Glucose does not activate nonadrenergic, noncholinergic inhibitory neurons in the rat stomach

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WE RECENTLY REPORTED THAT intravenous glucose infusion in anesthetized rats during insulin-induced hypoglycemia produces a robust and consistent inhibition of gastric motility (34). This inhibitory effect occurred immediately (within 30 s to 2 min) after the start of infusion. Most impressive was the small change in blood glucose level needed to evoke inhibition of gastric motility. In most studies, the change in blood glucose level required to evoke complete inhibition of hypoglycemia-induced increases in antral motility ranged between 3 and 5 mg/dl.

In the above-cited study (34) and in our laboratory’s earlier study (4), we concluded that intravenous glucose inhibited gastric motility via effects in the central nervous system (CNS) rather than in the periphery by modulating vagal outflow to the stomach. This conclusion fits with data indicating that the opposite state, namely hypoglycemia, increases gastric motility by a CNS action leading to increased vagal effects on the stomach (2, 35).

Vagal output from the CNS to the upper gastrointestinal tract is viewed by many investigators as comprising parallel excitatory and inhibitory pathways (3, 9, 12, 15, 27–30, 41, 42). The excitatory path consists of vagal preganglionic neurons synapsing onto cholinergic postganglionic neurons, and experimental results support the presence of an inhibitory path consisting of vagal preganglionic neurons synapsing onto nonadrenergic, noncholinergic (NANC) postganglionic neurons. The vagal preganglionic neurons originate in the dorsal motor nucleus of the vagus (DMV), and the signaling molecule for NANC neurons is thought to be nitric oxide (10, 14, 38, 40).

Data accumulated over the past 15 years suggest that endogenously occurring brain peptides, such as atrial natriuretic factor (18), oxytocin (19), corticotrophin releasing factor (15), and substance P (13), evoke a decrease in gastric motility because they act in the DMV to excite the inhibitory NANC pathway. Furthermore, vagovagal reflex activation, which inhibits gastric motility such as esophageal and antral distension, is also thought to engage the inhibitory NANC pathway (10, 28, 30). Recently, the inhibitory NANC pathway has also been implicated in mediating gastric motility changes associated with alterations in circulating glucose levels. Zhou et al. (47) suggested that glucose “facilitates” the vagal cholinergic pathway that synapses with the stomach nitric oxide pathway to cause gastric relaxation. Moreover, Yuan and Yang (45) described results showing that insulin-induced hypoglycemia causes vagally mediated changes in activity of nitric oxide neurons in the gastric plexuses.

The overall purpose of the present study was to increase our knowledge of how glucose alters vagal outflow to the stomach and decreases gastric motility. Specifically, we sought to determine whether intravenous glucose inhibits gastric motility, in part, or entirely, by activating a NANC inhibitory vagal pathway.

METHODS

Animals and Anesthesia

Adult male Sprague-Dawley rats weighing 300–400 g (Taconic, Germantown, NY) were housed in controlled conditions of room temperature (22°C) and light (12:12-h light-dark cycle) with free access to food and water. Before each experiment, food was withheld overnight, but water was provided ad libitum. Animals were anesthetized with an intraperitoneal injection of a mixture of urethane (800 mg/kg) and α-chloralose (60 mg/kg) dissolved in 0.9% saline. Toe-pinch and corneal reflexes were performed to assess the depth of

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anesthesia. Body temperature was monitored by a rectal thermometer and maintained at 37 ± 1°C with an infrared heating lamp. At the end of each experiment, all rats were killed with an overdose of pentobarbital sodium. Research described in this paper fully conforms to the requirements of the National Institutes of Health guidelines and was approved by the Georgetown University Animal Care and Use Committee.

