Gastric relaxation induced by hyperglycemia is mediated by vagal afferent pathways in the rat

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Zhou S-Y, Lu Y-X, Owyang C. Gastric relaxation induced by hyperglycemia is mediated by vagal afferent pathways in the rat. Am J Physiol Gastrointest Liver Physiol 294: G1158–G1164, 2008. First published March 20, 2008; doi:10.1152/ajpgi.00067.2008.—Hyperglycemia has a profound effect on gastric motility. However, little is known about the site and mechanism that sense alteration in blood glucose level. The identification of glucose-sensing neurons in the nodose ganglia led us to hypothesize that hyperglycemia acts through vagal afferent pathways to inhibit gastric motility. With the use of a glucose-clamp rat model, we showed that glucose decreased intragastric pressure in a dose-dependent manner. In contrast to intravenous infusion of glucose, intracisternal injection of glucose at 250 and 500 mg/dl had little effect on intragastric pressure. Pretreatment with hexamethonium, as well as truncal vagotomy, abolished the gastric motor responses to hyperglycemia (250 mg/dl), and perivagal and gastroduodenal applications of capsaicin significantly reduced the gastric responses to hyperglycemia. In contrast, hyperglycemia had no effect on the gastric contraction induced by electrical field stimulation or carbachol (10−5 M). To rule out involvement of serotonergic pathways, we showed that neither granisetron (5-HT3 antagonist, 0.5 g/kg) nor pharmacological depletion of 5-HT using p-chlorophenylalanine (5-HT synthesis inhibitor) affected gastric relaxation induced by hyperglycemia. Lastly, Nω-nitro-l-arginine methyl ester (l-NAME) and a VIP antagonist each partially reduced gastric relaxation induced by hyperglycemia and, in combination, completely abolished gastric responses. In conclusion, hyperglycemia inhibits gastric motility through a capsaicin-sensitive vagal afferent pathway originating from the gastroduodenal mucosa. Hyperglycemia stimulates vagal afferents, which, in turn, activate vagal efferent cholinergic pathways synapsing with intragastric nitric oxide- and VIP-containing neurons to mediate gastric relaxation.

Hypoglycemia has a wide range of effects on gastrointestinal functions. The inhibitory effect of hypoglycemia on gastric motility is well known. Bulato and Carlson (8) were the first to report that hypoglycemia inhibits hunger contractions in the fasted dog, and, conversely, insulin-induced hypoglycemia produces gastric hypermotility that can be inhibited by intravenous glucose administration. Similarly, in healthy human subjects, glucagon-induced hyperglycemia abolishes hunger contractions, as measured with an intragastric balloon, and, when blood glucose levels return to control levels, gastric contractions are restored (36). Aylett (2) showed that blood glucose elevation slows gastric emptying. Barnett and Owyang (4) used a glucose clamp study to demonstrate that acute hyperglycemia inhibits the occurrence of the gastric interdigestive migrating motor complex in healthy volunteers. During the 3-h hyperglycemic period, gastric contractions were significantly reduced at 140 and 175 mg/dl and were almost completely absent at a blood glucose level of 250 mg/dl. This observation has important clinical implications for patients with diabetic gastroparesis, as it may explain the common observation that stable gastric neuropathies often exhibit wide day-to-day fluctuations in gastric emptying rates and symptoms of nausea and vomiting (12, 14, 16, 17).

Little is known about the site and mechanism that sense alteration in blood glucose level. The vagovagal reflex plays an important role in the mediation of many digestive functions including gastrointestinal motility (23, 25, 37, 38). The primary vagal afferents transmit sensory information about the physiological status of the gastrointestinal tract, including mechanical and chemical stimulation and energy metabolism. The identification of glucose-sensing neurons in the nodose ganglia (13, 46) led us to hypothesize that hyperglycemia acts through vagal afferent pathways to inhibit gastric motility.

In this study, we examined the hypothesis that hyperglycemia stimulates vagal afferents that act by way of the brainstem to stimulate the vagal efferent cholinergic pathway synapsing with intragastric nitric oxide (NO)- and peptide VIP-containing neurons to mediate gastric relaxation.

MATERIALS AND METHODS

Ethical approval. All experiments involving animals were approved by the University Committee on Use and Care of Animals at the University of Michigan.

