Glucose acts in the CNS to regulate gastric motility during hypoglycemia

Min Shi,1 Allison R. Jones,2 Mark S. Niedringhaus,2 Rebecca J. Pearson,2 Ann M. Biehl,2 Manuel Ferreira Jr.,2 Niaz Sahibzada,2 Joseph G. Verbalis,1 and Richard A. Gillis2

Departments of 1Medicine and 2Pharmacology, Georgetown University Medical Center, Washington, District of Columbia 20057

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Glucose acts in the CNS to regulate gastric motility during hypoglycemia. Am J Physiol Regul Integr Comp Physiol 285: R1192–R1202, 2003. First published July 17, 2003; 10.1152/ajpregu.00179.2003.—Our purposes were to 1) develop an animal model where intravenously (iv) administered d-glucose consistently inhibited antral motility, and 2) use this model to assess whether iv glucose acts to inhibit motility from a peripheral or a central nervous system site and to elucidate the factor(s) that determine(s) whether stomach motor function is sensitive to changes in blood glucose. Rats were anesthetized with α-chloralose-urethane, and antral motility was measured by a strain-gauge force transducer sutured to the antrum. In some cases, antral motility and gastric tone were measured by monitoring intragastric balloon pressure. Increases in blood glucose were produced by continuous iv infusion of 25% d-glucose at 2 ml/h. Inhibition of antral motility and gastric tone was observed when gastric contractions were induced by hypoglycemia (subcutaneously administered insulin, 2.5 IU/animal). In contrast, no inhibition of gastric motor function was observed when glucose infusion was tested on gastric contractions that were 1) spontaneously occurring, 2) evoked by iv administered bethanechol in vagotomized animals, and 3) evoked by the TRH analog RX77368, microinjected into the dorsal motor nucleus of the vagus. Using the model of insulin-induced hypoglycemia to increase gastric motor activity, we found that neither sectioning the hepatic branch of the vagus (n = 5), nor treating animals with capsaicin to destroy sensory vagal afferent nerves (n = 5) affected the ability of iv d-glucose to inhibit gastric motor function. Our results indicate that an important factor determining whether stomach motor function will be sensitive to changes in blood glucose is the method used to stimulate gastric contractions, and that the primary site of the inhibitory action of iv glucose on gastric motility is the central nervous system rather than the periphery.

Address for reprint requests and other correspondence: R. A. Gillis, Dept. of Pharmacology, Georgetown Univ. Medical Center, Washington, DC 20057 (E-mail: gillisr@georgetown.edu).

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impair vagal activity to the stomach (8, 13, 16, 19, 34). Consistent with this proposal are studies showing that systemically administered glucose produces a decrease in efferent activity of the gastric vagus nerve (17, 33). In terms of site(s) of action, it has been proposed that glucose acts in the hepatic portal area to inhibit hepatic afferent nerve traffic to the brain, which results in a reflex reduction in efferent activity of the gastric vagus nerve (31, 33). It has also been proposed that glucose acts in the brain to alter control of vagally mediated gastric motility (2, 10, 18, 29, 32, 38).

The purposes of the present study were to 1) develop an animal model in which intravenously (iv) administered D-glucose consistently inhibited antral motility, and 2) use this model to assess whether glucose acts to inhibit motility from either a peripheral site or a central nervous system (CNS) site, and elucidate the factor or factors that determine whether stomach motor function is sensitive to physiological changes in blood glucose.

**MATERIALS AND 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 (ip) injection of a mixture of urethane (800 mg/kg) and α-chloralose (60 mg/kg) dissolved in 0.9% saline. Toe-pinches and corneal reflexes were performed to assess the depth of anesthesia. Body temperature was monitored by a rectal thermometer and maintained at 37 ± 1°C with an infrared heating lamp. Research described in this paper fully conforms to National Institutes of Health guidelines and has been approved by the Georgetown University Animal Care and Use Committee.

**General Procedures**

Rats were intubated to maintain an open airway and for instituting artificial respiration when necessary. The carotid artery and jugular vein were cannulated with polyethylene tubing (PE-50) to monitor blood pressure and to administer iv 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. For most experiments, a strain-gauge force transducer (Warren Research Products, Charlestown, SC) was sutured to the antrum of the stomach, parallel to the circular muscle, to measure phasic contractions. The two sutures were placed on the muscle at a distance from each other so that a baseline of 4–10 mmHg. Both the force and pressure transducers were connected to a bridge amplifier coupled to the Power Lab data-acquisition system (ADInstruments). Data were stored on a computer (Apple Macintosh G3 connected to Power Lab) for analysis at a later time.