**Experimental Protocols**

The effect of D-glucose administered intravenously was evaluated on gastric motility occurring under the following experimental conditions: 1) nitro-l-arginine methyl ester (l-NAME) inhibition of nitricergic transmission and 2) bethanechol-induced gastric motility. The protocols used for each of these experimental conditions are described below. Glucose infusion was performed by using a standard protocol for all studies, which was as follows: a 25% D-glucose solution was infused at a rate of 2 ml/h iv by a syringe pump (Razel Instruments, Mountain View, CA) data-acquisition system. An incision lateral to the midline was made, exposing the stomach and enabling removal of excess stomach contents through a small incision in the fundus. Tonic and phasic gastric contractions were measured with a latex balloon inserted into the stomach via the fundus and attached to a pressure transducer with polyethylene tubing (PE-160). The balloon was filled with warm deionized water (4–5 ml) to achieve a basal tone of 4–10 mmHg. The pressure transducers were connected to a bridge amplifier coupled to the Power Lab data-acquisition system (ADInstruments). Data were stored on a computer for analysis at a later time.

**General Procedures**

Rats were intubated to maintain an open airway and so that artificial respiration could be instituted when necessary. The carotid artery and jugular vein were cannulated with polyethylene tubing (PE-50) to monitor blood pressure and to administer intravenous drug and glucose infusions, respectively. Blood pressure was recorded by using a bridge amplifier connected to a Power Lab (ADInstruments, Mountain View, CA) data-acquisition system. An incision lateral to the midline was made, exposing the stomach and enabling removal of excess stomach contents through a small incision in the fundus. Tonic and phasic gastric contractions were measured with a latex balloon inserted into the stomach via the fundus and attached to a pressure transducer with polyethylene tubing (PE-160). The balloon was filled with warm deionized water (4–5 ml) to achieve a basal tone of 4–10 mmHg. The pressure transducers were connected to a bridge amplifier coupled to the Power Lab data-acquisition system (ADInstruments). Data were stored on a computer for analysis at a later time.

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Effect of Pretreatment with L-NAME on Glucose-Induced Inhibition of Insulin-Evoked Gastric Contractions

In three rats not pretreated with L-NAME (i.e., control animals), insulin administered subcutaneously increased gastric contractions, as reflected by an increase in total contraction amplitudes over 5 min and frequency of contractions (Table 1, Fig. 1A). These increases occurred on average 75.7 ± 7.3 min after insulin injection and were associated with a decrease of blood glucose from 87 ± 9 to 25 ± 5 mg/dl. Baseline intragastric pressures were not significantly altered by insulin (Table 1). At the time of the increases in gastric contractions, d-glucose (25% d-glucose at 2 ml/h iv) was administered and inhibited contractions within 3.3 ± 0.9 min (Fig. 1A). Blood glucose levels measured at the time of glucose-induced inhibition of gastric contractions averaged 36 ± 13 mg/dl.

In five additional rats, L-NAME was administered on average at 76.4 ± 5.6 min after subcutaneously administered insulin. At this time, insulin increased the total contraction amplitudes over 5 min from 0 to 66 ± 11 mmHg (Table 1, Fig. 1B). Contraction frequency at this time was 4.8 ± 0.6 contractions/min. No significant change was observed in intragastric pressure. L-NAME administered intravenously at a dose of 10 mg/kg produced an immediate increase in gastric contractions (Table 1, Fig. 1B). The sum of contraction amplitudes over 5 min increased to 127 ± 8 mmHg, and this increase was a significant change (P < 0.05) relative to the value obtained after insulin alone. Frequency of contractions attained a value of 6.0 ± 0.3 contraction/min, but this change was not statistically significant. Intragastric pressure increased after L-NAME and rose from 7.3 ± 0.6 to 8.4 ± 0.5 mmHg but was not statistically significant. Blood glucose levels averaged 89 ± 10 mg/dl before insulin and decreased to 23 ± 5 mg/dl after insulin administration. Addition of L-NAME did not further alter the blood glucose level (27 ± 5 mg/dl). On average, 10.5 ± 0.8 min after L-NAME was administered, d-glucose infusion was started and inhibited contractions within 4.1 ± 0.7 min (Table 1, Fig. 1B). Blood glucose levels measured at the time of glucose-induced inhibition of gastric contractions averaged 35 ± 5 mg/dl. Most important, the ability of d-glucose to inhibit insulin-evoked increases in gastric contractions was not significantly altered by pretreatment with L-NAME with regard to either the latency, 3.3 ± 0.9 min for the control group vs. 4.1 ± 0.7 min for the L-NAME-treated group, to inhibit gastric contractions or the magnitude of the inhibition (at the 6- to 11-min time period after glucose contractions were abolished in both groups) (Table 1).