Materials. The following materials were purchased: Nω-nitro-l-arginine methyl ester (l-NAME) and VIP antagonist (P-chloro-o-Phe6, Leu17)-VIP from Bachem (Torrance, CA); capsaicin, atropine sulfate, carbachol, p-chlorophenylalanine (PCPA), and hexamethonium bromide from Sigma-Aldrich (St. Louis, MO). Drugs were dissolved in physiological saline.

Animal preparation. Male Sprague-Dawley rats weighing between 250–300 g were fasted with water available ad libitum. The rats were anesthetized with urethane (1.0–1.5 g/kg ip). A tracheotomy was performed, and a tracheal tube was inserted through which the animals breathed room air spontaneously. Through a midline incision, a catheter with an attached rubber balloon was inserted into the stomach through an incision in the duodenum, as described in the next section. Cannulation of the jugular veins with polyethylene tubing (PE 50; BD Diagnostics, Sparks, MD) was performed in each rat.

Measurement of intragastric pressure. Intragastric pressure was measured with the use of a rubber balloon that was tied around a polyethylene tube (PE 160) and inserted into the body of the stomach through a small incision in the duodenum, as previously described (25). The balloon was secured at the pylorus with a suture to avoid

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movement, and the tube was connected to a pressure transducer (World Precision Instruments, Sarasota, FL), which was connected to a transducer amplifier (TB4M, World Precision Instruments). The balloon was filled with water at 37°C (1.2–2.0 ml, the volume determined to be the level necessary to induce an intragastric pressure of 5–10 cm H²O). Pressures were recorded and analyzed by Spike2, the data acquisition system for online analysis (Cambridge Electronic Design, Cambridge, UK). The exact location of the balloon was verified after each experiment.

**Hyperglycemia clamp studies.** Hyperglycemia was achieved with the use of a hyperglycemic clamp, as described by Ishiguchi et al. (18), who adapted the method from previous studies in humans (10). The clamp facilitates obtaining blood glucose concentrations at preset hyperglycemic levels up to 300 mg/dl and maintaining them for at least 30 min. The rats were anesthetized with urethane (1.0–1.5 g/kg ip). The right jugular vein was exposed, and a polyethylene catheter (PE 50) was surgically placed for glucose infusion. The animals were randomly divided into two groups: one group was given a saline infusion (control) and the other, a 20% dextrose infusion. Glucose concentrations in blood obtained from the tail were measured every 5–10 min with a glucose meter (Accu-Check; Roche, Mannheim, Germany). For blood sampling, the rat was held in a restrainer, and its tail was cleaned and poked with 26.5-gauge syringe needle. A drop of blood was collected and placed on the glucose test strip. Blood glucose levels were raised stepwise to preset concentrations by infusing a priming dose of 20% dextrose in the first 10 min with an infusion pump (SP 1000 syringe pump, World Precision Instruments) at the rate of 100 μl/min. After achieving hyperglycemia, the blood glucose concentration was maintained by adjusting the rate of the glucose infusion according to the blood glucose concentration measured every 5–10 min. Intragastric pressure was measured as described in the previous section.

**Bilateral subdiaphragmatic vagotomy.** To demonstrate that hyperglycemia acts by way of stimulation of the vagal pathways, acute bilateral subdiaphragmatic vagotomy was performed as previously described (25). A midline incision was made in the abdominal wall, and the stomach was carefully manipulated to expose the esophagus. The subdiaphragmatic vagal trunks were exposed by opening the diaphragm and the gastric cardia. Both anterior and posterior trunks of the vagal nerves were transected. For the control experiments, the abdominal vagal nerves were exposed but not cut. Hyperglycemia studies were performed as described in the previous section. To demonstrate the completeness of vagotomy, the gastric response to electrical stimulation of the vagus nerve was tested at the end of the experiments as described in the next section.

**Nerve stimulation and carbachol studies.** Through a midline incision on the anterior surface of the neck, the right cervical vagal nerve was dissected free. The peripheral cut end of the cervical vagus nerve was placed on an electrode and covered with liquid paraffin. The nerve was stimulated with a Grass stimulator (10 V; 1.25, 2.5, or 5 Hz; and 2 ms for 30 s) at 30 min before and 10 min after hyperglycemia was established. To determine whether hyperglycemia affects the muscle response to cholinergic stimulation, intragastric pressure response to carbachol (10⁻⁵ M, 0.1 ml given intravenously) was studied in the presence of hexamethonium (10 mg/kg iv). The study was repeated with intravenous infusion of glucose to induce hyperglycemia (250 mg/dl).

