (Plastics One, 22-G) with its tip positioned 2.0 mm above the fourth cerebral ventricle (coordinates: on the midline, 2.5 mm anterior to the occipital suture, and 4.5 mm ventral to the dura). Rats in experiment 2 received two cannulas: one as a guide for third-intracerebroventricular injections with its tip positioned 2.0 mm above the ventricle (coordinates: on the midline, 2.0 mm posterior to bregma and 7.5 mm ventral to dura) and the other (Plastics One, 19-G) for placement of the aqueduct plug (coordinates: 7.0 mm posterior to bregma, 1.0 mm lateral to the midline with an 11° angle toward...
sympathoadrenal mediated glycemic response to 5-thio-D-glucose ventricular cannula placement was assessed by measurement of the Procedure and closed with an obturator.

Cannula verification. At least 7 days after surgery, intracerebroventricular cannula placement was assessed by measurement of the sympathoadrenal mediated glycemic response to 5-thio-D-glucose ventricular cannula placement was assessed by measurement of the sympathoadrenal mediated glycemic response to 5-thio-D-glucose [210 μg in 2 μl of artificial cerebral spinal fluid (aCSF)] (3). The criterion for subject inclusion in the experiments was at least a doubling of plasma glucose level 1 h after injection. Habituation training. On several occasions rats were handled before lights out, and on one occasion they received intracerebroventricular and intraperitoneal vehicle injections to adapt them to these procedures.

Experiment 1: Effect of Fourth-Intracerebroventricular Exendin-(9–39) on LPS Anorexia

Rats received fourth-intracerebroventricular injections ~30 min before lights out, followed immediately by intraperitoneal injection. Three conditions were run across separate test days with at least 3 days between tests. All rats (n = 16) were tested under two control conditions: intracerebroventricular vehicle (2 μl aCSF) + intraperitoneal vehicle [isotonic saline (1.0 ml/kg)], and exendin-(9–39) (10 μg; California Peptide Research, Napa, CA) + intraperitoneal vehicle. These two conditions were presented in counterbalanced order across subjects. For one group of rats (n = 8), the last condition was a fourth-intracerebroventricular injection of exendin-(9–39), followed immediately by an intraperitoneal injection of LPS [Sigma-Aldrich, St. Louis, MO; 100 μg/kg body wt in saline vehicle (1.0 ml/kg)]. The last condition for a second group (n = 8) was a fourth-intracerebroventricular vehicle injection followed by LPS. Groups were matched for body weight. Tolerance to the ingestive effects of LPS (7) precluded a within-subjects comparison of these two injection conditions. The LPS dose selected is commonly tested (e.g., Refs. 7, 15), which, in our experience, yields ~60% suppression of 24-h intake. For each condition, cumulative food intake was measured 2, 4, 6, 8, and 24 h after injection.

Experiment 2: LPS Anorexia with Third-Intracerebroventricular Exendin-(9–39) and Aqueduct Occlusion

The conditions tested were identical to those of experiment 1, except for the intracerebroventricular placement and a variation in testing order. All rats (n = 19) received intracerebroventricular vehicle + intraperitoneal vehicle and intracerebroventricular exendin-(9–39) + intraperitoneal vehicle conditions, with condition order counterbalanced across rats. On the third test day the aqueduct was occluded by injection of silicon grease (5 μl). Separate groups of rats received intracerebroventricular vehicle + intraperitoneal LPS (n = 10) or intracerebroventricular exendin-(9–39) + intraperitoneal LPS (n = 9). Groups were matched for body weight. After the 24-h intake measure, the effectiveness of the aqueduct plug was verified by the absence of glycemic response to third-intracerebroventricular injection of 5-thio-D-glucose. Three subjects failed to meet this criterion and were excluded from the analysis.

Data Analysis

For each experiment, separate two-way ANOVAs (time × condition) were run for two-condition pairs as follows. The effect of exendin-(9–39) itself on cumulative intake was evaluated by within-subjects comparison of vehicle + vehicle and exendin + vehicle conditions. LPS anorexia was evaluated by within-subjects comparison of vehicle + vehicle and vehicle + LPS conditions. The ability of the antagonist to attenuate the effect of LPS was evaluated by between-subject comparison of vehicle + LPS and exendin + LPS conditions. In addition, within-subjects differences between vehicle + vehicle and exendin + LPS conditions were also evaluated.