Effect of d-Glucose-Induced Inhibition of Gastric Contractions on Bethanechol-Evoked Gastric Responses

Our rationale for these experiments was based on the idea that, if glucose acts to stimulate a vagal NANC inhibitory pathway to the stomach, gastric contractions evoked by bethanechol would be significantly reduced. Alternatively, if glucose acts primarily via another parallel pathway, i.e., inhibition of vagal cholinergic excitatory output, gastric contractions evoked by a peripherally acting agent such as bethanechol would not be diminished.

The test protocol in these studies was first to administer insulin subcutaneously to increase gastric contractions. Next, d-glucose was infused intravenously to inhibit gastric contractions. Finally, with d-glucose infusion continuing, bethanechol was administered at 30 μg·kg⁻¹·min⁻¹, and peak increases in gastric contractions were noted. The data obtained from five experiments are summarized in Table 2, and a representative
<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Baseline (min)</th>
<th>Insulin Effect (1–6 min)</th>
<th>L-NAME Effect (6–11 min)</th>
<th>Glucose Effect (1–6 min)</th>
<th>Glucose Recovery (6–11 min)</th>
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<tr>
<td></td>
<td>F, mmHg</td>
<td>IGP, mmHg</td>
<td>AMP, mmHg</td>
<td>IGP, mmHg</td>
<td>AMP, mmHg</td>
</tr>
<tr>
<td>Insulin and D-glucose</td>
<td>71 mg/dl</td>
<td>6.0</td>
<td>4.4</td>
<td>2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>L-NAME and D-glucose</td>
<td>&lt;20 mg/dl</td>
<td>n/a</td>
<td>n/a</td>
<td>7.5</td>
<td>11.0</td>
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</table>

Values are means ± SE. AMP, sum of amplitudes; F, frequency of phasic contractions; IGP, intragastric pressure. *Statistically significant difference compared with baseline values using one-way ANOVA, followed by Student-Newman-Keuls post hoc test.†Statistically significant difference compared with insulin effect values using one-way ANOVA, followed by Student-Newman-Keuls post hoc test.

Fig. 1. Representative chart recordings from two experiments in which 25% \( \text{D-glucose} \) infusion (2 ml/h) inhibited insulin-induced gastric contractions in an animal of the control group (A) and an animal given nitro-L-arginine methyl ester \( (\text{L-NAME}) \) after subcutaneously administered insulin (B). A: blood glucose (bgl) was 71 mg/dl before insulin administration. Next, at a recording break, 2.5 IU sc insulin was administered; 68 min later, the frequency of contractions had increased. At this time, bgl was <20 mg/dl, as indicated. D-Glucose was then infused (horizontal bar), inhibiting motility. B: L-NAME was administered after insulin-induced gastric contractions were present and before D-glucose infusion. Note that L-NAME increased contractions above those observed with insulin-induced hypoglycemia. D-Glucose was then infused (horizontal bar), inhibiting motility. IGP, intragastric pressure.

The experiment is presented in Fig. 2A. As described in the above section, insulin increased the sum of contraction amplitudes over 5 min and increased the frequency of contractions (Table 2, Fig. 2A). Blood glucose levels at this time dropped from 74 ± 9 to 21 ± 1 mg/dl. D-Glucose infusion, as described above, inhibited gastric contractions (Fig. 2A). Bethanechol infusion then stimulated gastric contractions. That is, on average, at 19.0 ± 4.4 min after the start of bethanechol infusion, both the sum of contraction amplitudes over 5 min and the frequency of contractions were significantly increased from 18 ± 8 mmHg and 1.7 ± 0.7 contractions/min to 112 ± 20 mmHg and 5.7 ± 0.5 contractions/min, respectively (Table 2).