**Experimental Protocols**

The effect of D-glucose administered iv was evaluated on gastric motility occurring under the following experimental conditions: 1) spontaneous activity, 2) insulin-evoked activity, 3) bethanechol-evoked activity in vagotomized animals, and 4) CNS-evoked vagal activity produced by microinjection of the TRH analog RX77368 into the dorsal motor nucleus of the vagus (DMV). The protocols used for each of these experimental conditions are described below.

**Studies of spontaneous activity.** Whereas most animals did not exhibit spontaneously occurring antral contractions, a minority of animals did exhibit a stable baseline of phasic contractions of at least 10-min duration (Fig. 1A). A blood glucose level was obtained during this stable period. Next, iv infusion of D-glucose was begun and maintained over a period of 25–30 min. These studies were performed by using the strain-gauge force transducer recording from the antrum. In addition, spontaneous activity was also studied by using...
tonic contraction of the stomach muscle as reflected by intragastric balloon pressure (IGBP). The lowest point of the intragastric pressure trace was obtained and used as an index of gastric tone. In animals with an inflated balloon placed in the stomach, spontaneous phasic antral contractions were also observed in the IGBP recording (Fig. 1B), and effects of glucose infusion were studied on both gastric tone and phasic antral contractions, as described above.

Studies of insulin-evoked activity. Once a 10-min duration of stable baseline of gastric motor activity was obtained by using either strain-gauge force transducer or intragastric balloon recordings, insulin (2.5 IU/animal) was administered subcutaneously (sc) to induce hypoglycemia. This insulin dose was based on results from a previous study (26) and adjusted downward to ensure that anesthetized animals would survive for a 3- to 5-h study duration. Blood glucose values were obtained before insulin was administered, at the time that robust increases in gastric motor activity were noted, during the 1- to 6-min period immediately after starting the glucose infusion, and during the recovery period after the termination of glucose infusion.

Studies of bethanechol-evoked activity in vagotomized animals. Our goal in these studies was to increase antral motor activity from a peripheral site of action. Hence, we utilized the muscarinic receptor stimulant drug bethanechol to directly activate receptors on the antrum. This agent, which has a quaternary charged nitrogen (6) and is presumably devoid of CNS effects, was administered by continuous iv infusion (30, 45, and 60 μg·kg⁻¹·min⁻¹) in vagotomized animals. Vagus nerves were sectioned bilaterally in the cervical region for the purpose of providing a model system in which gastric smooth muscle activity was driven entirely from peripheral cholinergic input. Blood glucose levels were obtained before glucose infusion was begun and 20 min after continuous infusion of glucose had been started.

CNS-evoked vagal activity produced by RX77368 into the DMV. The goal of these experiments was to increase antral motor activity by exciting the preganglionic vagal neurons projecting to the stomach. This was done by microinjecting the TRH analog RX77368 into the DMV. The detailed technique for doing this is described below. Glucose levels were not determined in this study, but the same glucose infusion protocol, as described for the other experimental conditions, was employed.

Hepatic Nerve Section

Animals underwent either hepatic nerve section or sham surgery for hepatic nerve section (controls). For these studies, the incision used for exposing the stomach was extended laterally past the midline to expose the liver. The liver was gently lifted up within the cavity, and the stomach was moved caudally to clearly expose the esophagus, the anterior vagal trunk, and the single hepatic branch exiting the anterior vagal trunk. The hepatic branch was carefully isolated from all connective tissue and blood vessels and severed with microscissors. The surgically exposed area was always examined afterwards to ensure that no tissue remained between the liver and the esophagus, between the levels of the esophageal plexus and the cardia of the stomach. This procedure followed closely the procedures described by Bellinger and Williams (4), Tordoff and Donald (39), and Latour et al. (23). Hepatic sham animals underwent the same procedures, except the hepatic branch was left intact.

Capsaicin Treatment

To test whether selective interruption of the vagal afferent innervation to the subdiaphragmatic viscera prevented n-glucose-induced inhibition of gastric motor function, we employed the drug capsaicin, which is known to damage vagal afferents responsible for CCK-induced satiety (30). Capsaicin was administered by ip injection. This drug was dissolved in a solution of 50% dimethyl sulfoxide and 50% NaCl (0.9% in sterile water). Rats were anesthetized with isoflurane before each capsaicin injection. The doses of capsaicin used were 25 mg/kg on day 1, followed by 50 mg/kg on each succeeding day for 4 days. This dosing regimen is a modification of the procedure described by Ritter and Landenheim (30) and identical to the procedure of McCann et al. (25). Control rats were treated in identical fashion, except that they received vehicle injections instead of capsaicin. Rats receiving capsaicin often exhibited a brief period (5–30 min) of respiratory arrest and required temporary artificial ventilation until breathing returned. Animals were allowed to recover 1 wk after the last capsaicin or vehicle ip injection, before the acute iv glucose experiments were undertaken.

