Inhibition of gastric motility by hyperglycemia is mediated by nodose ganglia K$_{ATP}$ channels

Shi-Yi Zhou,* Yuanxu Lu,* Il Song, and Chung Owyang

Division of Gastroenterology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan

Submitted 29 October 2010; accepted in final form 28 December 2010

Zhou S, Lu Y, Song I, Owyang C. Inhibition of gastric motility by hyperglycemia is mediated by nodose ganglia K$_{ATP}$ channels. Am J Physiol Gastrointest Liver Physiol 300: G394–G400, 2011. First published December 30, 2010; doi:10.1152/ajpgi.00493.2010.—The inhibitory action of hyperglycemia is mediated by vagal afferent fibers innervating the stomach and duodenum. Our in vitro studies showed that a subset of nodose ganglia neurons is excited by rising ambient glucose, involving inactivation of ATP-sensitive K$^+$ (K$_{ATP}$) channels and leading to membrane depolarization and neuronal firing. To investigate whether nodose ganglia K$_{ATP}$ channels mediate gastric relaxation induced by hyperglycemia, we performed in vivo gastric motility studies to examine the effects of K$_{ATP}$ channel activators and inactivators. Intravenous infusion of 20% dextrose induced gastric relaxation in a dose-dependent manner. This inhibitory effect of hyperglycemia was blocked by diazoxide, a K$_{ATP}$ channel activator. Conversely, tolbutamide, a K$_{ATP}$ channel inactivator, induced dose-dependent gastric relaxation, an effect similar to hyperglycemia. Vagotomy, perivagal capsaicin treatment, and hexamethonium each prevented the inhibitory action of tolbutamide. Similarly, N$^\omega$-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase, also blocked tolbutamide’s inhibitory effect. To show that K$_{ATP}$ channel inactivation at the level of the nodose ganglia induces gastric relaxation, we performed electroporation of the nodose ganglia with small interfering RNA of Kir6.2 (a subunit of K$_{ATP}$) and plasmid pEGFP-N1 carrying the green fluorescent protein gene. The gastric responses to hyperglycemia and tolbutamide were not observed in rats with Kir6.2 small interfering RNA-treated nodose ganglia. However, these rats responded to secretin, which acts via the vagal afferent pathway, independently of K$_{ATP}$ channels. These studies provide in vivo evidence that hyperglycemia induces gastric relaxation via the vagal afferent pathway. This action is mediated through inactivation of nodose ganglia K$_{ATP}$ channels.

AMONG THE WIDE RANGE OF EFFECTS of hyperglycemia on the gastrointestinal system, its inhibitory effect on gastric motility is well known. Bulatao and Carlson (5) first reported that hyperglycemia inhibited hunger contractions in the fasted dog and, conversely, that insulin-induced hypoglycemia produced gastric hypermotility. Similar observations have been made in humans (2, 3, 27). Barnett and Owyang (3) used a glucose clamp study to show that acute hyperglycemia inhibited the gastric interdigestive migrating motor complex in healthy volunteers. During the 3-h hyperglycemic period, gastric contractions were almost completely absent at a serum glucose level of 250 mg/dl and were significantly reduced at 175 and 140 mg/dl. This observation has important clinical implications, as it may explain the wide day-to-day fluctuations in gastric-emptying rates and symptoms of nausea and vomiting in patients with diabetic gastroparesis who have stable gastric neuropathies.

Although it is well demonstrated that hyperglycemia inhibits gastric motility, much less is known about the site(s) and mechanism(s) of action by which hyperglycemia modulates gastric motility. Our laboratory has identified the presence of glucose-excited neurons in rat nodose ganglia using retrograde labeling and in vitro intracellular recording studies (15). The uptake and metabolism of glucose in these neurons leads to closure of ATP-sensitive K$^+$ (K$_{ATP}$) channels, triggering membrane depolarization (15).