The protocol for the control experiments \((n = 4)\) was to administer insulin vehicle (physiological saline) subcutaneously, wait 66.3 ± 3.1 min, and then administer 25% \( \text{D-glucose} \) at 2 ml/h in place of \( \text{D-glucose} \) \((\text{L-glucose} \) at this dose exhibits no effect on gastric motor activity \((34)\). With \( \text{L-glucose} \) infusion continuing, bethanechol infusion \((30 \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})\) was started as described above. The increases in gastric motility observed at 19.0 ± 2.0 min after the start of bethanechol infusion were as follows: sum of contraction amplitudes over 5 min increased from 10 ± 2 to 70 ± 12 mmHg \((P < 0.05)\), and the frequency of contractions increased from 0.8 ± 0.3 to 4.2 ± 1.2 contractions/min \((P < 0.05)\). Thus, in the presence
Table 2. Effects of D-glucose on bethanechol-induced gastric motility

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Baseline</th>
<th>Insulin/Saline Effect</th>
<th>Glucose Effect</th>
<th>Bethanechol Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Σ AMP, mmHg</td>
<td>F, min⁻¹</td>
<td>IGP, mmHg</td>
<td>Σ AMP, mmHg</td>
</tr>
<tr>
<td>Saline and L-glucose (n = 4)</td>
<td>26±14</td>
<td>0.6±0.2</td>
<td>5.2±1.8</td>
<td>13±4</td>
</tr>
<tr>
<td>Insulin and D-glucose (n = 5)</td>
<td>10±4</td>
<td>1.0±0.4</td>
<td>8.4±0.8</td>
<td>76±10*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Statistically significant difference compared with baseline values using one-way ANOVA, followed by Student-Newman-Keuls post hoc test. †Statistically significant difference compared with glucose effect values using one-way ANOVA, followed by Student-Newman-Keuls post hoc test.

of D-glucose-induced inhibition of gastric contractions, the excitatory effect of bethanechol on gastric motor activity was not attenuated. Indeed, the excitatory effect of this muscarinic receptor agonist actually appeared to be somewhat enhanced compared with corresponding data obtained from the control experiments.

**Effect of D-Glucose Infusion on c-Fos Expression in DMV Neurons Projecting to the Stomach**

As mentioned in METHODS, these studies were performed in conscious rats. In our first study, insulin was administered subcutaneously and 60 min later, 25% D-glucose infusion was begun and continued for 3–5 min. This protocol was similar to the protocol used for the control studies carried out in anesthetized rats (see protocol for studies carried out in Table 1). One hour after D-glucose infusion was terminated, brains were harvested, and the DMV area was examined for c-Fos expression. Three experiments of this type were performed, and five control experiments were conducted where saline vehicle (instead of D-glucose) was infused for 3–5 min after subcutaneous insulin administration. Data obtained indicated that insulin increases c-Fos in the DMV to the same extent in both D-glucose-infused animals and the saline-infused animals. Assuming that insulin excites the vagal-cholinergic excitatory path to the stomach, we hypothesized that, if D-glucose activates the vagal-NANC pathway, then more c-Fos expression would be observed in the insulin plus D-glucose group compared with the control group. However, this did not occur: in five control rats treated with insulin followed by isotonic saline administration, the total number of cells expressing c-Fos in the dorsal vagal complex (NTS + DMV) was 2,084 ± 60, whereas in three rats treated with the same dose of insulin followed by D-glucose infusion, 1,953 ± 77 cells were activated to express c-Fos. It is possible that some activation of the vagal-NANC pathway did occur but was dwarfed by a more powerful excitatory effect of insulin on the vagal-cholinergic...
pathway. Hence, we next tried D-glucose infusion without insulin. Seven animals were given 25% D-glucose (n = 3) or saline (n = 4) infusion for 15 min. Sixty minutes after the termination of infusion, brains were harvested and examined for c-Fos and c-Fos plus Fluoro-Gold expression (Fluoro-Gold was administered intraperitoneally 5 days earlier to label DMV-projecting neurons as described in METHODS). Data are summarized in Table 3 and indicate that 15 min of 25% D-glucose infusion did not increase c-Fos expression in either the combined areas of the NTS and DMV or the DMV alone above that observed in control animals, i.e., in animals that received 15 min of saline infusion. Likewise, D-glucose infusion did not increase c-Fos expression in stomach-projecting DMV neurons as defined by Fluoro-Gold staining.

