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Mediation of Hyperglycemia-Evoked Gastric Slow-Wave Dysrhythmias by Endogenous Prostaglandins

WILLIAM L. HASLER, HANI C. SOUDAH, GARETH DULAI, and CHUNG OWYANG
Division of Gastroenterology, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan

Background/Aims: **Antral hypomotility and gastric dysrhythmias occur in diabetic gastroparesis. This study tested the hypothesis that acute hyperglycemia suppresses fed antral contractions and disrupts slow-wave rhythmicity via prostaglandin pathways.** ***Methods:*** **Six normal volunteers underwent electrogastrography and antroduodenal manometry under control, hyperglycemic clamp, and euglycemic, hyperinsulinemic clamp conditions before and after administration of indomethacin (50 mg orally three times daily for 3 days).** ***Results:*** **Hyperglycemic clamping to 230 mg/dL evoked a 4-fold increase in tachygastric activity and a 2.6-fold increase in arrhythmic activity ($P < 0.05$), whereas 140 and 175 mg/dL did not induce dysrhythmias. Antral motility indexes were reduced by 58% ± 14% at 175 mg/dL and 70% ± 8% at 230 mg/dL after a 750-kcal meal. Euglycemic, hyperinsulinemic clamping to insulin levels observed with the highest glucose infusions did not produce tachyarrhythmias or hypomotility. After indomethacin, hyperglycemic clamping to 230 mg/dL did not induce tachyarrhythmias. In contrast, indomethacin did not prevent the reduction in motility evoked by hyperglycemic clamping.** ***Conclusions:*** **Acute hyperglycemia, but not hyperinsulinemia, inhibits fed antral motility and induces gastric dysrhythmias at higher plasma glucose levels. Induction of dysrhythmias, but not hypomotility, is dependent on endogenous prostaglandin synthesis. These findings offer insight into the myoelectric disturbances of diabetic gastroparesis and suggest a possible therapeutic role for prostaglandin synthesis inhibitors for gastric dysrhythmias in this condition.**

Gastroparesis is a serious complication of diabetes mellitus that produces significant morbidity from nausea, vomiting, dehydration, and electrolyte disturbances.[1] Characteristic motor and electrical disturbances have been described in diabetic gastroparesis. Generally, delays in solid and/or liquid phase gastric emptying are observed in association with reduced postprandial antral motor activity and impaired cycling of the migrating motor complex, a fasting pattern believed to be responsible for clearance of undigested debris from the stomach.[2] Motor activity in the stomach is regulated by electrical pacemaker activity known as the slow wave. Abnormally high (tachygastric) or low (bradygastric) gastric slow-wave frequencies have been observed in diabetic gastroparesis and are associated with impaired antral motor activity.[3,4]

The pathophysiology of diabetic gastroparesis is poorly understood. It is known that induction of acute hyperglycemia in healthy volunteers can delay emptying of a meal from the stomach.[5,6] Furthermore, our laboratory has shown inhibition of normal migrating motor complex cycling in the stomach at plasma glucose levels as low as 140 mg/dL in healthy volunteers, indicating that some of the motor abnormalities observed in diabetic gastroparesis may be caused by elevations in plasma glucose levels irrespective of the concomitant presence of a visceral neuropathy or myopathy.[7] It is unknown what magnitude of increase in plasma glucose levels is required to blunt the antral motor response to a meal or if slow-wave disruption occurs at the same glucose levels as antral hypomotility. Furthermore, the role of secondary hyperinsulinemia in response to the glucose infusions in the mediation of motor and electrical disturbances in the stomach has not been studied. Finally, certain experimentally induced dysrhythmias in dogs are reversed by indomethacin treatment, suggesting mediation of slow-wave disruption by endogenous prostaglandins.[8] The role of endogenous prostaglandin synthesis in the induction of gastric dysrhythmias by hyperglycemia is unexplored.

The aims of this study were to determine if hyperglycemia produces motor and electrical disturbances similar to those observed postprandially in diabetic gastroparesis and to evaluate possible pathways involved in their mediation. Cutaneous electrogastrography (EGG) and antroduodenal manometry were performed in healthy volunteers at different plasma glucose levels to assess if the thresholds for induction of hypomotility and slow-wave disruption are the same, thus determining if gastric dysrhythmias are required for motor impairment. Euglycemic, hyperinsulinemic clamp studies showed if the disturbances observed were secondary to hyperglycemia per

Abbreviations used in this paper: EGG, electrogastrography.

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se or to insulin release evoked by elevations in plasma glucose levels. Finally, hyperglycemic clamp studies were repeated after administration of oral indomethacin to evaluate if endogenous prostaglandin pathways were involved in hyperglycemic induction of hypomotility or slow-wave dysrhythmias.

Materials and Methods

Subject Population

Six healthy volunteers (three men and three women), 20–27 years old, were recruited for cutaneous EGG and antroduodenal manometry. None of the volunteers were diabetic, none had a history of gastrointestinal symptoms or prior gastrointestinal surgery, and none were taking medications known to alter gastrointestinal motility or electrical activity. The studies were approved by the University of Michigan Human Use Committee, and each subject gave written informed consent before participation.