In the present study, we examined the hypothesis that inhibition of gastric motility by hyperglycemia is mediated by K$_{ATP}$ channels in the glucose-excited neurons of the nodose ganglia. In this manner, hyperglycemia stimulates vagal afferents that act by way of the brain stem to activate the vagal efferent cholinergic pathway, synapsing with intragastric nitric oxide-containing neurons to mediate gastric relaxation. To test this hypothesis, we performed in vivo gastric motility studies to examine the effects of K$_{ATP}$ channel activators and inactivators. To provide direct evidence that modulation of K$_{ATP}$ channels in glucose-excited nodose neurons is responsible for mediating hyperglycemia-induced gastric relaxation, in vivo gastric motility studies were performed after the K$_{ATP}$ channels were silenced by local application of small interfering RNA (siRNA) of Kir6.2 (a subunit of K$_{ATP}$ channels) into the nodose ganglia using electroporation. Success in knocking down Kir6.2 gene expression was verified by RT-PCR, Western blot, and immunohistochemistry.

MATERIALS AND METHODS

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

Chemicals. The following materials were purchased from Sigma-Aldrich (St. Louis, MO): diazoxide, tolbutamide, N$^\omega$-nitro-L-arginine methyl ester (L-NAME), capsaicin, and hexamethonium bromide. Drugs were dissolved in physiological saline. Capsaicin at a 1% solution was dissolved in 10% ethanol, 10% Tween 80, and 80% saline.

Animal preparation. Male Sprague-Dawley rats, weighing between 250 and 300 g, were fasted and given ad libitum access to water. The rats were anesthetized with an intraperitoneal injection of urethane (1.0–1.5 g/kg). A tracheotomy was performed, and a tracheal tube was inserted through which the animals spontaneously breathed room air. A catheter with an attached rubber balloon was inserted into the stomach through an incision in the duodenum, as previously described (21, 30). The jugular veins were cannulated with polyethylene tubing (PE 50; BD Diagnostics, Sparks, MD).

Hyperglycemia studies. Hyperglycemia was achieved with the use of a hyperglycemic clamp, as previously described (17, 30) and...
adapted from a method used in a human study (6). The clamp facilitates achieving serum glucose concentrations at preset hyperglycemic levels up to 400 mg/dl and maintaining them for at least 30 min. These levels are frequently observed in poorly controlled diabetics. In humans, hyperglycemia started to inhibit gastric motility at 140 mg/dl and abolished gastric contractions at 250 mg/dl (3). Hence we performed gastric motility studies by clamping blood glucose at 150, 200, 300, and 400 mg/dl. Each rat was anesthetized with urethane (1.0−1.5 g/kg, intraperitaloneal). The right jugular vein was exposed, and a polyethylene catheter (PE 50) was surgically placed for an infusion. The animals were randomly divided into two groups: one group was given a saline infusion (control), and the other group was given a 20% dextrose infusion. Glucose concentrations in blood obtained from the tail were measured every 5−10 min with a glucose meter (ACCUCHEK; Roche Diagnostics, Indianapolis, IN). Serum glucose levels were raised in increments to preset concentrations by infusing a priming dose of 20% dextrose at the rate of 50−100 μl/min in the first 10 min using a single-syringe infusion pump (SP100i, World Precision Instruments, Sarasota, FL). After hyperglycemia was achieved, the serum glucose concentration was maintained by adjusting the glucose infusion rate according to the serum glucose concentration measured every 5−10 min.

Measurement of intragastric pressure. Intragastric pressure was measured using a rubber balloon 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 (21, 30). The balloon was secured with a suture at the pylorus to prevent movement, and the tube was connected to a pressure transducer (World Precision Instruments). In this study, it was connected to a Transbridge transducer amplifier (SYS-TBM4M, World Precision Instruments). The balloon was filled with 1.2−2.0 mL water at 37°C, the volume determined to be the amount necessary to induce an intragastric pressure of 5−10 cmH2O. Pressures were recorded and analyzed by Spike2 (Cambridge Electronic Design, Cambridge, UK), the data-acquisition system for online analysis. The exact location of the balloon was verified after each experiment.