Another inhibitor of rhythmic gastric motility, CCK-8 (1), was also tested for evidence of stimulation of a vagal-NANC pathway. Two doses of CCK-8 (n = 6) and saline vehicle (n = 3) were injected as an intravenous bolus, and brains were harvested 60 min after intravenous injection. Both doses of CCK-8 (i.e., doses of 10 and 50 μg) produced similar effects; therefore, these data were combined. The data showed that CCK-8 activated significant amounts of c-Fos in the NTS and DMV combined area and in the DMV alone compared with the intravenous saline control group (Table 3). Most important, although significant c-Fos expression was detected within the DMV area after CCK-8, the c-Fos-positive cells were not DMV neurons that projected to the stomach as defined by Fluoro-Gold retrograde labeling (Table 3 and Fig. 3). Indeed, 11.5 ± 0.9% of the c-Fos expressing neurons in the NTS ± DMV area were located within the boundaries of the DMV, but only 0.2 ± 0.1% were in Fluoro-Gold-stained neurons. Finally, there were fewer DMV neurons labeled by Fluoro-Gold compared with the saline controls of the other two treatment groups (Table 3).

To confirm the ability of DMV neurons that project to the stomach to express c-Fos, animals that had already received Fluoro-Gold injection were given 2.5 IU insulin (n = 6) or the same volume of saline vehicle (n = 2) subcutaneously. After a period of 60 min for animals to develop hypoglycemia, another 60 min was allowed before brains were harvested for immunohistochemistry processing. Data obtained are presented in Table 3 and Figs. 3B and 4. As shown in Table 3, insulin-induced hypoglycemia increased c-Fos expression in neurons in the NTS and DMV combined area and in the DMV alone compared with saline controls. The double-labeled neurons occurred mainly in the medial portion of the nucleus. This was true of all animals studied. Figure 4 shows the rostral-caudal pattern of c-Fos expression present in the DMV along with the rostral-caudal pattern of double-labeled neurons. As can be
noted, c-Fos expression was greatest from 0.15 mm caudal to calamus scriptorius to 0.45 mm rostral to calamus scriptorius. The distribution pattern of double-labeled neurons followed that of the c-Fos expressing neurons. Finally, DMV neurons exhibiting c-Fos but not projecting to the stomach were fewer in number, and their percentage of the total c-Fos-labeled neurons was significantly different from that seen in the CCK-8 treatment group (Table 3).

DISCUSSION

The purpose of this study was to determine whether intravenously administered glucose inhibits gastric motility, in part, or entirely, by activating an inhibitory NANC vagal pathway, as has been suggested by a previous study (47). Three experimental approaches were taken. The first was based on the assumption that the NANC inhibitory output pathway includes a significant component of nitrergic neurons. This assumption was derived from findings demonstrating that drugs that inhibit nitric oxide synthase (NOS) counteract vagal nerve-induced inhibition of gastric motility (10, 14, 38, 40). In this case, prior treatment with a NOS inhibitor such as L-NAME should inhibit or decrease the response to intravenously administered glucose if this were in fact mediated by NANC (nitrergic) neurons. The second approach was based on assessing gastric contractions evoked by the peripherally acting muscarinic receptor agonist bethanechol. We reasoned that if glucose acts to stimulate a vagal NANC inhibitory pathway to the stomach, gastric contractions evoked by bethanechol would be significantly reduced regardless of the neurotransmitters comprising the NANC neuron. On the other hand, if glucose acts primarily via the other parallel pathway, i.e., inhibition of vagal cholinergic excitatory output, gastric contractions evoked by a peripherally acting agent such as bethanechol would not be diminished. The third approach was based on the assumption that if glucose acts to stimulate vagal NANC inhibitory output, then c-Fos expression should be evident in a subset of stomach-projecting DMV neurons after intravenous glucose administration.

Our findings were as follows: 1) prior treatment with L-NAME in a dose that others have shown will block NANC influence on gastric motility, i.e., 10 mg/kg iv (10, 38), had no effect on glucose-induced inhibition of gastric motility; 2) bethanechol-induced increases in gastric motility were not attenuated by the presence of gastric motility-inhibiting doses of glucose (and, indeed, bethanechol-induced increases in gastric motility were enhanced); and 3) no significantly appreciable c-Fos expression in stomach-projecting DMV neurons occurred after glucose dosing. These results lead us to conclude that glucose does not activate NANC neurons in the rat stomach. By exclusion, we suggest that glucose inhibits hypoglycemia-induced gastric motility primarily by acting in the CNS and inhibiting the excitatory path consisting of vagal preganglionic neurons synapsing onto cholinergic myenteric neurons.