**Peripheral application of capsaicin.** To investigate the role of the vagal afferent pathway in the mediation of the effect of hyperglycemia, we examined the effect of perivaginal application of capsaicin (22, 25). Following anesthetization with pentobarbital sodium (50 mg/kg ip), an upper midline laparotomy was performed and the abdominal vagal nerve trunks were exposed and isolated with a piece of parafilm. A small piece of gauze soaked in 1% capsaicin solution (0.2 ml per rat) was applied to the vagal trunks for 30 min. After capsaicin treatment, the gauze was removed. The nerve trunks were rinsed with warm saline, and the parafilm was removed. Vehicle alone was applied to the vagal trunks of the control rats. Hyperglycemia studies as described in the previous section were performed 5 days after surgery in the capsaicin-treated and control rats.

**Gastrroduodenal mucosal application of capsaicin.** To determine whether the glucose-sensitive afferent nerve endings originate from the gastrroduodenal mucosa, we examined the effects of the mucosal application of capsaicin in the stomach and duodenum (25). Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). After laparotomy, the stomach and duodenum were isolated and temporarily ligated at both ends. Capsaicin (2 ml) (6 mg/ml dissolved in 10% Tween, 80% in olive oil) was applied topically to the gastrroduodenal mucosa for 30 min. A saline application was used in control rats. Hyperglycemia studies were performed 7 days after local capsaicin application. Rats were checked for normal eye wiping movement, which is an indication that local mucosal treatment of capsaicin has no systemic effect (41).

**Effects of 5-HT₃ receptor antagonist and PCPA treatments.** Our previous studies indicated that luminal glucose stimulates the vagal afferent pathway through the release of intestinal 5-HT, which acts as a paracrine substance to mediate vagal signal transduction by way of the 5-HT₃ receptor (24, 47). To rule out involvement of the 5-HT₃ pathway in the mediation of the gastric motility response to hyperglycemia, we measured the effect of the 5-HT₃ receptor antagonist granisetron (15). Gastric motility studies in response to hyperglycemia (250 mg/dl) were performed 15 min after intravenous infusion of granisetron (0.5 mg/kg). To further exclude any role for endogenous 5-HT in the mediation of gastric motility responses to hyperglycemia, we examined the effects of PCPA, a 5-HT synthesis inhibitor. PCPA inhibits tryptophan hydroxylase, the enzyme acting at the rate-limiting step of 5-HT synthesis. Depletion of 5-HT stores in the brain, intestinal tissue, and blood occurs after PCPA treatment (20, 42, 47). PCPA (500 mg/kg) suspended in 2 ml gum arabic solution was administered intraperitoneally on two consecutive days. Controls received only gum arabic. Studies of the gastric motility response to hyperglycemia (250 mg/dl) were conducted as described in the previous section.

**Antagonist studies.** To determine the role of nitric oxide receptors in the mediation of gastric relaxation induced by hyperglycemia, hexamethonium (20 mg/kg bolus) was injected and continuously infused at a rate of 10 mg·kg⁻¹·h⁻¹ for 20 min before the administration of glucose, as previously described (37). To determine the role of NO and VIP, l-NAME (10 mg/kg) and a VIP antagonist (30 nmol/kg) were injected 10 min before the infusion of glucose. These specific doses of l-NAME and the VIP antagonist have been shown to abolish rapid and prolonged relaxation, respectively, in response to vagal stimulation in vivo (37, 47).

**Effect of central hyperglycemia on gastric motility.** To rule out the possibility that hyperglycemia may act at a central site to induce gastric relaxation, we examined the effect of intracisternal injection of glucose on gastric motility. We adapted the method from a previous study by Adelson et al. (1). Briefly, the rats were anesthetized, and the head of each rat was fixed in a stereotactic holder. A midline incision was made along the back of the head and neck, and the underlying muscles were separated and retracted. The atlanto-occipital membrane was carefully exposed. A small incision was made in the membrane with a 25-gauge needle. A PE 10 polyethylene tube (BD Diagnostics) filled with saline was inserted through the incision into the cisterna magna. The cannula was secured with a drop of cyanoacrylate glue, and the incision was closed.