Bilateral subdiaphragmatic vagotomy. To show that toltubatide, a KATP channel activator, acts by way of stimulation of the vagal pathway, acute bilateral subdiaphragmatic vagotomy was performed, as previously described (30). A midline incision was made in the abdominal wall, and the stomach was carefully manipulated to expose the esophagus. The subdiaphragmatic vagus trunks were exposed halfway between 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.

Perivagal application of capsaicin. To investigate the role of the vagal afferent pathway in the mediation of the effect of toltubatide, we examined the effect of a perivagal application of capsaicin, as previously described (21, 30). After anesthetization with pentobarbital sodium (50 mg/kg, intraperitaloneal), 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/rat) was applied to the vagal trunks for 30 min, after which 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. Toltubatide studies were performed 5 days after surgery in the capsaicin-treated and control rats.

Antagonist studies. To determine the role of nicotinic receptors in the mediation of toltubatide-induced gastric relaxation, hexamethonium (10 mg/kg) was injected 20 min before the administration of toltubatide, as previously described (21, 30). To determine the role of nitric oxide, L-NAME (10 mg/kg) was injected 10 min before the infusion of toltubatide. These Specific doses of hexamethonium and L-NAME have been shown to abolish gastric contraction and relaxation, respectively, in response to vagal stimulation in vivo (28, 30).
Western blot analysis. Protein from the rat nodose ganglia was extracted as previously described (15). Briefly, nodose ganglia were obtained and pooled for Western blot analysis. The tissues were lysed and centrifuged at 14,000 g for 10 min. Protein samples were run on Ready Gel 12% Tris-HCl (Bio-Rad, Hercules, CA) for 1.5 h at 80 V and then transferred to polyvinylidene difluoride membranes for 1 h at 80 V. The membranes were blocked with StartBlock buffer T20 (ThermoFisher Scientific, Waltham, MA) for 1 h at room temperature, probed overnight with primary antibodies against Kir6.2 (Santa Cruz Biotechnology) at 1:1,000 dilution, 4°C, and washed for 1 h in Tris-buffered saline. The membranes were probed with corresponding horseradish peroxidase-conjugated secondary antibodies at 1:2,000 dilution. The resulting bands were scanned with an Epson Stylus Photo R2400 and analyzed using ImageJ (NIH).

Analysis of data. Results were expressed as means ± SE. Statistical analysis was performed using one-way ANOVA, followed by the Kruskal-Wallis test or the Student’s t-test, depending on the particular study design. Significance was accepted at the level of P < 0.05.

RESULTS

Hyperglycemia-induced gastric relaxation. After an overnight fast, the basal serum glucose level was 86 ± 9 mg/dl (n = 12). Intragastric pressure was measured in anesthetized rats by a balloon attached to a catheter placed in the body of the stomach. Hyperglycemia was induced using a glucose clamp protocol. The intragastric pressure was set at 5–10 cmH2O with balloon distention and was stable for at least 60 min before the glucose infusion. Intravenous (iv) infusion of 20% dextrose produced a dose-dependent inhibition of gastric motility (Fig. 1). Increasing serum glucose levels reduced stomach muscle tone and progressively inhibited phasic gastric activities (Fig. 1A). Maximum relaxation was observed at a serum glucose level of 250–300 mg/dl (Fig. 1). A decrease in serum glucose levels from 300 to 150 mg/dl partially restored phasic gastric activities (Fig. 1A). The subsequent studies were performed with hyperglycemia maintained with a serum glucose of 250–300 mg/dl.

