Gastric Effects of Galanin and Its Interaction with Leptin on Brainstem Neuronal Activity

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Received August 30, 2001; accepted January 3, 2002 This article is available online at http://jpet.aspetjournals.org

ABSTRACT

Galanin is a 29-amino acid peptide that is widely distributed throughout the central nervous system, peripheral nervous system, and gastrointestinal and genitourinary tracts. Leptin is a hormone secreted from adipose tissue and the gut and other tissues. In this study, using an in vitro neonatal rat preparation, we investigated the gastric effects of galanin and its interaction with leptin on nucleus tractus solitarius (NTS) neurons receiving gastric vagal inputs. We showed that peripheral gastric galanin (300 nM) produced a mean inhibition response of 53.2 ± 2.1% compared with the control level of 100% (P < 0.01) in 27 of 58 neurons tested. A concentration-dependent effect of galanin on NTS neuronal activity was observed. The galanin receptor antagonist [galanin-(1–12)-Pro3-(Ala-Leu)2-Ala amide], or M40, significantly reversed the galanin-induced inhibition effect (P < 0.01). In contrast, we showed that the peripheral gastric effect of leptin (10 nM) produced a mean activation response of 167.4 ± 8.2% compared with the control level. The NTS neurons that we recorded could respond to both galanin and leptin or respond to only one of them. Subsequently, we evaluated gastric interactions between galanin and leptin on NTS unitary activity when galanin (100 nM) and leptin (10 nM) were applied together in the gastric compartment. We observed that the effect of leptin when applied alone (168.8 ± 7.7%) was reduced to 146.2 ± 4.7% after coapplication of both compounds (P < 0.05 compared with leptin alone; P < 0.01 compared with galanin alone, 55.1 ± 3.2%). Our data suggest that galanin modulates the leptin signals, which regulate the ingestive process in neonates.

Both central and peripheral signals play roles in the complicated neuronal circuitry that regulates feeding and energy homeostasis. Peripheral signals are relayed via afferent sensory fibers, which are the primary neuroanatomical link between the gastrointestinal tract and central neural substrates (Altschuler et al., 1989; Berthoud et al., 1990). The vagus is a major visceral sensory nerve conveying information from the gastrointestinal tract to the brainstem. We previously reported that leptin, an adipose tissue-derived circulating hormone, activated brainstem neurons responding to gastric vagal stimulation (Yuan et al., 1999). We also observed gastric interaction between leptin and cholecystokinin, a neuuropeptide that regulates food intake, on brainstem neuronal activity via gastric vagal afferents (Yuan et al., 2000a). These results led to a question concerning gastric interactions between leptin and other neuropeptides, such as galanin.

Galanin is a 29-amino acid peptide that is widely distributed throughout the central nervous system, peripheral nervous system, and gastrointestinal and genitourinary tracts. It mediates a wide spectrum of effects, including regulation of gastrointestinal smooth muscle and stimulation of feeding behavior (Fathi et al., 1997). The neural center controlling food intake is primarily composed of catecholaminergic, serotoninergic, and peptidergic systems (Leibowitz and Shor-Posner, 1986; Gibbs and Smith, 1992; Sahu and Kalra, 1993; Hirschberg, 1998). Several gastrointestinal peptides including galanin can modulate food intake (Clark et al., 1985; Leibowitz, 1991). These peptides regulate appetite via both central and peripheral mechanisms (Clark et al., 1985; O'Donohue et al., 1985). Experimental studies demonstrated that neuropeptide Y and galanin strongly stimulated the appetite (Clark et al., 1985; Clark and Kalra, 1990; Leibowitz, 1990). These gastrointestinal peptides may affect the central control of appetite via the vagal and spinal nerves (Berelowitz et al., 1992; Gibbs and Smith, 1992; Leibowitz, 1995). Baranowska et al. (2000) observed that the release of gastrointestinal peptides, including galanin, is disturbed in obesity and in anorexia nervosa. These findings suggest that dysfunction of the brain-gut axis may also be an important factor in the abnormal control of appetite.

