Glucagon-like peptide (GLP-1) is involved in the central modulation of fecal output in rats

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Gülpinar, M. Ali, Ayhan Bozkurt, Tamer Coşkun, Nefise B. Ulusoy, and Berrak Ç. Yeğen. Glucagon-like peptide (GLP-1) is involved in the central modulation of fecal output in rats. Am J Physiol Gastrointest Liver Physiol 278: G924–G929, 2000.—In addition to its insulinotropic action, exogenously administered glucagon-like peptide (GLP-1) inhibits gastropancreatic motility and secretion via central pathways. The aims of the present study were to evaluate the effects of exogenous GLP-1-(7–36) amide on fecal output and to investigate the role of endogenous GLP-1 on stress-induced colonic activity. With the use of a stereotoxic instrument, adult male Sprague-Dawley rats weighing 200–250 g were fitted with stainless steel cerebroventricular guide cannulas under ketamine anesthesia. A group of rats were placed in Bollman-type cages to induce restraint stress. Fecal output monitored for 2 h was increased significantly by intracerebroventricular GLP-1 to 500, 1,000, and 3,000 pmol/rat (P < 0.05–0.01), whereas intraperitoneal GLP-1 had no effect. Intracerebroventricular administration of the GLP-1 receptor antagonist exendin-(9–39) (10 nmol/rat) reversed the increases induced by GLP-1 (500 pmol/rat; P < 0.01). Similar results were also observed with the injection of corticotropin-releasing factor receptor antagonist astressin (10 μg/rat; ivc). The significant increase in fecal pellet output induced by restraint stress was also decreased by both intracerebroventricular exendin (10 nmol/rat) and astressin (10 μg/rat; P < 0.01–0.001). These results suggest that GLP-1 participates in the central, but not peripheral, regulation of colonic motility via its own receptor and that GLP-1 is likely to be a candidate brain-gut peptide that acts as a physiological modulator of stress-induced colonic motility.

Glucagon-like peptide 1; exendin; astressin

THE GLUCAGON-LIKE PEPTIDE (GLP-1)-(7–36) amide is a gastrointestinal regulatory peptide formed and secreted from open-type endocrine cells (L cells) in the small (24, 27) and large (26) intestines in response to absorbed nutrients (7, 28). In addition to its well-known glucoregulatory effects, GLP-1 strongly inhibits pentagastrin- and meal-induced gastric acid secretion and gastric emptying of nonnutrient (17) and nutrient liquid meals (34, 41). It also inhibits pancreatic exocrine and endocrine secretions, such as pancreatic bicarbonate, protein, and pancreatic polypeptide secretion (42). The inhibitory effects of GLP-1 on the upper gastrointestinal and pancreatic functions are exerted via vagal afferent-mediated central mechanisms (17, 42), in which the dorsal vagal complex and the blood-brain barrier-free brain site, the area postrema (AP), are probably of major importance (18, 33). Together with peptide YY, GLP-1 is thought to represent one of the enterogastrones of the “ileal brake” mechanism (40). But it is unknown whether exogenously administered GLP-1 has any effect on colonic motility.

The ileal L cells express the preproglucagon gene that undergoes posttranslational processing, forming glucagon, GLP-1, and GLP-2. It was also shown that the preproglucagon gene expression found in the large intestine is in similar concentration to that reported for the small intestine and is high enough to have a physiological role (5). Gel chromatographic analysis of hypothalamic and brain stem tissue extracts revealed the presence of preproglucagon precursor, which undergoes posttranslational processing, similar to that found in the intestine. Many of the retrogradely labeled neurons in the caudal portion of the nucleus of the solitary tract (NTS) that project to many brain regions, including paraventricular nuclei (PVN), contain GLP-1 (20). Immunohistochemical studies demonstrate that GLP-1-immunoreactive nerve fibers are found in the forebrain, including hypothalamic, thalamic, and cortical areas, with the highest densities in the hypothalamic dorsomedial and PVN. Receptor autoradiographic studies have shown that these areas also have GLP-1 binding sites in high densities. NTS and the blood-brain barrier-free areas like the subfornical organ, the organum vasculosum laminae terminalis, and AP also have high densities of GLP-1 binding sites, although GLP-1 immunoreactive nerve fibers are in low density (9, 20).