Effects of KATP channel activators and inactivators on hyperglycemia-induced gastric relaxation. Our in vitro studies showed that a subset of nodose ganglia neurons is excited by rising ambient glucose levels, an action that involves inactivation of KATP channels, leading to membrane depolarization and neuronal firing (15). To determine whether nodose ganglia KATP channels mediate hyperglycemia-induced gastric relaxation, we examined the effects of KATP channel activators and inactivators on gastric motility in vivo. Increasing the serum glucose level reduced stomach muscle contractions and resulted in gastric relaxation and a reduction in intragastric pressure. The iv administration of diazoxide (30 mg/kg), a KATP channel activator, had little or no effect on gastric phasic activities or basal tone, but blocked the inhibitory effect of hyperglycemia on gastric motility. Diazoxide at 30 mg/kg reduced gastric relaxation induced by hyperglycemia (serum glucose, 250 mg/dl) by 83.7 ± 3.0% (n = 6; P < 0.05) (Fig. 2).

Conversely, iv administration of tolbutamide, a KATP channel inactivator, induced dose-dependent gastric relaxation, a similar effect to that induced by hyperglycemia. Tolbutamide at 20, 30, and 40 mg/kg produced gastric relaxation and lowered intragastric pressure by 1.65 ± 0.28, 2.75 ± 0.25, and 3.65 ± 0.4 cmH2O, respectively (n = 4, P < 0.05) (Fig. 3).
Effects of tolbutamide are mediated by the vagal afferent pathway. Vagotomy or administration of hexamethonium blocked the inhibitory action of tolbutamide. Administration of hexamethonium (10 mg/kg) reduced basal intragastric pressure. Intragastric pressure returned to basal level within 30 min after the administration of hexamethonium. Hexamethonium reduced the inhibitory action of tolbutamide (30 mg/kg) by 72 ± 5% (n = 4; P < 0.05) (Fig. 4). This observation indicates that tolbutamide acts at a presynaptic site of the cholinergic pathway to induce gastric relaxation. Similar to hexamethonium, bilateral subdiaphragmatic vagotomy reduced the gastric response to tolbutamide by 77.2 ± 11.4% (n = 4; P < 0.05) (Fig. 4). This suggests that the vagal pathway is the primary site of action of tolbutamide to inhibit gastric motility.

In a separate study, we showed that perivagal capsaicin treatment also prevented gastric relaxation induced by tolbutamide (Fig. 4). Perivagal capsaicin reduced gastric relaxation induced by tolbutamide by 81.2 ± 14.7% (30 mg/kg) (n = 4, P < 0.05). This suggests that the tolbutamide-induced gastric relaxation is mediated by the vagal afferent pathway.

Effects of tolbutamide are blocked by L-NAME. To determine if nitric oxide plays a role in mediating gastric relaxation induced by tolbutamide, we studied the effects of L-NAME. Nitric oxide has been shown to be a major mediator of gastric relaxation induced by vagal stimulation (22, 28). Administration of L-NAME at 10 mg/kg, which abolishes the rapid transient relaxation induced by electrical vagal stimulation (22), reduced gastric relaxation induced by tolbutamide (30 mg/kg) by 86.0 ± 13.9% (n = 4, P < 0.05) (Fig. 5). This suggests the involvement of a nonadrenergic, noncholinergic inhibitory vagal pathway.

Effects of electroporation of the nodose ganglia with siRNA of Kir6.2 on hyperglycemia-induced gastric relaxation. To provide direct evidence that inactivation of the nodose ganglia KATP channel induces gastric relaxation, we performed bilateral electroporation of the nodose ganglia with siRNA of Kir6.2 (a subunit of KATP) and plasmid pEGFP-N1 carrying the GFP gene. Immunocytochemistry of GFP performed 5 days after electroporation showed diffuse presence of GFP in nodose neurons of nodose ganglia transfected with either Kir6.2 siRNA or control siRNA. In nodose ganglia transfected with Kir6.2 siRNA, immunostaining signals for anti-Kir6.2 antibody were weak or absent, whereas, in nodose ganglia transfected with control siRNA, the anti-Kir6.2 antibody signal was intense (Fig. 6A). Kir6.2 gene expression was measured by RT-PCR and Western blot analysis. At 5 days after electroporation, Kir6.2 gene expression was reduced to 35% of control, and Kir6.2 protein level was decreased to 20% of control (Fig. 6, B and C), confirming that gene and protein expression of Kir6.2 in the nodose ganglia was successfully knocked down.