Consistent with our findings are several additional findings. First, studies of the effects of glucose on spontaneously occurring efferent vagal preganglionic nerve activity revealed that glucose injected into the carotid artery of anesthetized rats immediately suppressed the efferent activity in the gastric vagus nerve (8, 33). Second, studies of neural activity in the DMV of rats indicated that intravenously administered glucose infusion significantly reduces this activity (37). In that study, extracellular single unit activity was recorded from the DMV area, and glucose infusion reduced neural firing from 16.2 ± 2.6 to 5.0 ± 1.4 discharges/s. Third, findings of the present study demonstrated that CCK-8, a neuropeptide well known to inhibit rhythmic gastric motility in part by reflexively influencing parasympathetic outflow to the stomach (39), also did not increase c-Fos expression in stomach-projecting DMV neurons. This latter finding agrees with the earlier report of Rinaman et al. (25), who found relatively little c-Fos expression in the DMV of rats after CCK-8 administration. Furthermore, Takahashi and Owyang (39) found that prior treatment of rats with the NOS inhibitor L-NAME had no effect on CCK-8-induced inhibition of gastric motility, consistent with a lack of engagement of NANC neurons with this inhibitor of rhythmic gastric motility as well.

The lack of a role for the NANC pathway in the stomach-relaxing effect of glucose and CCK-8 is also in agreement with our data obtained with nicotine administration (5) and esophageal distension (31). In these studies, both intravenously administered nicotine and esophageal distension in the rat cause gastric relaxation that appears to be mediated by vagal efferent neurons. However, analogous studies carried out following intravenous nicotine administration and esophageal distension similarly revealed a lack of significant expression of c-Fos in stomach-projecting DMV neurons, suggesting predominantly decreased rather than increased vagal activity.

Our inability to show a positive role for activation of NANC neurons in the rat stomach using experimental interventions that inhibit gastric motility, such as intravenous glucose and CCK-8 in hypoglycemic rats, intravenous nicotine, and esophageal distension, compels us to examine the strength of evidence from previous studies supporting a role of this pathway. The NANC pathway has been implicated in the gastric relaxation produced by centrally administered oxytocin (19). However, Verbalis and colleagues (22) indicated that oxytocin, like
intravenous glucose and CCK-8, does not increase c-Fos expression in the DMV. Atrial natriuretic factor, substance P, and corticotrophin-releasing hormone have all been described as activating the vagal preganglionic neurons synapsing onto NANC myenteric neurons (12, 15, 18). Evidence for this conclusion was based on experiments showing that gastric relaxation caused by locally applied drug to the DMV could be prevented by prior bilateral cervical vagotomy. Anatomically, the DMV is in close proximity to the medial subnucleus of the tractus solitarius (mNTS), making it difficult to be sure that a gastric response evoked by microinjecting a drug into the DMV originates solely from the DMV. In fact, bilateral cervical vagotomy will also prevent gastric relaxation evoked from local drug application to mNTS (6). A more definitive test of a direct DMV effect is the demonstration that ipsilateral vagotomy will prevent an inhibitory effect on gastric motility, but ipsilateral vagotomy was not performed in these studies. [Note: ipsilateral vagotomy does not appear to block gastric motility responses evoked from the mNTS (6).] Evidence that esophageal distension induced gastric relaxation-invoked activation of the NANC pathway was based, in part, on the finding that esophageal distension excites DMV neurons that comprise fibers of the efferent cervical vagus nerve (28). The difficulty in interpreting these data is that it is not clear whether the nerves identified in this study actually innervate the stomach. Finally, Ishiguchi et al. (10) concluded that distension of the antrum results in reflex activation of a NANC pathway. Their evidence was based on data showing that intravenous L-NAME blocked reflex-induced inhibition of the pylorus. Unfortunately, in this study, reflex function was tested 15 min after L-NAME administration. By this time, L-NAME accumulates in the NTS to such an extent that it reduces spontaneous firing of mNTS neurons (17). Because nitric oxide in the NTS is essential for the function of the vagovagal reflex (5, 31), one cannot conclude from these results that NANC neurons in the rat stomach are involved in the response.