Leptin, the secreted product of the obese (ob) gene, regulates food intake and energy balance. Leptin is not only

ABBREVIATIONS: ob, obese; NTS, nucleus tractus solitarius; GAL-IR, galanin immunoreactivity; M40, [galanin-(1–12)-Pro3-(Ala-Leu)2-Ala amide].
expressed in adipose tissue (Zhang et al., 1994) but also expressed in gastric mucosa and fundic glands (Bado et al., 1998; Mix et al., 1999). Whether gastric galanin interacts with leptin to modulate brainstem neuronal activity, which may lead to changes in long-term feeding behavior, has not been explored.

In this study, an in vitro neonatal rat preparation was used. This preparation retains the functional circuitry of the brainstem vagal-neuronal link with the gastric system, providing a unique opportunity to test the peripheral gastric interactions among peptides on the central nervous system. We evaluated the peripheral gastric effect of galanin on unitary activity in the nucleus tractus solitarius (NTS) and then investigated the effect of gastric interaction between galanin and leptin on brainstem neurons.

**Materials and Methods**

**Animal and Surgical Preparation.** The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Chicago. Experiments were performed on 32 Sprague-Dawley neonatal rats of 1 to 6 days old purchased from Harlan (Indianapolis, IN). After the animal was deeply anesthetized with halothane, a craniotomy was performed, and the forebrain was ablated at the caudal border of the pons by transection. The caudal brainstem and cervical spinal cord were isolated by dissection in modified Krebs’ solution that contained 128.0 mM NaCl, 3.0 mM KCl, 0.5 mM NaH2PO4, 1.5 mM CaCl2, 1.0 mM MgSO4, 21 mM NaHCO3, 1.0 mM mannitol, 30.0 mM glucose, and 10.0 mM HEPES. The stomach, connected to the esophagus with the vagus nerves linking it to the brainstem, was kept, and all the other internal organs were removed. The preparation was then isolated and pinned, with the dorsal surface up, on a layer of Sylgard resin (Dow Corning Corp., Midland, MI) in a recording chamber. An incision was made in the lateral surface of the stomach wall to minimize possible leakage to the other compartment. Peptides were applied only to the gastric compartment, and their effects on the NTS neuronal activity were evaluated.

Each test compound was first dissolved in a small volume of Krebs’ solution. The concentrated solution was then applied to the gastric compartment. The final drug concentration in the gastric compartment was calculated based on the amount of concentrated solution and the total Krebs volume in the gastric compartment. Drug solution was applied 5 min prior to any pharmacological observation to provide sufficient time for drug delivery to reach a steady-state level. To observe galanin-leptin interaction, solutions were added simultaneously as described under Results. After each observation, drug was washed out from the compartment. The NTS neuronal responses observed during pretrial or pretreatment (control) were compared with post-trial (washout) to confirm that brainstem neuronal activity returned to the control level after washout.

**Peripheral Gastric Effects of Galanin.** Peripheral gastric effects of galanin (300 nM) produced a mean inhibition of 21.3% compared with the control level (100%) in 27 of 58 neurons tested. There was a concentration-dependent effect of galanin on NTS neuronal discharge fre-
Gastric effect of M40, a galanin receptor antagonist, was evaluated in 12 NTS neurons that responded to galanin. Galanin (100 nM) produced a mean inhibition response of 57.7 ± 5.9% compared with control. Subsequently, galanin (100 nM) and M40 (100 nM) were applied together into the gastric compartment. M40 significantly reversed galanin-induced effect (89.6 ± 6.0%; P < 0.01).

Peripheral Gastric Effects of Leptin. Twenty units that showed activation responses to galanin, as noted in the preceding section, were also tested after leptin application. As shown in Table 1, peripheral effects of leptin (10 nM) produced a mean activation response of 167.4 ± 8.2% of the control level in 17 neurons tested. The difference in the NTS neuronal activity between the control and the recording after leptin was significant (P < 0.01). The remaining three units that responded to galanin were not affected by leptin (Table 1).