Gastric motility studies were performed as described in the previous section. After a 30-min recording, 100 μl of glucose (250 or 500 mg/dl) dissolved in 1,000 μl saline was administered with a Hamilton syringe into the cisterna magna over 30 s, followed by a 5-μl saline flush. Intragastric pressure was monitored for 20 min after each injection.

**Analysis of data.** Results were expressed as means ± SE. Statistical analysis was performed using one-way ANOVA, followed by the
RESULTS

Glucose clamp studies. After an overnight fast, the basal blood glucose level was 90 ± 7 mg/dl (n = 10). The intragastric pressure was set at 5–10 cm H2O with balloon distension, and this level remained stable for at least 60 min before the infusion of glucose. Intravenous infusion of dextrose (20%) produced a dose-dependent inhibition of gastric motility (Fig. 1). At blood glucose concentrations of 150, 200, 250, 300, and 350 g/dl, intragastric pressure decreased by −0.76 ± 0.34, −1.85 ± 0.38, −1.91 ± 0.28, −2.31 ± 0.23, and −2.59 ± 0.38 cm H2O, respectively. As shown in Fig. 2, increasing blood glucose levels reduced stomach muscle tone and progressively inhibited phasic gastric activities. Decreasing blood glucose levels from 300 to 150 mg/dl partially restored phasic gastric activities. Subsequent studies were performed with hyperglycemia maintained at 250–300 mg/dl.

Effects of hexamethonium and vagotomy. Administration of hexamethonium (10 mg/kg) reduced basal intragastric pressure by 25 ± 3%; intragastric pressure returned to basal level within 30 min. Hexamethonium markedly reduced the inhibitory action of hyperglycemia maintained at 250 mg/dl (P < 0.05) (Fig. 3). This observation indicates that hyperglycemia acts at a presynaptic site of the cholinergic pathway.

Immediately after truncal vagotomy, basal intragastric pressure was reduced by 26 ± 4%. The intragastric pressure returned to basal level within 30 min, and the glucose infusion experiment was started. Similar to hexamethonium, truncal vagotomy markedly inhibited the gastric response to hyperglycemia at 250 mg/dl (P < 0.05) (Fig. 3). This suggests that the vagal pathway is the primary site of action of glucose to inhibit gastric motility.

Effect of perivagal and gastroduodenal mucosal applications of capsaicin. Perivagal application of capsaicin markedly reduced gastric relaxation in response to hyperglycemia (250 mg/dl) (P < 0.05) (Fig. 3). Similar to perivagal application of capsaicin, gastroduodenal application of capsaicin also significantly reduced the gastric responses to hyperglycemia (P < 0.05) (Fig. 3). In separate studies, we showed that neither perivagal nor gastroduodenal mucosal applications of capsaicin affected gastric responses to electrical vagal stimulation (n = 6) (10 V, 5 Hz, 2 ms for 30 s) (data not shown), indicating that administration of capsaicin does not affect efferent vagal transmission.

Effect of hyperglycemia on gastric contraction induced by electrical vagal stimulation and intravenous administration of carbachol. Electrical vagal stimulation (10 V, 1.25, 2.5, or 5 Hz; 2 ms for 30 s) induced a frequency-dependent increase in gastric pressure. This contraction was completely abolished by atropine (100 μg·kg−1·h−1). Electrical stimulation (10 V, 5 Hz, 2 ms for 30 s) of the peripheral cut end of the vagus nerve produced an increase in intragastric pressure (3.32 ± 0.33 cm H2O) (n = 4). Infusion of d-glucose to achieve a blood level of 250 mg/dl failed to affect the gastric contraction induced by vagal stimulation (3.26 ± 0.39 cm H2O) (n = 4, P > 0.05).

To determine whether hyperglycemia affects the muscle response to cholinergic stimulation, we showed that carbachol (10−5 M, 0.1 ml iv) produced an increase in intragastric pressure (6.97 ± 0.91 cm H2O) (n = 4) in the presence of hexamethonium (10 mg/kg iv). Infusion of glucose to induce hyperglycemia (250 mg/dl) failed to inhibit the contractile response induced by carbachol (6.62 ± 1.36 cm H2O) (n = 4, P > 0.05).