In these acute experiments, gastric motility responses to CCK were always evaluated before glucose was tested. The purpose of this was to ensure ourselves that selective afferent vagotomy had occurred with capsaicin treatment. The specific protocol used was as follows. 1) Animals were anesthetized and set up for antral recording with a strain-gauge force transducer and then received insulin sc as described above. Once a robust increase in antral motility was observed, three iv bolus doses of CCK octapeptide (CCK-OP) were administered at 5-min intervals. Doses of CCK-OP were 3.3, 12, and 50 ng, and these doses administered to capsaicin vehicle-treated animals inhibited antral motility for 68 ± 16, 78 ± 24, and 103 ± 34 s, respectively (n = 7). In capsaicin-treated animals, the lower two iv bolus doses of CCK-OP had no motility-inhibiting effect, and the effect of the highest dose was attenuated (duration of inhibition was only 30 ± 60 s) (n = 5). 2) Once CCK-OP testing was complete and we were convinced that significant selective afferent vagotomy had taken place, iv glucose was evaluated for its ability to inhibit insulin-evoked increases in antral motility.

Microinjection of RX77368 into the DMV

TRH and its longer-acting analog RX77368 [gGlu-His-(3,3′-dimethyl)-Pro-NH₂] both stimulate gastric function through vagal-dependent pathways on microinjection into either the dorsal vagal complex or the nucleus ambiguous (20). The dose of RX77368 that is effective is 7.7 pmol (20). To microinject RX77368 into the DMV in our studies, we dissolved the drug in 0.9% saline and used double-barreled pipettes with a tip diameter between 30 and 60 μm. All microinjections were given unilaterally. Injections were given in volumes of 60 nl, and the dose of RX77368 used was somewhat higher than that used by Ishikawa et al. (20), namely, 30 pmol. Microinjections were given within 5 s by hand-controlled pressure. Calamus scriptorius was used as a zero reference point. Stereotoxic coordinates are taken from our laboratory’s previously published microinjection studies of the DMV (12) and ranged from 0.3 to 0.5 mm rostral to calamus scriptorius, medial-lateral 0.3–0.5 mm from the midline, and dorsal-ventral 0.5–0.7 mm from the dorsal surface of the medulla. Animals were also given dexamethasone (0.5 ml sc) before brain surgery to prevent swelling in the brain.
Histological Verification

At the end of the experiment, all rats were killed with an overdose of pentobarbital. Brains were removed and fixed in a mixture of 4% paraformaldehyde and 20% sucrose for at least 24 h. The brain was cut into 40-µm-thick coronal sections and stained with neutral red. The location of the microinjection site was studied in relation to the location of the DMV by using the atlas of Paxinos and Watson (28).

Analysis of Gastric Motor Activity and Statistical Analysis

To examine the motor activity when strain-gauge force transducer recordings were made, the frequency of contractions over a 5-min period was determined and converted to contractions per minute. Contractions were counted only if their amplitude equaled or exceeded 1 g. For quantitating the strength of contractions, we used the method of Kihara et al. (22), which was to sum the amplitude of contractions over a specified time period, namely, over a 5-min time period. To examine motor activity when IGBP recordings were made, gastric tone was determined by using the lowest points of the intragastric pressure trace. An approximate average value was obtained by drawing a line, by eye, through the lowest points occurring during a 5-min period. Because IGBP correlates well with fundic activity (11), we assumed that any further inhibition by D-glucose could not be responsible for generating IGBP (14). Antral contractions were usually superimposed on the tonic contraction, and these antral contractions were analyzed in the same manner as described for the strain-gauge force transducer recordings. Only IGBP changes of ≥1.0 mmHg were considered as significant contractions and were analyzed. Means ± SE were calculated by using Sigmastat. Data were analyzed by using either Student’s paired t-test or ANOVA (in the case of multiple comparisons) followed by the Newman-Keuls post hoc test. Differences were considered statistically different when P < 0.05.

Drugs and Source

Insulin (Humulin) was purchased from Eli Lilly (Indianapolis, IN). Isoflurane was purchased from Abbott Laboratories (North Chicago, IL). CCK-OP was purchased from Peninsula Laboratories (San Carlos, CA). Dexamethasone was purchased from Elkins-Sinn (Cherry Hill, NJ). TRH analog RX77368 was a gift from Dr. Yvette Taché, University of California at Los Angeles. All other drugs were purchased from Sigma (St. Louis, MO). All drugs were dissolved in 0.9% saline. The pH of iv drug solutions was between 7.0 and 7.4, and microinjection drug solution pH was 7.0–7.2.