In support of these findings, intracerebroventricular injection of GLP-1 has induced c-fos expression in a number of hypothalamic neuroendocrine areas, including magnocellular neurons of the PVN, supraoptic nuclei, and parvcellular neurons of the PVN, whereas slight induction of c-fos immunoreactivity was seen in the arcuate nucleus, NTS, and AP. Moreover, ~80% of the corticotropin-releasing factor (CRF)-positive neurons in the parvcellular part of the PVN have coexpressed c-fos activity after intracerebroventricular GLP-1 injections. GLP-1-induced c-fos expression was almost completely abolished by the GLP-1 antagonist...
exendin-(9–39). These results have suggested that at least one of the actions of GLP-1 in the central nervous system is via its specific receptors and through the activation of the hypothalmo-pituitary-adrenocortical axis by the stimulation of CRF neurons (20, 21).

It is well known that exposure to stressful stimuli alters gastrointestinal motility and secretions in different species, including human beings (3, 35). CRF is the main mediator and serves to integrate endocrine, autonomic, and behavioral responses to stress (6). Histamine (22, 30) and thyrotropin-releasing hormone (36) are other neurotransmitters released during stress, whereas CCK-8 and neuropeptide Y have antistress properties (11, 13, 15). CCK is shown to block the emotional stress- and CRF-induced colonic motor alterations (13). On the other hand, it is unknown whether GLP-1 and GLP-1-containing neurons have any role in the stress-induced visceral responses.

The aim of the present study was to evaluate the role of GLP-1 as a potential peripheral and central regulator of colonic motility in the rat. This study also aimed to investigate the participation of GLP-1, as a neurotransmitter, and GLP-1-containing neurons in the stress-induced colonic motor changes.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats weighing 200–250 g were housed individually in a light- and temperature-controlled room on a 12:12 h-light-dark cycle where the temperature (22 ± 2°C) and relative humidity (65–70%) were kept constant. The animals were fed a standard pellet lab chow, and access to water was allowed ad libitum. They were not fasted before experiments, but food was withdrawn while fecal pellet outputs were monitored. Experiments were approved by The Marmara University, School of Medicine, Animal Care and Use Committee.

Intracerebroventricular cannulation. The rats were anesthetized with ketamine (100 mg/kg ip) and chlorpromazine (0.75 mg/kg ip) and placed on a stereotaxic instrument (Stoelting Lab). According to the atlas of Paxinos and Watson (29), stainless steel cerebroventricular guide cannulas (22-gauge; Plastic Products, Roanoke, VA) were inserted into the right lateral cerebral ventricles (1.1 mm caudal and 1.5 mm lateral to the bregma, 3.2 mm ventral to the surface of the skull) and fixed to the skull with dental acrylic cement anchored around the three stainless steel screws. Experiments were performed at least one wk after the cannulation. Each rat received three different doses with a 3-day washout period between each dose. At the end of each experiment, animals were decapitated after injection of methylene blue to verify correct placement of the cannulas.

Stress model. The stress model used in the present study was a mild, nonnocarcinogenic restraint stress. In this model, a group of rats were lightly stressed in Bolman-type cages (5 cm wide, 17 cm long, and 8 cm high) for 2 h, allowing slight movement of head, legs, and trunk (25). Drugs were injected 10 min before exposure to restraint stress, and the number of fecal pellets expelled by each stressed animal was monitored for 2 h, starting at 5 min after the induction of stress. In the stress group, each animal was used on only one occasion to rule out the possible adaptation of rats to the stress procedure, which might be seen with repeated exposure (8). Control rats were kept in their home cages for 2 h.

Administration of drugs. All intracerebroventricular injec-
tions were administered in a 5-μl volume over a period of 1 min using a Hamilton syringe, whereas intraperitoneal injections were given in 0.3-ml volumes. When the injections of the antagonists or the vehicle (BSA) preceded that of GLP-1, a 10-min (in intracerebroventricular injections) or a 15-min (in intraperitoneal injections) interval was given between the injections. In the stress groups, antagonists were administered 10 min before the placement of rats into the stress cages. The effective doses of GLP-1 on fecal pellet output were determined by using different GLP-1 doses both centrally and peripherally (300–3,000 pmol/rat icv or ip). The solutions of GLP-1 (Sigma, St. Louis, MO) and exendin-(9–39) amide (Peninsula Laboratories Europe) were dissolved in 0.1% BSA. CRF receptor antagonist astressin (kindly provided by Jean Rivier, The Clayton Foundation Laboratory for Peptide Biology, San Diego, CA) was dissolved in bidistilled water at pH 7.0 warmed to 37°C and was injected at a dose of 10 μg/rat.