Gastric Interaction between Galanin and Leptin on NTS Unitary Activity. To evaluate the interaction between galanin and leptin, we tested three groups of NTS neurons, which were different from the units reported above. The first group consisted of 15 units that showed activity change in response to both galanin (100 nM) and leptin (10 nM). The second group consisted of 14 units that did not respond to galanin (100 nM) but showed activity change in response to leptin (10 nM). The third group consisted of four units that showed inhibition response to galanin (100 nM) but did not respond to leptin (10 nM).

In the first group of 15 units, galanin (100 nM) and leptin (10 nM) were applied together to the gastric compartment. As shown in Fig. 2, the effect of leptin when applied alone (168.8 ± 7.7%) was reduced to 146.2 ± 4.7% after coapplication of both compounds (P < 0.05 compared with leptin alone; P < 0.01 compared with galanin alone, 55.1 ± 3.2%). In the second group, the same concentrations of galanin and leptin were used to test the gastric compartment of 14 units that did not respond to galanin but showed activity change in response to leptin when they were applied alone. The effect of leptin alone (165.1 ± 9.0%) was reduced to 153.8 ± 7.5% after coapplication of both compounds. However, this reduction did not reach a statistically significant level. In the third group, the same concentrations of galanin and leptin were applied together to evaluate four units that showed inhibition response to galanin alone (59.2 ± 11.6%) but did not respond to leptin application. Coapplication of both compounds increased the activity to 75.5 ± 7.2%. Some data from these three groups are summarized in Table 2.

In addition, interaction observation was made in another eight NTS neurons in response to which neither galanin (100 nM) nor leptin (10 nM) changed their activity. When both galanin (100 nM) and leptin (10 nM) were applied to the gastric compartment, no noticeable neuronal activity change was recorded in any of these units.

Discussion

In this study, gastric effects of galanin and its interaction with leptin on NTS units processing gastric vagal inputs were investigated. A neonatal rat brainstem-stomach preparation was used, in which we have previously demonstrated gastric neurochemical effects on gastric vagally evoked brainstem neuronal activity (Barber et al., 1995; Yuan et al., 1999). Galanin and leptin are peptides that have central and peripheral effects. This preparation allows us to restrict galanin and leptin to the gastric compartment and to observe peripheral effects without interfering with brainstem functions. The development of obesity in rodent models is concomitant with effects from hormonal and metabolic changes on leptin homeostasis (Saladin et al., 1995). Our experiments were performed on nonobese preweaned animals to avoid the complicating effects of metabolic patterns on leptin activity as seen in adults.

We used 1- to 6-day-old rats to demonstrate interactions between gastric galanin and leptin on neurons in the medial subnucleus of the NTS. In a series of retrograde transynaptic neuronal viral infection studies of rats in this age group, Rinaman et al. (1999, 2000) demonstrated synaptic connectivity between gastric vagal afferents, neurons in the medial subnucleus of the NTS, and preganglionic vagal motor neurons. In rats, the leptin system, with respect to ob gene expression and leptin production, is operational 1 day after birth (Rayner et al., 1997). In our recent study, we showed that intraperitoneally injected leptin-modulated feeding behavior led to a significant decrease in weight gain in neonatal rats (Yuan et al., 2000b). Thus, our experimental model is appropriate for the present investigation.

Our results demonstrated that neurons located in the NTS were responsive to both gastric galanin and leptin. Peripheral gastric galanin had an inhibitory effect on brainstem neuronal activity. In our investigation, we observed that peripheral gastric effects of galanin produced significant inhibition response compared with the control. M40, a galanin receptor antagonist, also reversed the gastric effect of galanin in NTS neurons. In addition, our results indicated that peripheral gastric effects of leptin produced significant activation response. We also evaluated the interaction between
galanin and leptin in three groups of NTS neurons. In the first group, we observed that the effect of leptin when applied alone was reduced after coapplication of both compounds. In the second group, we tested galanin and leptin in the gastric compartment on NTS units that did not respond to galanin but did respond to leptin. We observed that the effect of leptin alone was reduced by some extent after coapplication of both compounds. This is probably due to galanin's subthreshold inhibition activity in extracellular recording. In the third group, we evaluated galanin and leptin in the gastric compartment on NTS units that did not respond to leptin but did respond to galanin. Coapplication of both compounds reduced galanin's inhibition effect.