RESULTS

Effect of IV D-Glucose Infusion on Spontaneously Occurring Gastric Motility and Gastric Tone

The main purpose of our study was to determine the site of action where D-glucose acts to decrease gastric motility. To accomplish this, we studied whether experimental interventions that eliminate GI vagal afferent neurons (i.e., hepatic vagal nerve section and capsaicin treatment) would influence the ability of iv administered D-glucose to inhibit antral motility. As a first step, it was imperative that we utilized a neurally intact model in which iv D-glucose consistently inhibited antral motility; based on our search of the literature, it was not clear what model would be suitable (see Introduction). For our initial studies, we attempted to develop this model by testing the effects of iv administered D-glucose on spontaneously occurring antral motility. Two problems occurred during this attempt: 1) spontaneous activity was so low in most experiments that any further inhibition by D-glucose could not be reliably detected, and 2) in the minority of experiments, in which robust spontaneous antral contractions were present, continuous iv administered D-glucose infusion (25% solution administered at 2 ml/h) given over a period of 25–30 min had no appreciable effects on antral motility. Five animals comprised this group, and the data are summarized in Table 1. The magnitude of spontaneously occurring antral motility, as reflected by the strain-gauge force transducer recordings, was 36.8 ± 13.5 g tension (sum of amplitudes of contractions over 5 min), and the frequency of contraction was 2.6 ± 0.7 contractions/min. Baseline blood glucose levels averaged 90 ± 16 mg/dl. The data on the effect of D-glucose were obtained during two 5-min periods, namely, 1–6 and 25–30 min after the start of the D-glucose iv infusion. As can be noted from Table 1,

Table 1. Effect of intravenous D-glucose infusion on spontaneously occurring gastric contractions and gastric tone

<table>
<thead>
<tr>
<th>Method of measuring gastric contractions</th>
<th>Baseline</th>
<th>Glucose Effect (1–6 min after start of infusion)</th>
<th>Glucose Effect†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Contraction amplitudes</td>
<td>Frequency, contractions/min</td>
</tr>
<tr>
<td>Strain-gauge force transducer on the antrum, g tension</td>
<td>5</td>
<td>36.8 ± 13.5</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>IGBP, mmHg</td>
<td>4</td>
<td>42.2 ± 28.8</td>
<td>1.7 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE, n, no. of experiments. Contraction amplitudes are the sum of amplitudes of contractions (≥1 g) over 5 min. Contraction amplitudes are measured in grams for strain-gauge force transducer method and in mmHg for intragastric balloon pressure (IGBP) method. n/a, Does not apply. †Glucose effect is 25–30 min after start of infusion for strain-gauge force transducer method and 11–16 min after start of infusion for IGBP method. *P < 0.05 compared with baseline values.
Effect of IV D-Glucose Infusion on Insulin-evoked Increases in Antral Motility

Because spontaneous activity of the stomach was lacking in most experiments that used strain-gauge force transducer recordings and because IV D-glucose, when it was able to be tested on spontaneous activity, was without effect (Table 1, Fig. 1), we used the technique of insulin-induced hypoglycemia, described by Sakaguchi and Shimojo (31), to create a neurally intact model in which IV D-glucose consistently inhibited antral motility. This can be seen in the data tabulated for IV administered D-glucose on IGBP recordings of spontaneously occurring gastric tone and motility appears in Fig. 1B.

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Table 2. Effect of either hepatic nerve section or capsaicin treatment on intravenous D-glucose-induced inhibition of insulin-evoked antral motility

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Percentage of Baseline Amplitudes, %</th>
<th>Percentage of Baseline Frequency, %</th>
<th>Experimental Group</th>
<th>Percentage of Baseline Amplitudes, %</th>
<th>Percentage of Baseline Frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>Hepatic nerve cut</td>
<td>150</td>
<td>120</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>70</td>
<td>70</td>
<td>Capsaicin</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>Glucose</td>
<td>70</td>
<td>70</td>
<td>Glucose</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>Glucose + Atropine</td>
<td>70</td>
<td>70</td>
<td>Glucose + Atropine</td>
<td>100</td>
<td>90</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of experiments. Amplitude, contraction amplitudes are sum of amplitudes of contractions (≤1 g) over 5 min. *P < 0.05 compared with the insulin effect using one-way ANOVA and Student-Newman-Keuls test.
complete inhibition of antral motility. This effect is best reflected in the change in the 5-min sum of antral contractions, which decreased from 59.8 ± 12.4 to 0.4 ± 0.4 g (P < 0.05, Table 2). Blood glucose levels rose from 20 ± 3 to 44 ± 6 mg/dl at the time of inhibition of antral motility. Once it was established that D-glucose infusion abolished most of the activity in the gastric antrum, the D-glucose infusion was terminated (i.e., D-glucose infusion was stopped after 6 ± 1 min of infusion). Recovery from the D-glucose effect was rapid; antral motility was fully restored 13 ± 1 min after the D-glucose infusion was terminated (Table 2). At this time, blood glucose levels had decreased back to 20 ± 2 mg/dl, presumably reflecting the continuing effect of the sc administered insulin. A representative experiment illustrating the immediate inhibitory effect of D-glucose on antral motility is depicted in Fig. 2A.

