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

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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. It mediates a wide spectrum of effects, including regulation of gastrointestinal smooth muscle and stimulation of feeding behavior. 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; Leibowitz, 1990). 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; Leibowitz, 1990). 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; Leibowitz, 1990). 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; Leibowitz, 1990). 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; Leibowitz, 1990). 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; Leibowitz, 1990). 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; Leibowitz, 1990). 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; Leibowitz, 1990). 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; Leibowitz, 1990). 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; Leibowitz, 1990). 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; Leibowitz, 1990). 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 neope...
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 on the lateral surface of the stomach wall to minimize possible gastric vagal fiber damage. The stomach was opened, and its contents were removed. The stomach was then pinned down, and both mucosa and serosa were exposed to Krebs’ solution in the gastric compartment. The preparation was superfused with Krebs’ solution at 23 ± 1°C. The bathing solution was aerated continuously with a mixture of 95% O₂ and 5% CO₂ and adjusted to pH 7.35 to 7.45 (Barber et al., 1995; Yuan et al., 1998).

Stimulation and Recording Methods. A suction microelectrode was placed on the gastric vagal branch from the subdiaphragmatic vagi for electrical stimulation, and units in the medial subnucleus of the NTS receiving gastric vagal inputs were evaluated in this study. The gastric vagal fibers were stimulated with single or paired pulses of 200 μA for 0.2 ms at a frequency of 0.5 Hz by a Grass stimulator (model S8800; Grass Instruments, Quincy, MA) coupled to a stimulus isolation unit (SIU 5B). This current provided a stimulus intensity 1.5 to 2.0 times greater than that required to produce maximal amplitude of the C-wave in the vagal nerve action potential (Yuan et al., 1998).

Single tonic unitary discharges were recorded extracellularly in the medial subnucleus of the NTS by glass microelectrodes filled with 3 M NaCl, with an impedance of 10 to 20 MΩ (for unitary discharge recordings see Barber et al., 1995). A collision test was applied to identify orthodromic inputs (Lipski, 1981) to ensure that only second- or higher-order NTS neurons in the gastric vagal afferent system were used in this study.

The NTS unitary discharges were amplified with high-gain AC-coupled amplifiers (Axoprobe-1A; Axon Instruments, Union City, CA), displayed on a Hitachi digital storage oscilloscope (model VC-6525; Hitachi Dentshi, Ltd., Tokyo, Japan) and recorded on a Vetter PCM tape recorder (model 200; A.R. Vetter Co., Rebersburg, PA).

For histological identification purposes, some glass microelectrodes were filled with 2% pontamine sky blue in 0.5 M sodium acetate solution. After each unitary recording, current was applied at 5 μA in 5-s on/10-s off cycles for approximately 5 min, with the negative lead connected to the microelectrode.

Experimental Protocols. Galanin and leptin have both peripheral and central actions. To investigate the peripheral gastric effects of these peptides on brainstem neurons without interfering with central nervous system functions, a partition was made at the mid-thoracic level of the preparation. An agar seal separated the recording bath chamber into a brainstem compartment and a gastric 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 pretreatment (control) were compared with post-trial (washout) to confirm that brainstem neuronal activity returned to the control level after washout.

Concentrations of galanin and [galanin-(1–12)-Pro3-(Ala-Leu)2-Ala amide] (M40) used in this study (see Results) were based on previous reports (Wang et al., 1997; Koegler et al., 1999) and our pilot experiments. We selected a concentration of 10 nM leptin in this study according to our previous observation (Yuan et al., 1999). Tachyphylaxis was tested by reapplying the test compound to the gastric compartment and observing whether the response to a given concentration of the compound varied by less than 5%.

At the end of eight experiments, after the NTS neuronal responses to test compounds were observed, the vagus nerve was severed at the low thoracic level. For all eight units that responded to peptides prior to vagal discontinuation, gastric effects disappeared after the vagus was severed. Also, at the completion of each experiment, colored solution was applied to one compartment to confirm that there was no leakage to the other compartment.

Drugs. Drugs used in this study were galanin, obtained from Bachem California (Torrance, CA), the galanin receptor antagonist M40, obtained from Peninsula Laboratories (Belmont, CA), and methionyl murine leptin, or leptin, obtained from Amgen Biologicals (Thousand Oaks, CA).

Data and Statistical Analysis. The data from the NTS unitary activity were expressed as mean ± S.E. and analyzed on the basis of action potential discharge rate and drug concentration-related effects. The number of action potentials in a given duration was measured under pretrial, trial, and post-trial conditions. The control data (pretrial) were normalized to 100%, and the NTS neuronal activities 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.

Analysis of variance and Student’s t test with P < 0.05 considered statistically significant.

Results

A total of 99 tonic, gastric, vagally evoked NTS units were recorded. Their mean basal firing rate was 0.8 ± 0.2 Hz. There was no significant difference in basal firing rate between units that responded and did not respond to gastric galanin and/or leptin.

Peripheral Gastric Effects of Galanin. Peripheral gastric effects of galanin (300 nM) produced a mean inhibition response of 53.2 ± 2.1% 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-
The difference in the NTS neuronal discharge frequency between the control recording and the recording after galanin (100 nM) applications was significant ($P < 0.01$). 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 $\pm$ 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 activation 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 $\pm$ 7.7%) was reduced to 146.2 $\pm$ 4.7% after coapplication of both compounds ($P < 0.05$ compared with leptin alone; $P < 0.01$ compared with galanin alone, 55.1 $\pm$ 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 $\pm$ 9.0%) was reduced to 153.8 $\pm$ 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 $\pm$ 11.6%) but did not respond to leptin application. Coapplication of both compounds increased the activity to 75.5 $\pm$ 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 comitant 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, Rinnaman 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 peripheral gastric effects of leptin produced significant activation response. We also evaluated the interaction between...
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</th>
<th>P Value (Compared with Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galanin (100 nM)</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 (10 nM)</td>
<td>20</td>
<td>17</td>
<td>85.0</td>
<td>167.4 ± 8.2%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
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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 (Kyrkouri 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-
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.

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References


Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL,


Leibowitz SF and Shor-Pesner G (1986) Brain serotonin and eating behavior. Appetite 7 (Special Issue).


Sahu A (1998) Leptin decreases food intake induced by melanin-concentrating hormone (MCH), galanin (GAL) and neuropeptide Y (NPY) in the rat. Endocrinology 139:4739–4742.


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