In addition to its endocrine, exocrine, and autocrine functions (Wang et al., 1997; Kisfalvi et al., 2000), galanin plays an important role in the regulation of fat intake (Leibowitz, 1991). It increased food intake when injected into specific brain regions (Crawley et al., 1990; Corwin et al., 1993). In the hypothalamus, galanin acted on neurons in the paraventricular nucleus, the medial preoptic area, and the median eminence to regulate feeding behavior (Leibowitz, 1994). Koegler et al. (1999) observed that M40 was most effective at reducing deprivation-induced food intake when injected into the hindbrain. In another study, Koegler and Ritter (1998) observed that galanin receptors in the NTS region mediate feeding in response to galanin and that the galaninergic nerve terminals innervating these receptors may originate in part from cell bodies in the paraventricular nucleus. So far, studies on the effects of galanin related to eating behavior, nutrient partitioning, and body weight gain have focused on a central mechanism of action involving hypothalamic neuronal circuits (Kyrkouli et al., 1986; Leibowitz and Kim, 1992). Previous studies did not show whether galanin activates the peripheral terminals of visceral afferent neurons and initiates neuronal activity change in the central nervous system, as observed in this study.

Moderate to dense galanin immunoreactivity (GAL-IR) has been observed in the NTS (Boissonade et al., 1996), the primary brainstem relay for visceroceptive information from the gastrointestinal system. GAL-IR has been observed in the dorsal motor nucleus of the vagus, one of the recipients of axonal projections from the NTS (Boissonade et al., 1996). Sweerts et al. (2000) observed galanin binding sites in the human inferior vagal (nodose) ganglion. In addition, galanin has been demonstrated in vagal sensory neurons. Galanin production in vagal sensory neurons increased in response to a reduction in fatty acid oxidation, a known stimulant of fat ingestion (Calingasan et al., 1992).

Galanin is also widely distributed throughout the gastrointestinal tract (Kuwahara et al., 1990; Lee et al., 1994). GAL-IR has been observed in nerve cell bodies and nerve fibers in all layers of the canine lower esophagus, gastric antrum, pylorus, ileum, and colon, and in the sphincters of the lower esophagus and pylorus (Wang et al., 1995; Fathi et al., 1997). Galanin immunoreactivity is present predominantly in the myenteric and submucosal plexi (Melander et al., 1985; Ekblad et al., 1989). Results of structure-function studies show that two subtypes of receptors (GALR1 and GALR2) may mediate galanin's actions in the gut (Gu et al., 1995).

In our study, we demonstrated that galanin, when applied to the stomach, can stimulate activity in NTS neurons receiving gastric vagal inputs. These results suggest that galanin can activate the peripheral terminals of gastric vagal afferents and modulate physiological action at the level of the brainstem. In addition, we observed that M40 reversed most of the inhibitory activity of galanin. M40 is a GALR1 antagonist, and a weak GALR2 agonist (Bartfai et al., 1993), whereas GALR2 mRNA has a widespread peripheral distribution and is highly expressed in the stomach (Fathi et al., 1997). This may explain why M40 was unable to completely reverse gastric galanin effects in our experiments. El-Salhy et al. (2000) studied the effects of cervical vagotomy on the content of several neuroendocrine peptides in different parts of the murine gastrointestinal tract, which are known to receive vagal innervation, and observed an increased level of galanin after vagotomy. In this regard, although the evidence that galanin controls food intake by acting on peripheral vagal receptors is strong, the observation by El-Salhy et al. (2000) suggests the existence of additional mechanisms.

Galanin interacts with other peptides, leptin and cholecys-

### Table 1

Gastric effects of galanin and leptin on NTS neuronal activity in neonatal rats

Activity change indicates the level of inhibition or activation (mean ± S.E.M.) compared with control (100%).