Five experiments were then performed after hepatic nerve section. Hepatic nerve section had no effect on D-glucose-induced inhibition of antral motility (Table 2). A representative experiment illustrating the immediate inhibitory effect of D-glucose on antral motility is shown in an animal with hepatic nerve section in Fig. 2B.

Identical studies performed with insulin and D-glucose infusion on five capsaicin-treated animals and seven control animals for capsaicin treatment showed no alteration in the capacity of iv D-glucose infusion to inhibit antral motility (Table 2). In these studies, evidence that capsaicin treatment had eliminated GI vagal afferent neurons was obtained by testing the effects of low doses of CCK-OP on antral motility in control and capsaicin-treated animals. Whereas CCK-OP iv produced complete inhibition of antral motility in control animals, this peptide was ineffective at inhibiting antral motility in capsaicin-treated animals (see MATERIALS AND METHODS).

In three neurally intact animals, L-glucose was evaluated by using the identical protocol described above for D-glucose. Data obtained are as follows: baseline sum of amplitudes of contractions over 5 min was 1.8 ± 1.8 g tension, and frequency of contraction was 0.2 ± 0.2 contractions/min. Blood glucose level was 94 ± 11 mg/dl. After sc insulin, corresponding values for sum of amplitudes of contractions, frequency of contractions, and blood glucose levels were 49.1 ± 16.0 g, 3.5 ± 0.9 contractions/min, and 26 ± 3 mg/dl, respectively. The iv L-glucose infusion had no effect on antral motility. Values for sum of amplitudes of contractions and frequency of contractions at 1–6 min and 11–16 min after infusion were 56.8 ± 16.6 g and 3.9 ± 1.0 contractions/min (1–6 min), and 81.8 ± 14.2 g and 4.9 ± 1.4 contractions/min (11–16 min), respectively. Blood glucose levels at the 11- to 16-min time period averaged 27 ± 3 mg/dl. A representative experiment is shown in Fig. 3. Thus L-glucose infusion had no inhibitory effect on insulin-induced increases in antral motility.

Effect of IV D-Glucose Infusion on Insulin-evoked Changes in Gastric Function Using IGBP as an End Point

In four neurally intact animals, D-glucose was evaluated by using the identical insulin-glucose protocol...
described in the previous section, except, instead of using strain-gauge force transducer recordings obtained from the antrum as an end point, IGBP changes were used as the end point. Of primary interest in these studies was the question of whether iv d-glucose infusion could counteract tonic contraction of the stomach that is reflected by measures of IGBP. Data on phasic contractions superimposed on the IGBP recordings were also collected. Baseline values for IGBP, sum of amplitudes of contractions over 5 min, frequency of contractions, and blood glucose levels were 5.5 ± 1.8 mmHg, 21.1 ± 7.8 mmHg, 1.4 ± 0.3 contractions/min, and 78 ± 11 mg/dl, respectively. These values were 5.9 ± 2.0 mmHg, 70.5 ± 21.8 mmHg, 3.7 ± 0.9 contractions/min (P < 0.05), and 24 ± 3 mg/dl (P < 0.05), respectively, at 84 ± 10 min after sc insulin. D-Glucose infusion after insulin-induced hypoglycemia had a statistically significant effect on IGBP. IGBP decreased to 4.3 ± 1.5 mmHg and represented a decrease in IGBP of 31.6 ± 6.9% (P < 0.05, paired comparison). Comparative data obtained with the muscarinic receptor blocker atropine methylbromide averaged a 25.2 ± 8.5% decrease in IGBP (n = 4). Additionally, d-glucose infusion abolished the phasic gastric contractions that were evoked by insulin. The sum of contractions over 5 min was reduced to 0.6 ± 0.6 mmHg, and the frequency of contractions was reduced to 0.1 ± 0.1 contractions/min. Blood glucose level at this time period of 1–6 min after the start of iv d-glucose infusion was 30 ± 4 mg/dl.

Once the d-glucose iv infusion was terminated, some recovery of gastric tone, as reflected by IGBP (4.9 ± 1.7 mmHg), was noted. Also, full recovery of phasic contractility was observed (sum of contractions over 5 min was 67.2 ± 16.9 mmHg, and frequency of contractions was 3.8 ± 0.9 contractions/min). These recovery values were obtained at 19 ± 3 min after d-glucose iv infusion had been terminated. At this time, blood glucose level was 24 ± 4 mg/dl. A representative experiment illustrating these inhibitory effects of d-glucose is shown in Fig. 4.