Effects of 5-HT3 receptor antagonist and PCPA treatments. Intravenous infusion of granisetron (0.5 g/kg) had no effect on intragastric pressure. Fifteen minutes after the administration...
of granisetron, intravenous infusion of 20% dextrose to achieve a blood glucose level of 250 mg/dl produced gastric relaxation similar to that observed under control conditions without granisetron (2.90 ± 0.41 vs. 3.21 ± 0.65 cm H2O, n = 4, P > 0.05).

PCPA (500 mg/kg) was injected intraperitoneally into 6 rats on two consecutive days, and 4 rats were pretreated with vehicle (gum arabic) solution. Hyperglycemia (250 mg/dl) induced by glucose infusion stimulated a similar degree of gastric relaxation in both PCPA- and vehicle-treated rats (3.18 ± 0.45 vs. 2.94 ± 0.54 cm H2O, n = 6, P > 0.05).

Effects of L-NAME and VIP antagonist. To determine whether NO and VIP play a role in mediating gastric relaxation induced by hyperglycemia, we examined the effects of L-NAME and a potent VIP antagonist. NO and VIP have been shown to mediate vagally induced gastric relaxation (28). Administration of L-NAME (10 mg/kg), which abolished the rapid transient relaxation induced by electrical vagal stimulation (28), reduced the gastric relaxation induced by hyperglycemia (250 mg/dl) by 76 ± 9% (P < 0.05) (Fig. 4). Intravenous administration of the VIP antagonist (30 nmol/kg) also decreased gastric relaxation in response to hyperglycemia (250 mg/dl) by 53 ± 12% (P < 0.05) (Fig. 4). A combination of L-NAME and the VIP antagonist completely abolished the gastric relaxation stimulated by hyperglycemia (250 mg/dl) (Fig. 4).

Effect of intracisternal injection of glucose. In contrast to intravenous infusion of glucose, intracisternal injection of glucose at 250 or 500 mg/dl did not significantly affect intragastric pressure. A tracing from one gastric pressure recording is shown in Fig. 5. Blood glucose remained euglycemic after intracisternal injection of glucose.

DISCUSSION

This study showed that hyperglycemia reduced gastric motility in a dose-dependent manner. This effect was abolished by perivagal or gastroduodenal mucosal application of capsaicin. Furthermore, we provided evidence that hyperglycemia stimulated vagal afferent pathways, which, in turn, activated vagal efferent cholinergic pathways synapsing with intragastric NO and VIP neurons to mediate gastric relaxation. This is the first demonstration that hyperglycemia inhibits gastric motility by activating the vagal afferent pathways innervating the gastroduodenal mucosa.

The infusion of concentrated dextrose as performed in the present study caused an elevation in blood insulin levels. It has been shown that insulin injection activated neurons in the enteric plexus in the stomach. The action is vagally mediated since vagotomy abolished the c-Fos expression in the stomach in response to insulin-induced hypoglycemia (45). Although it is conceivable that hyperinsulinism, secondary to hyperglycemia, is responsible for the inhibition of gastric motility, it is unlikely because previous work indicates a stimulatory role for insulin on gastrointestinal tract motility. Prasad and Sarna (29) reported that administration of insulin to fasted dogs results in premature migrating myoelectric complex phase III activity. Other investigators observed disruption of the migrating myoelectric complex and development of intestinal hyperactivity characteristic of a fed-like pattern during insulin infusion (7).

Although the presence of glucose-sensing neurons in the hypothalamus was first reported in 1953 (27), little is known about the site and mechanism that sense alteration in blood glucose level and modulate gastric motility. In general, the glucose-sensing neurons located in the brain are involved in the control of neuroendocrine function, nutrient metabolism, and energy homeostasis (21). These central nervous system neurons are unlikely to play a major role in mediating digestive functions in response to changes in circulating glucose under physiological conditions because the glucose level in the cerebrospinal fluid ranges between 10 and 30% of blood glucose levels, and, hence, these neurons are too insensitive to detect physiological changes in blood glucose concentrations (34). In this study, we further demonstrated that intracisternal injection of glucose into the cisterna magna failed to affect gastric motility, thus ruling out a central site of action of glucose to inhibit gastric motility.