In rats with nodose ganglia treated with Kir6.2 siRNA, no gastric response to hyperglycemia (265 ± 25 mg/dl) or tolbutamide (30 mg/kg) was observed (Fig. 7). However, these rats responded to secretin (10 pmol/kg iv), which acts by way of the vagal afferent pathway, independently of KATP channels (21) (Figs. 7 and 8). Kir6.2 gene silencing of the nodose ganglia prevented gastric relaxation induced by hyperglycemia (265 ± 25 mg/dl) and tolbutamide (30 mg/kg) by 80.8 ± 9.3% (Fig. 8A) and 86.4 ± 16.3% (Fig. 8B), respectively.

These studies provided in vivo evidence that hyperglycemia induces gastric relaxation by way of the vagal afferent pathway, an action mediated by inactivation of the KATP channels in the nodose ganglia.

DISCUSSION

Our studies showed that iv infusion of 20% dextrose induced gastric relaxation in a dose-dependent manner. The inhibitory effect of hyperglycemia was blocked by diazoxide, a KATP channel activator. Conversely, administration of tolbutamide, a KATP channel inactivator, induced dose-dependent gastric relaxation, a similar effect to that induced by hyperglycemia. Furthermore, we showed that, in rats with nodose ganglia treated with Kir6.2 siRNA to silence KATP channel expression, gastric responses to hyperglycemia and tolbutamide were not observed. These observations strongly support our hypothesis that inhibition of gastric motility by hyperglycemia is mediated by KATP channels in the nodose ganglia. In glucose-excited neurons, increased glucose stimulates glucokinase-mediated ATP production, raises the ATP-to-ADP ratio, and inactivates the KATP channel, leading to membrane depolarization and neuronal firing (15). This, in turn, activates the vago-vagal pathways to mediate the gastric relaxation.

Glucose sensing to date has been considered to be the domain of the central nervous system. Glucose-sensing neurons in the hypothalamus generally are involved in the control of neuroendocrine function, nutrient metabolism, and energy homeostasis (20). However, this group of neurons is unlikely to play a major role in mediating digestive function in response to changes in circulating glucose levels, because changes in the glucose level in the cerebrospinal fluid range between only 10–30% of the serum blood glucose level and, therefore, may not be rapid or sensitive enough to reflect changes in peripheral glucose levels (20).
Several studies suggest that acute hyperglycemia affects a subpopulation of neurons in the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus (DMV) (10, 19, 22). Sakaguchi et al. (24) showed that injection of glucose into the DMV of anesthetized rats decreases gastric motility. Other investigators have shown that glucose administration into the NTS modulates gastric motor function (10). However, these studies failed to show that neurons of the NTS and the DMV are true primary sensors of peripheral glucose, thus rendering the physiological significance of the observations unclear. We have shown that hyperglycemia stimulates vagal afferents, which, in turn, activate vagal efferent cholinergic pathways synapsing with intragastric nitric oxide-containing neurons to mediate gastric relaxation (30). Furthermore, our laboratory identified the presence of glucose-excited and glucose-inhibited neurons in rat nodose ganglia (15). In glucose-excited neurons, the uptake and metabolism of glucose leads to closure of KATP channels, triggering membrane depolarization (15), whereas the inhibitory action of glucose-inhibited neurons is mediated by an ATP-independent, two-pore K⁺ channel, TRESK, that uses d-myo-inositol-1,4,5-trisphosphate signaling pathways (13–15). The present study is an extension of the above observations and is an attempt to show that the inhibition of gastric motility by hyperglycemia is mediated by KATP channels of the nodose ganglia.