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of NTS Units</th>
<th>No. of Responses</th>
<th>Response</th>
<th>Activity Change (Compared with Control)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galanin</td>
<td>58</td>
<td>27</td>
<td>46.6</td>
<td>53.2 ± 2.1%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Leptin</td>
<td>20</td>
<td>17</td>
<td>85.0</td>
<td>167.4 ± 8.2%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Fig. 2. Gastric interactions between galanin and leptin on 15 nucleus tractus solitarius units receiving gastric vagal inputs. All these units responded to both galanin (100 nM) and leptin (10 nM). A change of neuronal activity level was observed as galanin (100 nM) and leptin (10 nM) were applied together. Control is normalized to 100%. Brackets indicate the mean ± S.E.M. *, P < 0.01 compared with after wash. **, P < 0.05 compared with leptin alone and P < 0.01 compared with galanin alone.
Activity levels (mean ± S.E.M.) are compared with control (100%).

<table>
<thead>
<tr>
<th>No. of NTS Units</th>
<th>Galanin (100 nM)</th>
<th>Leptin (100 nM)</th>
<th>Galanin (100 nM) plus Leptin (10 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>15</td>
<td>55.1 ± 3.2%</td>
<td>168.48 ± 7.7%</td>
</tr>
<tr>
<td>Group 2</td>
<td>14</td>
<td>No effect</td>
<td>165.1 ± 9.0%</td>
</tr>
<tr>
<td>Group 3</td>
<td>4</td>
<td>59.2 ± 11.6%</td>
<td>No effect</td>
</tr>
</tbody>
</table>

* P < 0.01 compared with galanin (100 nM) alone; P < 0.05 compared to leptin (10 nM) alone.

In animal models, leptin reduces appetite and increases energy expenditure (Zhang et al., 1994; Halaas et al., 1995; Pellemounter et al., 1995). Several observations suggest that leptin may have specific functions in the gastrointestinal tract. Upon a single injection, leptin reduced food intake in ob/ob or lean mice only after several hours (Cohen et al., 1996; Barrachina et al., 1997). The effect of leptin may require the presence of food-related gastric or postgastric signals (Barrachina et al., 1997). In addition, leptin has been observed in the stomach mucosa of rats and humans (Bado et al., 1998; Cinti et al., 2001). Sobhani et al. (2000) observed the presence of leptin and leptin receptor proteins in the human stomach and suggested that gastric epithelial cells may be a direct target for leptin. Cinti et al. (2001) also concluded that three important pathways, i.e., endocrine, exocrine, and autocrine, for the action of leptin are present in human stomach. It has also been shown that leptin injected into the portal vein of rats results in a sustained increase in vagal hepatic afferent activity, which indicates that feeding-suppression effects of leptin are mediated by its effects on signal transmission through both the central and the peripheral nervous systems (Shiraishi et al., 1999). In this study, we observed that activation effect of gastric leptin on brainstem neurons was reduced by coapplication of galanin. Our data demonstrate that there is a peripheral gastric interaction between galanin and leptin. Exact sites at which leptin, alone and with galanin, interacts to influence gastric vagal afferent discharges remain to be determined. In addition, the duration of interaction between leptin and galanin needs to be explored.

It would be beneficial to know whether future in vivo observation can demonstrate that peripherally injected galanin increases weight gain in neonatal rats and whether this effect is modified by peripheral leptin administration (cf. Yuan et al., 2000b). Peripheral gastric interaction between galanin and leptin may provide a useful concept for understanding multifactorial control of ingestive behavior and open new avenues for obesity and eating disorders such as anorexia nervosa and bulimia.

In summary, we observed peripheral gastric effects of galanin and its interaction with leptin on brainstem neuronal activity. Our results indicate that gastric galanin interacts with leptin at the level of the stomach to decrease afferent neural signals to the NTS. Thus, our data suggest that galanin modulates the potency of leptin signals that modify food intake in the neonatal rat.

Acknowledgments

We thank Spring A. Maleckar for technical assistance.

References


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