However, it should be noted that, in contrast to peripheral hyperglycemia, insulin hypoglycemia on gastrointestinal functions appears to be centrally mediated (43). These investigators showed that hypoglycemia induced by insulin induced neuronal activation in the brain vagal-regulatory nuclei including the paraventricular nucleus of the hypothalamus, locus coeruleus, dorsal motor nucleus of the vagus, and nucleus tractus solitarii (NTS). These, in turn, altered gastrointestinal functions by activating the vagal efferent pathways. This action of hypoglycemia is independent of the vagal afferents (45), as bilateral cervical vagotomy did not influence insulin-induced Fos expression in the brain. Thus it appears that both glucose-
sensitive neurons and glucose sensors are located both centrally and peripherally. Our present studies indicate that peripheral hyperglycemia causes gastric relaxation via its action on vagal afferents. Others show that insulin hypoglycemia exerts its action mainly in the CNS to alter gastric functions. These suggest that central and peripheral glucose-sensitive neurons may have differential sensitivity to hypo- and hyperglycemia.

Recent studies suggest that acute hyperglycemia affects a subpopulation of neurons in the NTS and the dorsal motor nucleus of the vagus (3, 11, 19, 28, 43). These investigators demonstrated a prominent action of glucose to increase vagal afferent excitatory synaptic transmission to NTS neurons. Sakaguchi et al. (31, 32) reported that glucose injection into the dorsal motor nucleus of the vagus of anesthetized rats decreases gastric motility. In addition, Ferreira et al. (11) demonstrated that administration of glucose into the NTS modulates gastric motor and secretory functions. The physiological relevance of these observations is unknown; these investigators did not demonstrate that neurons in the NTS or the dorsal motor nucleus of the vagus are true primary sensors of changes in peripheral glucose levels.

Our study showed that the nicotinic receptor antagonist hexamethonium markedly reduced the inhibitory effect of hyperglycemia on gastric motility, suggesting that hyperglycemia is acting on a presynaptic site along the cholinergic pathway. To further identify the location of action, we examined the effect of bilateral subdiaphragmatic vagotomy. Similar to hexamethonium, vagotomy also markedly reduced the inhibitory action of hyperglycemia, suggesting that hyperglycemia inhibits gastric motility via a vagal pathway. To determine whether hyperglycemia exerts its action via an afferent or an efferent vagal pathway, we examined the effect of perivagal treatment with the sensory neurotoxin capsaicin. Capsaicin has been widely used as a tool to investigate the role of afferent C fibers in many physiological processes (5, 6, 25, 30, 35). Systemic administration of capsaicin affects neurotransmission in all somatic and visceral capsaicin-sensitive fibers. In this study, we applied capsaicin directly to the vagal trunks to avoid any damage to the afferent nerve terminals in the peripheral and central nervous systems, which has been observed with systemic administration of capsaicin. Previous studies have shown that perivagal capsaicin treatment interrupts the vagal afferent pathways that mediate the action of CCK on satiety (35), gastric motility and emptying (30), and pancreatic enzyme secretion (22). Our studies showed that perivagal pre-treatment with capsaicin markedly reduced the gastric response to hyperglycemia, an effect similar to that observed with vagotomy. Similar observations have been made with secretin, which, at physiological doses, inhibits gastric motility by way of a vagal afferent pathway (25).

To further localize the site of action of hyperglycemia on the vagal afferent pathway, we examined the effect of the mucosal application of capsaicin in the gastroduodenal region. This technique has been used to demonstrate chemically the sensory fibers in the duodenal mucosa (41). We showed that, similar to perivagal capsaicin treatment, gastroduodenal application of capsaicin markedly reduced the inhibitory effect of hyperglycemia, indicating that glucose-sensitive afferent fibers originate from the vagal branch in the mucosa of the stomach and duodenum. These observations were further confirmed with the use of an in vitro vagus stomach preparation that completely eliminated any influence of the central nervous system and/or systemic responses (44). We have previously identified subsets of gastric vagal afferents that are glucose responsive (46).