The KATP channel is formed from Kir6.2 channel-forming units and the regulatory sulfonylurea receptor (8). Our laboratory has shown Kir6.2 protein and sulfonylurea receptor-1 protein are abundant in the rat nodose ganglia (15). KATP channels have been implicated as the underlying mechanism responsible for the glucose-sensing mechanism in pancreatic β-cells and in neurons of the ventromedial hypothalamus (20). Opening the KATP channel produces a K⁺ current that hyperpolarizes the cell and reduces electrical excitability. Conversely, KATP channel closure increases membrane excitability (26). In the present study, we investigated the effects of the

---

**Fig. 6.** Kir6.2 expression in nodose ganglion after electroporation with small interfering RNA (siRNA) targeting Kir6.2. A: green fluorescent protein (GFP) and Kir6.2 fluorescence images in a rat nodose ganglion 5 days after in vivo electroporation. Top: immunohistochemical staining for enhanced GFP (EGFP; green) and Kir6.2 (red) in a rat nodose ganglion injected with EGFP vector and control siRNA. Bottom: immunohistochemical staining of EGFP and Kir6.2 in a rat nodose ganglion injected with EGFP vector and siRNA targeting Kir6.2. Diffuse staining of EGFP in nodose ganglion neurons was observed in the nodose ganglion treated with control siRNA and in the nodose ganglion treated with Kir6.2 targeted siRNA, indicating successful transfection. Five days after electroporation, nodose ganglion neurons treated with Kir6.2 siRNA showed little staining for Kir6.2. In contrast, nodose ganglion neurons treated with control siRNA expressed both EGFP and Kir6.2 [yellow on merged image (A, top)]. Calibration bar = 100 μm. Success in knocking down Kir6.2 gene expression in the nodose ganglia was validated by RT-PCR (B) and Western blot analysis (C). GAPDH and actin were used as the loading controls. Treatment with siRNA targeting Kir6.2 resulted in a 65 and 80% reduction in Kir6.2 gene expression and protein level, respectively.

**Fig. 7.** Representative recordings showing absence of gastric responses to hyperglycemia and tolbutamide in rats treated with siRNA targeting Kir6.2. Representative recordings show that, in rats with nodose ganglia treated with Kir6.2 siRNA, no gastric response to hyperglycemia (274 mg/dl) or tolbutamide (30 mg/kg) was observed. However, these rats responded to secretin (10 pmol/kg iv), which acts by way of the vagal afferent pathway, independent of KATP channels.
The inhibitory effect of tolbutamide was also blocked by vagotomy, perivagal hyperglycemia. Similar to hyperglycemia, the effect of tolbutamide on gastric relaxation was abolished (26). Conversely, iv infusion of tolbutamide, which depolarizes the cell and reduces electrical excitability (30) of hyperglycemia on gastric motility. Diazoxide and tolbutamide were used as pharmacological activation and inactivation of KATP channels. One may argue that KATP channel modulators may act on a diverse group of cells containing KATP channels, not specifically on neurons in the nodose ganglia. However, we showed that the inhibitory action of the KATP channel activator tolbutamide was abolished by vagotomy and perivagal capsaicin treatment, whereas diazoxide, the KATP channel activator, blocked the relaxatory effect of hyperglycemia on gastric motility, which is mediated by the vagal afferent pathway (30).

To provide direct evidence that inhibition of gastric motility by hyperglycemia is mediated by nodose ganglia KATP channels, we silenced the gene expression of the KATP channels by local electroporation of siRNA of Kir6.2 (a subunit of KATP) and plasmid pEGFP-N1 carrying the GFP gene. Electroporation uses short, high-voltage pulses to overcome the barrier of the cell membrane (23). This transient permeabilization can be used to load cells with a variety of different molecules, such as drugs, tracers, RNA, and DNA. This approach is safe for animals and humans. We have successfully applied this technique to introduce siRNA into nodose ganglia cells (15) and to mediate gastric relaxation induced by hyperglycemia. We validated success in knocking down Kir6.2 gene expression by Western blot analysis and immunohistochemistry. In rats whose nodose ganglia were treated with Kir6.2 siRNA to silence the expression of KATP channels, no gastric motility response to hyperglycemia was observed (Figs. 7 and 8). Similarly, the gastric response in these rats to tolbutamide, which acts via modulation of the KATP channels, was also abolished (Figs. 7 and 8).