**Effect of IV d-Glucose Infusion on Bethanechol-evoked Increases in Antral Motility Using Strain-gauge Force Transducer Recordings**

In five animals, instead of increasing antral motility by insulin, the muscarinic receptor agonist drug bethanechol was infused continuously iv. The animals were treated with bilateral cervical vagotomy to examine d-glucose in a model in which activity in the gastric antrum was due solely to a peripheral mechanism. Bethanechol, infused at doses of either 45 μg·kg⁻¹·min⁻¹ (n = 2) or 60 μg·kg⁻¹·min⁻¹ (n = 3) produced a marked increase in antral motility (Table 3 and Fig. 5). (Note: results obtained with both bethanechol doses were similar; hence, data from the five animals were analyzed together.) Administration of iv d-glucose had no effect on bethanechol-induced increases in antral motility. This was true at the 1- to 6-min time period after iv d-glucose infusion was started (Table 3, Fig. 5) and at the 15- to 20-min time period after iv glucose infusion was started (Table 3, Fig. 5).

Because the antral motility response to the 45 and 60 μg·kg⁻¹·min⁻¹ iv bethanechol infusion was of greater magnitude than what was obtained with sc administered insulin (Table 2), we reduced the bethanechol iv infusion dose to 30 μg·kg⁻¹·min⁻¹ in three animals in an attempt to evoke a maximal antral motility response that was similar to that obtained with sc administered insulin. As can be noted from the data tabulated in Table 3, 30 μg·kg⁻¹·min⁻¹ bethanechol did evoke antral motility responses, at least with regard to the sum of contractions over 5 min, that were similar to those obtained with sc administered insulin. Yet iv administered d-glucose had no significant effect on bethanechol-evoked increases in motility at the 1- to 6-min time period after iv d-glucose infusion was begun, or at the later time period of 15–20 min after iv glucose was begun (Table 3).

**Effect of IV d-Glucose Infusion on Phasic Antral Activity Evoked by RX77368 Microinjected into the DMV Using Strain-gauge Force Transducer Recordings**

An inhibitory effect of d-glucose was always noted in our study when gastric motility and tone were evoked by insulin administration. Insulin-evoked increases in
CNS GLUCOSE INHIBITS STOMACH MOTILITY

Table 3. Effect of intravenous D-glucose on bethanechol-evoked phasic antral motility

<table>
<thead>
<tr>
<th>Glucose Effect (15-30 min after start of infusion)</th>
<th>Glucose Effect (20 min after start of infusion)</th>
<th>Glucose Effect (45-60 min after start of infusion)</th>
<th>Baseline</th>
<th>Frequency, contractions/5 min</th>
<th>Frequency, contractions/5 min</th>
<th>Frequency, contractions/5 min</th>
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</thead>
<tbody>
<tr>
<td>Contraction amplitude, mg</td>
<td>Glucose concentration, mg/dl</td>
<td>Contraction amplitude, mg</td>
<td>Glucose concentration, mg/dl</td>
<td>Contraction amplitude, mg</td>
<td>Glucose concentration, mg/dl</td>
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<td>0.0±0.0</td>
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<tr>
<td>74±7</td>
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<tr>
<td>Values are means ± SE; n, n, n of experiments. Contraction amplitudes are sum of amplitudes of contractions (≤1 g) over 5 min. *P &lt; 0.05 compared with baseline using one-way ANOVA and Student-Newman-Keuls test.</td>
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DISCUSSION

Our purpose was to develop an animal model in which hyperglycemia would consistently inhibit antral motility and to use that model to determine whether a peripheral or a brain site of action of glucose was responsible for inhibition of gastric motility. In developing this model, we became aware that acute changes in blood glucose concentration modulation gastric motor function only under specific experimental conditions, namely, only under the condition of hypoglycemia. When hypoglycemia was produced by sc administered insulin, the inhibitory effect of iv D-glucose on antral motility was consistent and robust. Antral motility was completely inhibited in nearly all animals, and the effect was fairly immediate (within 30 s to 2 min). The inhibitory effect was present throughout the period of D-glucose infusion and was lost ~15 min after termination of the glucose infusion. The effect was selective for D-glucose, because L-glucose in the same dose exerted no inhibitory effect. Most impressive was the small change in blood glucose level needed to evoke inhibition. In most studies, the change in blood glucose level required to produce complete inhibition of insu-
lin-induced increases in antral motility ranged between 3 and 5 mg/dl.