Measurement of fecal pellet output. Because of the short half-life of GLP-1 (16), the number of fecal pellets expelled by each animal, after intracerebroventricular or intraperitoneal GLP-1 injections, was monitored by counting stool excretion every 30 min for 2 h. Fecal pellet outputs during injections were disregarded. When the injection of the antagonists preceded the injection of GLP-1, fecal output monitoring was started after GLP-1 injection. In the stress groups, counting of the fecal pellet was also continued for 2 h with 30-min intervals, but it was started 5 min after the placement of rats into the stress cages to avoid the possible stress of handling.

Statistics. Results are expressed as means ± SE. One-way ANOVA was used for multiple comparisons, whereas Student’s t-test was chosen for comparison of paired results. Differences were considered statistically significant if P < 0.05.

RESULTS

Effect of central and peripheral administration of GLP-1 on fecal output in nonstressed rats. Intracerebroventricular injection of GLP-1 at doses of 500, 1,000, and 3,000 pmol/rat significantly increased 2-h fecal pellet output (P < 0.05–0.01) compared with intracerebroventricular vehicle (BSA) group (1.28 ± 0.84), whereas a dose of 300 pmol/rat did not alter the number of stool excretions significantly. However, intraperitoneal GLP-1 injection did not affect fecal output even in a higher dose range (1,000–3,000 pmol/rat) (Fig. 1 and Table 1). The lowest effective dose of 500 pmol/rat was used in the groups in which antagonists were administered. In these groups, intracerebroventricular administration of exendin-(9–39) at a dose of 10 nmol/rat reversed the increase in fecal pellet output (2.00 ± 1.00) induced by intracerebroventricular GLP-1 (P < 0.01; Fig. 2), whereas intracerebroventricular injection of exendin-(9–39) alone had no effect on defecation of the rats. Similar results were also observed by the intracerebroventricular injection of CRF receptor antagonist astressin (10 μg/rat; P < 0.01). Neither intracerebroventricular exendin-(9–39) at a dose of 10 nmol/rat nor intracerebroventricular injection of astressin at 10 μg/rat reversed fecal pellet output induced by the higher dose of GLP-1 (1,000 pmol/rat; Table 1).

Blockade of stress-induced defecation. In nonstressed rats injected with intracerebroventricular vehicle, the number of fecal pellets was 1.28 ± 0.84 h. The
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restraint stress led to an increase in fecal pellet output by fivefold during a 2-h stress exposure ($6.00 \pm 0.78$) compared with the intracerebroventricular vehicle-treated group (Fig. 3). In rats pretreated with intracerebroventricular astressin (10 µg/rat), restraint stress did not induce a significant increase in fecal pellet output ($2.00 \pm 0.89$; $P < 0.01$). Similarly, stress-induced increase in stool excretion was abolished by 10 nmol/rat intracerebroventricular exendin ($0.2 \pm 0.2$; $P < 0.001$). No significant difference was observed among the effects of the two antagonists.

**DISCUSSION**

In addition to its known inhibitory effects on gastrointestinal functions, the results of the present study suggest that GLP-1 may also have a role in the regulation of colonic motility. Intracerebroventricular GLP-1 increased the number of stools excreted in a 2-h period, whereas the peripheral administration of GLP-1 over the same dose range had no significant effect. These results provide indirect evidence for central mechanisms involving GLP-1 in the regulation of colonic motility.