RESULTS

Experiment 1

Figure 1 shows the overall effects of treatment condition on cumulative intake during the 24 h after injections. The intake suppression with LPS relative to the vehicle condition was pronounced overall [F(1,7) = 19.02, P < 0.004] and grew in magnitude over time [interaction: F(4,28) = 7.93, P < 0.0003], amounting to ~45% of the baseline value 24 h after treatment. Comparison between the LPS and the LPS + exendin conditions revealed a significant antagonism of the anorexic response [F(1,14) = 5.09, P < 0.05], which was most pronounced at the later time points [interaction: F(4,56) = 5.28, P < 0.002]. The reversal of LPS anorexia, however, was not complete; overall, the difference in cumulative intakes between vehicle and LPS + exendin condition was not signifi-
observations that within the central system mediating LPS anorexia awaits observation; alternatively, may be indirect; i.e., transmitted by neurons with the GLP-1 neuron itself. The activation of the GLP-1 neuron, the action of cytokine (or other mediators of LPS anorexia) on the GLP-1 neurons is direct, i.e., driven by the suppression requires release of the native peptide from GLP-1 neurons. Therefore, the results were comparable to those of experiment 1.

DISCUSSION

The results of experiment 1 establish a role for GLP-1 in the mediation of LPS anorexia. The GLP-1 antagonist, delivered to the fourth ventricle, itself had no effect on feeding behavior but substantially reduced the anorexic response to LPS. These data are consistent with the hypothesis that the maximal intake action of cytokine (or other mediators of LPS anorexia) on the GLP-1 neuron itself is the activation of the GLP-1 neuron, alternatively, may be indirect; i.e., transmitted by neurons with other neurochemical phenotypes. Central elements upstream with respect to the position of the GLP-1 neuron in the system may include proopiomelanocortin (POMC) and corticotropin-releasing hormone (CRH) neurons, the activity of which also has been shown to be necessary for LPS anorexia (5, 22). An adequate characterization of the position of GLP-1 neurons within the central system mediating LPS anorexia awaits observations that 1) define the set of relevant cytokine, prosta- glandin, or other signals that constitute the central trigger for the ingestive effect of LPS, 2) determine whether GLP-1 and other peptide neurons that can be implicated in the behavioral response to LPS express receptors for these signals, and 3) outline the pattern of interconnections among the relevant neuronal types.

The present study highlights, for the ingestive response to LPS, the significance of GLP-1 projections intrinsic to the caudal brain stem and discounts a role for GLP-1 projections to receptor populations in the forebrain. The role of the brain stem is indicated by the effectiveness of the fourth-intracerebroventricular route of antagonist administration. Antagonist delivered and limited to forebrain, by contrast, yielded no attenuation of LPS anorexia. With unrestricted flow of CSF, we would predict that third-intracerebroventricular antagonist administration would indeed attenuate the anorexic response but infer on the basis of the present results that the reversal would reflect action on GLP-1-R in the caudal brain stem. It is important to note, first, that the negative results concerning basal forebrain contributions were based on third-intracerebroventricular delivery of antagonist, which may not have effectively accessed tissue at appreciable distances from the ventricle. Thus, although the present results minimize roles for the paraventricular and arcuate hypothalamic nuclei and other sites proximal to the ventricle, further work in which antagonist is delivered to yet other sites, such as the dorsomedial hypothalamus and central nucleus of the amygdala, should be pursued.

Our results indicating a minimal contribution of forebrain GLP-1-Rs should not be readily applied to discussions of GLP-1 contributions to feeding control more generally or to the GLP-1-R mediation of the anorexic effects of other treatments. GLP-1-Rs are expressed in hypothalamic neurons of demonstrated importance for energy homeostasis. Direct action of GLP-1 on POMC neurons in arcuate nucleus and on oxytocin neurons (24) and CRH neurons in paraventricular hypothalamus (18), for example, may modulate neuroendocrine, autonomic, and/or feeding responses influenced by activity at these sites. We had noted that intake suppression was observed when GLP-1 itself was delivered at low doses to the hypothalamic paraventricular nucleus (10). Interestingly, a recent study focusing on GLP-1 mediation of LiCl anorexia yielded conclusions precisely opposing those clearly drawn from our analysis of intake suppression after LPS treatment. It had already been shown (14) that GLP-1-R antagonist delivered to the lateral ventricle attenuates the intake suppressive effects of systemic LiCl administration. Given the caudal flow of CSF, those results did not address brain stem vs. forebrain receptor mediation. Billes and colleagues (1), however, went further to show that fourth-intracerebroventricular antagonist administration did not influence the anorexic response, indicating a forebrain GLP-1-R mediation of LiCl anorexia. It appears the GLP-1 system plays multiple roles in the control of feeding behavior. A better understanding of the system will require identification of the relevant circuits in both forebrain and caudal brain stem and an appreciation of their relative contributions under different physiological conditions.

REFERENCES

The work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-21397 and DK-42284.