One critical issue related to electroporation is whether the electrical stimulation itself can induce significant changes in neuronal function or even neuronal death. In our experiments, we used the same electrodes that were used to deliver an identical stimulation intensity to brain slices for in vitro experiments (29). This type of stimulation did not cause any neuronal injury when delivered at either low or high frequency (1–100 Hz, for durations of a few seconds to 15 min) (29). Furthermore, in our preliminary studies, we performed terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) staining to label cells undergoing apoptosis. Double staining for GFP and TUNEL revealed no evidence of extensive cell death between the stimulation electrodes within the nodose ganglia. However, TUNEL-positive staining was detected in areas around the tracks of the stimulators, which serve as a positive control for TUNEL under these experimental conditions (data not shown). To show that the nodose ganglia retained functional integrity after electroporation, we showed that the stomachs of rats treated with KATP siRNA responded normally to secretin (10 pmol/kg iv), which acts via the vagal afferent pathway, independently of KATP channels (21) (Figs. 7 and 8).

We have shown that most neurons in the rat nodose ganglia contain KATP channels, but only ~26% exhibit glucose excitatory properties (15). This finding is similar to observations in the hypothalamus (7, 18), which suggests that other elements may contribute to regulating the sensitivity of the KATP channel to glucose stimulation. Among these, phosphatidylinositol bisphosphate (PIP2) has been shown to reduce the sensitivity of KATP channels to ATP (4, 16). Our laboratory has shown that depletion of intracellular PIP2 using wortmannin increases the fraction of glucose-excited neurons from 26 to 80%, suggesting that the intracellular PIP2 level affects the glucose-sensing ability of nodose ganglia neurons (15). This may explain variations in the gastric response among rats undergoing hyperglycemia challenges (25, 30).

In conclusion, using a pharmacological and gene deletion approach, we have provided direct evidence that inhibition of glucose-sensing neurons in the hypothalamus (7, 18) may contribute to regulating the sensitivity of the KATP channel to glucose stimulation.

![Gastric responses to hyperglycemia and tolbutamide in rats treated with siRNA targeting Kir6.2. A: in rats with nodose ganglia treated with electroporation of Kir6.2 siRNA, no gastric response to hyperglycemia (250 mg/dl) was observed. However, these rats responded to secretin (10 pmol/kg iv), which acts by way of the vagal afferent pathway, independent of KATP channels. *P < 0.05, compared with hyperglycemia alone. B: in rats with nodose ganglia treated with electroporation of siRNA targeting Kir6.2, there was little or no gastric response to tolbutamide (30 mg/kg). *P < 0.05, compared with tolbutamide alone. Values are means ± SE; n = 6.](Image)
gastric motility by hyperglycemia is mediated by KATP channels in the nodose ganglia. We showed that hyperglycemia induced by clamping blood glucose levels at 150, 200, 300, and 400 mg/dl dose-dependently inhibited gastric motility. These levels are frequently observed in poorly controlled diabetes. Similar observations were made in humans in whom hyperglycemia started to inhibit gastric motility at 140 mg/dl and abolished gastric contractions at 250 mg/dl (3). This observation has important clinical implications: hyperglycemia alone, in the absence of neuropathy or myopathy, can alter gastric motor functions. 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 symptoms, depending on serum glucose levels (11). The demonstration that KATP channels in the nodose ganglia play a critical role in the mediation of gastric relaxation induced by hyperglycemia may lead to the identification of therapeutic targets to prevent the gastric motor abnormality induced by hyperglycemia commonly observed in diabetes.

**GRANTS**

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants RO1 DK48419, RO1 DK58913, and P30 DK34933 and American Diabetes Association Awards I-06-JF-58 and I-09-IN-44.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**