### Table 1. The effect of GLP-1 on fecal pellet output

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>n</th>
<th>Fecal Pellet Output, no./2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle, ip)</td>
<td>6</td>
<td>1.00 \pm 1.00</td>
</tr>
<tr>
<td>GLP-1 (1000 pmol/rat ip)</td>
<td>7</td>
<td>1.86 \pm 0.86</td>
</tr>
<tr>
<td>GLP-1 (3000 pmol/rat ip)</td>
<td>6</td>
<td>0.75 \pm 0.75</td>
</tr>
<tr>
<td>Control (vehicle, icv)</td>
<td>7</td>
<td>1.28 \pm 0.84</td>
</tr>
<tr>
<td>GLP-1 (300 pmol/rat icv)</td>
<td>7</td>
<td>2.14 \pm 0.96</td>
</tr>
<tr>
<td>GLP-1 (500 pmol/rat icv)</td>
<td>13</td>
<td>4.15 \pm 0.59*</td>
</tr>
<tr>
<td>GLP-1 (1000 pmol/rat icv)</td>
<td>12</td>
<td>5.42 \pm 0.81**</td>
</tr>
<tr>
<td>Exendin (10 nmol/rat icv) + GLP-1</td>
<td>6</td>
<td>2.00 \pm 1.00†</td>
</tr>
<tr>
<td>GLP-1 (1000 pmol/rat icv)</td>
<td>6</td>
<td>5.40 \pm 1.50**</td>
</tr>
<tr>
<td>Exendin (10 µg/rat icv) + GLP-1</td>
<td>10</td>
<td>2.40 \pm 0.86††</td>
</tr>
<tr>
<td>Exendin (10 µg/rat icv) + GLP-1</td>
<td>8</td>
<td>7.13 \pm 1.59**</td>
</tr>
</tbody>
</table>

Values are means \pm SE. Either exendin or astressin was administered intracerebroventricularly (icv) 10 min before icv administration of glucagon-like peptide (GLP-1). *$P < 0.05$ and **$P < 0.01$ compared with intracerebroventricular vehicle (BSA) group; ††$P < 0.01$ compared with group treated with same dose of GLP-1.
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Mechanisms involved in the stimulatory action of GLP-1 on colonic motility.

Studies of mRNA transcripts for the GLP-1 receptors, Northern blot analysis, and RT-PCR studies revealed that the GLP-1 receptor gene is also widely distributed in the large intestine as well as the different regions of the brain of mice (4). In addition, the presence of both mRNA transcripts encoding the preproglucagon precursor and proglucagon-derived peptides in the brain has been demonstrated by several studies (14, 32, 38), and the pattern of the post-translational processing in rat brain stem and hypothalamus was indistinguishable from that seen in porcine and human small intestine. In situ hybridization histochemistry revealed that a population of GLP-1-producing cells is found in the caudal portion of NTS and that these cells contribute to the terminal field of at least one hypothalamic target, especially to the PVN. There are numerous sites in the central nervous system that possess GLP-1-like immunoreactive fibers (GLP-1-IR), such as the forebrain areas, including hypothalamic, thalamic, and cortical areas. But the densest innervation of GLP-1-IR nerve fibers was seen in the hypothalamic drosomedia nuclei and PVN. It is important to note that the content and density of GLP-1-IR fibers in the hypothalamic nuclei correlate well with the distribution and density of GLP-1 binding sites (9). Central administration of GLP-1 was shown to induce c-fos immunoreactivity, largely in a number of hypothalamic neuroendocrine areas, including magnocellular and parvicellular neurons of the PVN and supraoptic nuclei, whereas a slight induction of c-fos expression was seen in the arcuate nucleus and NTS, including AP (21). Moreover, the amount of c-fos reactivity was well correlated with the distribution of GLP-1-IR fibers in different regions of the brain, and the induction of c-fos activity was completely inhibited by the injection of GLP-1 receptor antagonist exendin-(9–39).

In the present study, intracerebroventricular GLP-1 increased the fecal pellet output, and this induction was reversed by intracerebroventricular injection of exendin-(9–39). Together with previous reports, these data provide a strong support for the hypothesis that GLP-1, as a central neurotransmitter, has a role in the regulation of colonic function by acting directly via the autoreceptors, possibly within the hypothalamic nuclei. In addition to this direct mechanism, GLP-1 may also act on the interneurons, especially in hypothalamic nuclei and between hypothalamic and other nuclei that are important in the regulation of gastrointestinal functions. Further explanations of the mechanisms involved in the action of central GLP-1 and its precise acting site(s) need to be elucidated using different study models. Regarding the mechanisms of action, in the present study, it is also shown that GLP-1-induced stool excretion is reversed by intracerebroventricular injection of astressin. This finding is consistent with the results of a study in which GLP-1 induced c-fos activity in ~80% of the corticotropin-releasing hormone (CRH)-positive neurons in the medial parvicellular part of the PVN (21).