Using this model, we are able to obtain evidence suggesting that iv D-glucose-induced inhibition of gastric motility is due to D-glucose acting in the CNS and not in the periphery. Three types of evidence are provided, namely, 1) sectioning the hepatic nerve had no effect on D-glucose-induced inhibition of antral motility; 2) capsaicin pretreatment to remove peripheral vagal afferent nerve fibers had no effect on D-glucose-induced inhibition of antral motility; and 3) levels of D-glucose effective at inhibiting CNS-induced increases in antral motility (caused by insulin; see Ref. 2) were ineffective in inhibiting peripherally induced increases in antral motility (caused by bethanechol in vagotomized animals). We also observed that iv D-glucose, in the presence of insulin-induced hypoglycemia, reduced IGBP. IGBP is due, in part, to the tonic contractile activity present in the fundus (14). Hence, these IGBP data suggest that D-glucose inhibits not only antral activity but fundic activity as well. We assume that the inhibition of fundic activity by D-glucose was also due to a CNS site of action of D-glucose, because it responded to the same iv dose of D-glucose as the antrum and followed the same characteristic time course of effect. It should be noted that emerging evidence indicates that the activity of antral and fundic smooth muscle of the stomach is controlled by two separate CNS brain stem pathways (11), suggesting that iv D-glucose acts at a CNS site that inhibits both of these circuits.

Consistent with a CNS site of action of glucose is the time required for penetration of peripheral glucose into cerebrospinal fluid (CSF) in rats. Steffens and colleagues (37) reported that rats receiving an iv infusion of 10 mg/min glucose exhibited a rise in CSF glucose of 125 ± 22.3 mg/dl from 93 ± 7.6 mg/dl by 10 min after the start of glucose infusion. Assuming a linear rise in CSF glucose over the first 10 min of glucose infusion, an increase in CSF glucose would have taken place at the time that inhibition of gastric motility took place. Furthermore, the glucose inhibitory effect was over at 15 min after terminating the glucose infusion, and, according to the data of Steffens et al., CSF glucose declined to 94 ± 13.6 mg/dl 10 min after termination of glucose infusion. Also consistent with a CNS site of action of glucose are our earlier findings, indicating that microinjection of glucose into the medulla oblongata, specifically into the nucleus tractus solitarius, inhibits gastric motility (10). Additionally, Ishiguchi et al. (18) reported that glucose acts in the CNS to inhibit gastric distension-induced pyloric relaxation. Whereas evidence from the present study, combined with other findings described above, supports a CNS site of action of iv glucose, it is possible that iv glucose induces the release of an endogenous substance (i.e., a hormone) that acts in the CNS to inhibit gastric motor function.

In contrast to our evidence of a CNS site of action of iv D-glucose, there is also evidence that iv D-glucose may act at a peripheral site, specifically at responsive elements of vagal afferent nerves to inhibit gastric motility. Sakaguchi and Shimojo (31) showed that hepatic portal vein injections of D-glucose in the rat in bolus doses of 30 and 60 mg/kg inhibited insulin-induced increases in gastric tone and motility. Hepatic vagus nerve section abolished the inhibitory effect of the 60 mg/kg bolus dose of D-glucose. When 60 mg/kg D-glucose was given as a bolus injection into the jugular vein, it had no effect on gastric motility. However, the crucial experiment would have been to find an iv D-glucose continuous infusion dose that would inhibit gastric motility and then determine whether hepatic vagus nerve section would prevent the inhibition. Without this information, it is uncertain as to whether an iv glucose infusion could produce a blood concentration of glucose high enough to equal the bolus effect of 60 mg/kg D-glucose injected directly into the portal vein.

Table 4. Effect of intravenous D-glucose infusion on phasic antral activity evoked by TRH analog RX77368 microinjected into the dorsal motor nucleus of the vagus

<table>
<thead>
<tr>
<th>Effect of TRH</th>
<th>Glucose Effect (1-6 min after start of infusion)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Glucose Effect (1-6 min after start of infusion)</td>
<td>Recovery</td>
</tr>
<tr>
<td>Contraction amplitudes, g</td>
<td>Frequency, contractions/min</td>
<td>Contraction amplitudes, g</td>
</tr>
<tr>
<td>0.0 ± 0.0</td>
<td>41.6 ± 9.3*</td>
<td>44.8 ± 9.7</td>
</tr>
<tr>
<td>0.0 ± 0.0</td>
<td>4.1 ± 0.5*</td>
<td>4.3 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3 experiments. Contraction amplitudes are sum of amplitudes of contractions (≥1 g) over 5 min. *P < 0.05 compared with baseline using paired t-test.