The absence of c-Fos expression in DMV neurons that project to the stomach after intravenous D-glucose infusion as stand-alone evidence for D-glucose not activating the DMV pathway connected NANC neuron is insufficient evidence for drawing a conclusion. The reason for this is that absence of c-Fos expression does not always indicate an absence of increased neural activity because not all brain cells express c-Fos in response to stimulation (22). However, despite this caveat, the L-NAME and bethanechol data nonetheless do provide evidence against glucose-induced activation of an inhibitory vagal pathway. Also, our c-Fos methodology clearly was able to detect excitation of DMV neurons that project to the stomach following insulin-induced hypoglycemia. Hypoglycemia as shown by others (22, 45) activated c-Fos expression in ~22% of DMV neurons that project to the stomach. The DMV neurons activated by hypoglycemia were located in the medial part of the nucleus (Fig. 3). DMV neurons projecting to the antrum are located mainly in the medial part of the nucleus, whereas DMV neurons projecting to the proximal stomach (e.g., fundus) are located primarily in the lateral part of the nucleus (21, 23). These findings correlate nicely with the effect of insulin-induced hypoglycemia on gastric motility. Hypoglycemia in this study, as well as in our previous study (34), increased phasic antral motility but did not affect tonic contractions that are known to be generated from the proximal stomach (7). In our studies, tonic contractions were reflected in the intragastric pressure values, and these were not significantly affected by insulin-induced hyperglycemia. A separation of functional neural pathways to the antrum and fundus was also noted by Ferreira et al. (5), with nicotine microinjected into the mNTS of the rat. A low dose of nicotine microinjected into the mNTS affected gastric tone, whereas a higher dose was required for evoking effects on phasic antral contractions. Nicotine-induced decreases in gastric tone engaged a CNS circuit that used norepinephrine at α2-adrenoceptors of the DMV, whereas the nicotine-induced decreases in phasic contractions engaged a CNS circuit that used GABA at the DMV. It is tempting to speculate that insulin-induced hypoglycemia might activate antral-projecting DMV neurons by a mechanism involving inhibition of GABA. This would not be surprising, since our previous findings indicate that glucose inhibited antral motility probably by release of GABA at the DMV (4).

Although CCK-8 did not appear to activate DMV neurons that project to the stomach, nonetheless, some cells in the DMV were activated as determined by c-Fos expression (Table 3 and Fig. 3). It is known that two populations of small neurons likely exist in the DMV that do not exit the brain via the vagus nerves (20). One population consists of interneurons or neurons that project for only short distances (20). The other population may project to other regions of the brain. There is evidence of afferent projections to the paraventricular nucleus of the hypothalamus (36), the parabrachial nucleus (11), and cerebellum (46). Two of these three projection areas are known to influence gastric motility (paraventricular nucleus (32) and cerebellum (16)) and might be involved in the CNS pathway mediating the effect of CCK-8 on gastric motility. The data obtained with CCK-8 graphically emphasize the importance of using a retrograde tracer like Fluoro-Gold before drawing conclusions about experimental interventions that appear to induce c-Fos expression in DMV neurons, since c-Fos expression may occur in quite substantial numbers of DMV cells that do not actually project to the stomach (Table 3). In this regard, it is particularly striking that intravenously administered glucose did not activate any neurons in the DMV, whether projecting to the stomach or not.

In summary, our goal was to determine whether glucose-induced inhibition of gastric motility in hypoglycemic rats was caused in part or entirely by activation of the DMV pathway connected NANC neurons. By pretreating animals with the NOS inhibitor L-NAME, by assessing the ability of bethanechol to stimulate gastric motility during glucose exposure, and by examining DMV neurons for c-Fos expression during exposure to glucose, we found no evidence in support of glucose-induced activation of an inhibitory vagal pathway. Instead, our data are most consistent with a predominant effect of glucose to inhibit excitatory vagal preganglionic neurons synapsing onto cholinergic myenteric neurons.

GRANTS

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