The blood-brain barrier-free areas of the third ventricle (subfornical organ, organum vasculosum laminae terminalis) and the AP were postulated to be responsible for the effects of peripherally administered GLP-1 on gastric secretory and motor responses (17), since high densities of the binding sites are observed in these areas despite the low GLP-1-IR nerve fibers. However, in contrast to gastric findings, the results of the present study do not indicate any effect of peripherally administered GLP-1 on colonic motility. The underlying discrepancy between the responses, which may be due to different pathways or receptors, needs to be further elucidated. Since a sixfold higher peripheral dose reaching 3 nmol/rat had no effect on fecal output, the effect appears to be centrally mediated.

It is well known that CRF plays a major role in mediating stress-induced alteration of gastrointestinal motor functions, including colonic motility. By regulating CRF release, a number of neurons and neurotransmitters in and between the hypothalamus, particularly PVN, and amygdala participate in stress-induced responses (1, 10, 19, 21, 25). For example, neuropeptide Y with its dense cell bodies and terminals within the amygdala might be an endogenous agent that buffers against the stressor-induced responses, possibly by regulating hypothalamic CRF release (15). Endogenous 5-HT is suggested to be another substance mediating stress-induced colonic motility. Both intracerebroventricular injection of CRF and stress peripherally promote the release of 5-HT from enterochromaffin cells and enteric serotonergic neurons and lead to the enhancement of colonic motility (23). Stress also activates dopamine metabolism in mesolimbic and mesocortical structures, as well as in striatum, and the release of CRF due to stress exposure involves the activation of central dopaminergic neurons (12). Another neurotransmitter that has an antistress effect is vasopressin (AVP), which is coexpressed with CRF in parvocellular neurons of the PVN. In one study, it was shown that intracerebroventricular injection of AVP significantly reduced the effects of stress- and CRF-induced colonic motility and that the involvement of AVP in the stress-induced alteration of colonic motility was dependent on previous activation of CRF neurons and/or its release (2).

It was previously reported that centrally infused GLP-1 inhibits feeding/drinking behavior (37) and gastric emptying in rats (17). GLP-1-induced c-fos expression is abolished by prior administration of the antagonist exendin-(9–39). Furthermore, exendin-(9–39) also induced c-fos expression in a number of central sites, implying a constant GLP-1-mediated inhibitory tone released by the antagonist (37). In further studies, high densities of GLP-1 binding sites were demonstrated within the medial parvocellular subset of the PVN, and c-fos expression was triggered in the majority of the CRH-containing perikarya after central GLP-1 injection. Thus it was speculated that at least one of the central effects of GLP-1 is exerted by a direct influence on the CRH-containing motor neurons of the hypothalamo-pituitary-adrenocortical axis (9). Intracerebro-
ventricular GLP-1 also induced c-fos expression in oxytocin- as well as CRH-containing neurons in the PVN and stimulated circulating levels of AVP and corticosterone (33, 39).

Together with the results of the present study, these aforementioned observations support the hypothesis that central GLP-1 is involved in stress-induced responses by regulating CRF release either directly or through the participation of other interneurons. Our results demonstrate that GLP-1 may be one of the central neurotransmitters that are involved in the central regulation of colonic motility. Inhibition of both stress- and intracerebroventricular GLP-1-induced fecal pellet output by previous administration of exendin-(9–39) suggests that the stimulatory effect of GLP-1 on colonic motility is mediated by its own receptors and involves interaction with the CRF-containing neurons. These findings are in parallel with the study in which central administration of GLP-1 has induced c-fos immunoreactivity in CRF-positive neurons (20). However, the precise sites of action and the mechanisms of this stimulatory effect of GLP-1 on colonic motility necessitate further studies.

In conclusion, the present study demonstrates that GLP-1 acts centrally, but not peripherally, to regulate colonic motility. The results also suggest that the GLP-1 receptors on the GLP-1-containing neurons are involved in stress-induced colonic motility. Thus GLP-1 is likely to be a candidate neurotransmitter that acts as a central modulator of colonic motility after stress exposure.

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