Fig. 6. Representative chart recording from 1 experiment recorded with a strain-gauge force transducer, in which antral motility induced by the TRH analog RX77368 (30 pmol/60 nl), microinjected into the dorsal motor nucleus of the vagus, was not affected by 25% D-glucose infusion (2 ml/h). Blood glucose levels are indicated below the trace.
Zhou et al. (42) reported preliminary findings, suggesting that iv administered dextrose in rats acts on the vagal afferent pathways in the periphery to cause reduction of GABAergic inhibitory influence on the DMV. This enables facilitation of a vagal cholinergic pathway to occur, which synapses onto an intragastric nitric oxide (NO) pathway to cause gastric relaxation. Some of their evidence is based on the finding that inhibitory effects of hyperglycemia were blocked by L-NAME (10 mg/kg), an inhibitor of NO synthase. However, it is difficult to interpret data obtained with L-NAME because, whereas this drug will inhibit NO synthase in an intragastric NO pathway, it will also act in the nucleus of the tractus solitarius pathways that comprise the central components of the vago-vagal reflex (11). Furthermore, earlier studies by Ferreira et al. (10) reported that L-glucose causes an increase (not a decrease) of GABAergic inhibitory influence on the DMV.

Our study, in addition to providing evidence for a CNS site of action of iv d-glucose to inhibit gastric motility, reveals that d-glucose modulation of gastric motor function is dependent on the type of mechanism responsible for ongoing antral motility. In the case of increased antral motility caused by insulin-induced hypoglycemia, d-glucose is always effective in relaxing the stomach. Other instances in which d-glucose are effective are against antral phase II and III of the migrating motor complex (3, 13, 5), hunger contractions (7), and feeding- and sham feeding-induced antral contractions (19). What all of these inducers of antral activity have in common with insulin-induced hyperglycemia is that they appear to affect the antrum through the vagus nerve. Furthermore, glucose has been demonstrated to produce a decrease in efferent vagal nerve discharge (17, 33). Hence, it is tempting to conclude that the responsiveness of antral motor activity to d-glucose depends on the degree to which efferent vagal activity is responsible for the stomach contractions. We attempted to address this question in the present study by testing the ability of iv d-glucose to counteract RX77368-induced increases in antral motility evoked from the DMV. This method increases motor activity in the antrum by exciting the cholinergic vagal neurons from the hindbrain that innervate the stomach (20). However, under this experimental condition, d-glucose had no effect. Hence, we hypothesize that iv d-glucose acts in a CNS pathway that controls the activity of DMV neurons, rather than directly at DMV neurons. One such pathway that is both excited by insulin-induced hypoglycemia (35, 41) and affected by glucose (9) that projects to the DMV (35) is the paraventricular nucleus of the hypothalamus. Another major excitatory pathway to the DMV is from the raphe obscurus (27), which is not excited by insulin-induced hypoglycemia (41). Future studies will focus on the possibility of an interaction between d-glucose and insulin-induced hypoglycemia at the paraventricular nucleus.

Finally, we were surprised at the lack of effect of d-glucose on spontaneously occurring antral motility and tone of the fundus, as reflected by IGBP recordings of animals not receiving insulin. Ishiguchi and colleagues (19) also reported a lack of effect of iv administered d-glucose on spontaneously occurring antral motility in conscious rats using an extraluminal miniature force transducer sutured on the serosal surface of the antrum to record circular muscle contraction. In their studies, rats were fasted overnight and received a continuous infusion of d-glucose (50% at 0.01–0.03 ml/min) via a cannula placed in the jugular vein. Motility index of the antral contractions for 30 min of iv glucose infusion (blood glucose level increased to 13.8 ± 1.9 mM; n = 7) was not significantly affected compared with parallel data obtained with 30 min of saline infusion. Similarly, in a recent human study, hyperglycemia resulting in blood glucose concentrations of 18.05 ± 0.23 mM/l in diabetic patients had no significant effect on the numbers of antral pressure waves and their amplitude, as recorded from the individual side holes of a manometric perfused catheter (34). We assume that the lack of any effects of iv d-glucose infusions in these studies was due to the lack of involvement of a glucose-sensitive circuit projecting to DMV neurons.

In summary, iv administered d-glucose consistently and very sensitively inhibits antral motility and fundic tone in the setting of insulin-induced hypoglycemia. This inhibition primarily is due to d-glucose acting in the CNS to inhibit vagal outflow to the stomach. Based on the failure of d-glucose to affect motility induced by a TRH receptor agonist, we speculate that the site of the glucose effect in the brain is on neurons projecting to the DMV rather than on DMV neurons themselves. Our results also indicate that the method used to stimulate gastric contractions is an important determinant of whether d-glucose will affect gastric motility. In this regard, the experimental model that we employed for our studies has utility for future studies on the location of CNS pathways that regulate glucose effects on gastric motility.

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DISCLOSURES

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