Hyperglycemia reportedly inhibits cholinergic transmission (40), which may contribute to a delay in gastric emptying (9). To rule out this possibility, we showed that hyperglycemia fails to inhibit gastric contraction induced by electrical field stimulation. This suggests that hyperglycemia does not have a direct inhibitory effect on the vagal release of acetylcholine, which is the principal postsynaptic neuronal neurotransmitter mediating muscle contraction induced by electrical vagus stimulation. Hence, it argues against hyperglycemia-induced inhibition of the vagal efferent pathways to mediate gastric relaxation. Moreover, the failure of hyperglycemia to inhibit gastric contraction induced by carbachol indicates that hyperglycemia does not have a direct inhibitory effect on gastric smooth muscle cells.

Luminal stimuli, such as glucose or maltose, induce 5-HT release from mucosal enterochromaffin cells, and this, in turn, activates 5-HT7 receptors on mucosal vagal afferent terminals (47). In this manner, 5-HT acts as a paracrine substance to inhibit gastric motility through a vagal cholinergic pathway. To rule out involvement of the 5-HT7 pathway in the mediation of the gastric motility response to hyperglycemia, we showed that the 5-HT7 antagonist granisetron failed to affect gastric relaxation induced by hyperglycemia. In separate studies, we showed that pharmacological depletion of 5-HT stores with the use of a 5-HT synthesis inhibitor p-chlorophenylalanine, which markedly reduces duodenal mucosal 5-HT levels and abolishes pancreatic secretion induced by luminal administration of glucose (23), also had no effect on gastric relaxation induced by hyperglycemia (250 mg/dl). This suggests that hyperglycemia inhibits gastric motility by way of a 5-HT-independent pathway.

Vagal stimulation produces two modes of relaxation in the rat stomach, rapid relaxation followed by prolonged relaxation (39). Rapid relaxation is antagonized by a NO inhibitor and prolonged relaxation is blocked by a VIP antagonist (39), which suggests that different neurotransmitters mediate different modes of relaxation. In rats, gastric distension induces a vagovagal reflex that stimulates the NO-containing gastric myenteric plexus (37). On the other hand, secretin-induced gastric relaxation is mediated by a VIP pathway (26). In this study, we showed that L-NAME and the VIP antagonist each partially reduced gastric relaxation induced by hyperglycemia. A combination of the two antagonists completely abolished hyperglycemia-induced gastric relaxation. These observations indicate that activation of vagal afferents by hyperglycemia stimulates vagal efferent cholinergic pathways synapsing with intragastric NO- and VIP-containing neurons to mediate gastric relaxation.

Our finding that acute hyperglycemia inhibits gastric motility differs from that reported by Shi et al. (33), who showed that hyperglycemia failed to inhibit spontaneous or bethanechol-induced gastric contractions in rats, as measured by a strain-gauge force transducer sutured to the antrum. On the other hand, intravenous infusion of glucose inhibited the antral contractions stimulated by insulin-induced hypoglycemia. This inhibitory action of glucose was not affected by sectioning the hepatic branch of the vagus nerve nor by capsaicin treatment.
These differences in findings may be related to the methods used to stimulate and record gastric pressure. We measured gastric pressure with a water-filled balloon inserted into the body of the stomach, whereas Shi et al. (33) measured antral motility using a strain-gauge transducer sutured to the antrum. It is not surprising that the inhibitory effect of glucose on antral contractions induced by hyperglycemia was not sensitive to capsaicin treatment. Since insulin-induced hypoglycemia acts centrally to stimulate gastric contractions, one would not expect this action to be affected by glucose acting on the vagal afferent fibers. In fact, we also observed that hyperglycemia did not affect gastric contractions induced by direct electrical stimulation of the vagus nerve. This further emphasizes the importance of the methods used to stimulate stomach contraction because they will determine the sensitivity of gastric motor function to changes in blood glucose levels.

The demonstration that hyperglycemia inhibits gastric motility has obvious clinical importance. It suggests that hyperglycemia alone, in the absence of underlying neuropathy or myopathy, can alter gastric motor function. This may explain the common clinical observation that diabetic patients with stable motor defects often exhibit wide-day-to-day variations in the severity of their symptoms depending on blood glucose control (14). In type I and type II diabetes, there is a strong correlation between delayed gastric emptying of liquids and blood glucose levels >270 mg/dl (16, 17). Similarly, delays in solid emptying occur during periods of hyperglycemia in type I diabetes, which improve during periods of euglycemia (12). This study identifies the site and neural pathways that sense alteration in blood glucose level and modulate gastric motility.

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