Cutaneous EGG

Cutaneous EGG was performed according to the method of Stern et al.[9] Subjects were placed in a semirecumbent position in a quiet, warm room without visual or auditory distractions. After gentle skin abrasion to enhance electrical conduction, four Ag-AgCl electrodes (Accutac Diaphoretic ECG Electrodes; NDM, Dayton, OH) were affixed to the abdomen. The first electrode was placed in the midclavicular line below the left costal margin. The third electrode was placed midway between the xiphoid and umbilicus. The second electrode was placed equidistant between the first and third electrodes. A fourth reference electrode was affixed in the right upper quadrant of the abdomen. Electrodes were connected via direct nystagmus couplers (model 9859; SensorMedic Corp., Anaheim, CA) to a chart recorder for continuous display of the slow wave activity. Time constants were set at 10 seconds and high frequency cutoffs at 0.3 Hz to minimize interference from nongastric signals. Respirations were monitored by a belt pneumograph connected to an indirect blood pressure coupler (model 9863B; SensorMedic Corp.) on the chart recorder, and any signals showing clear respiratory artifact were excluded from analysis. The chart recorder was interfaced with a personal computer (4DX2-66V; Gateway 2000, North Souix City, ND) via an analog-to-digital converter (DAS-16; Metrabyte Corp., Taunton, MA).

The three channels of EGG recording were initially analyzed visually to determine which lead provided the signal most free of noise. This lead was then subjected to quantitative computer analysis. All tracings were analyzed in blinded fashion so that the investigator did not know either the volunteer or the test conditions being studied. Signals were digitized at 4 Hz by the analog-to-digital converter and filtered above 15 cpm and below 0.5 cpm to remove high- and low-frequency noise. Fast Fourier transformation was performed on 4-minute segments of recording using commercially available software (Fourier Perspective III; Alligator Technologies, Fountain Valley, CA). Running power spectral analyses were calculated across the frequency range from 0.5 to 10 cpm in 2-minute intervals in overlapping fashion. Each line in the running power spectral analysis plot represented the amplitude of the signal at the different frequencies. Data from the power spectral analyses were imported in spreadsheet format to commercially available software (Lotus 1-2-3, Release 2; Lotus Development Corp., Cambridge, MA), in which the dominant frequency was determined in the postprandial period by assessment of the largest signal amplitude. If the maximal signal amplitude for a given 4-minute recording segment occurred at a frequency of ≥ 2 and ≤ 4.5 cpm, the dominant frequency was defined as within the normal range. If the maximal signal amplitude was present at a frequency of >4.5 and ≤ 9 cpm, the dominant frequency for that recording segment was defined as tachygastria. A dominant frequency of >0.5 and <2 cpm was defined as bradygastria. Data were expressed as the percentage of time that the dominant frequency was in either the tachygastric or bradygastric frequency range in the postprandial period. To provide a quantitative measure of slow-wave arrhythmic activity, a frequency instability index (cpm) was calculated from the standard deviation of the dominant frequencies in all of the 4-minute recording segments during the fed period.

Gastroduodenal Manometry

Manometric evaluation of antral and duodenal motor activity was performed concurrently with cutaneous EGG. After an overnight fast, a water-perfused eight-lumen polyvinyl manometric catheter (Arndorfer Medical Specialities, Greendale, WI) was placed in the small intestine perorally under fluoroscopic guidance in all volunteers so that six pressure ports spanned the distal antrum and two were positioned in the duodenum. The catheter was connected by means of a pneumohydraulic water-perfusion apparatus (perfusion rate, 0.25 mL/min; Arndorfer Medical Specialities) to force transducers (model P23xL; Gould, Oxnard, CA) that relayed information to a chart recorder (model R611; Beckman Instruments, Schiller Park, IL) for continuous monitoring of motor activity.

Basal motor activity was recorded in the healthy volunteers until completion of one complete cycle of the MMC had progressed through the distal duodenal site. For control studies, a 750-kcal mixed solid-liquid meal was ingested during phase I of the migrating motor complex, the period of motor quiescence. For hyperglycemic and hyperinsulinemic clamp studies (see later), intravenous infusions were begun during phase I. After maintenance of a selected blood glucose for 2 hours, the 750-kcal mixed meal was given, and hyperglycemic or hyperinsulinemic clamping was continued in the postprandial period. For all studies, postprandial motor activity was measured for 45 minutes after completion of the meal.

Antral motility indexes in the postprandial period were determined by calculating the areas under the pressure curves with a Bit Pad Two digitizer (Summagraphics, Fairfield, CT) connected to a personal computer with the program Easydij (version 3.0; Geocomp, Golden, CO). The manometric re-

cording was placed on the digitizer tablet and traced with an electric stylus to transfer the digitized signal to the computer. The pressure-time relations for the fed motor complexes were integrated to calculate areas under the pressure curves. Motility indexes were expressed as millimeters of mercury multiplied by minutes of recording.

Test Conditions

Each volunteer underwent EGG and manometric recording under six separate test conditions on different days. On each day, venous blood samples were withdrawn for determination of plasma glucose and insulin levels before and during the studies.

For control studies, two venous blood samples were obtained before meal ingestion and were analyzed for plasma glucose and insulin levels. The mean of these results represented the fasting glucose and insulin levels. Venous blood was removed and analyzed 15, 30, and 45 minutes after completion of the meal. The mean results from these samples represented the postprandial glucose and insulin levels.

Hyperglycemic clamping studies were performed according to the method of DeFronzo et al. to fix plasma glucose at 140, 175, and 230 mg/dL on separate days.[10] An intravenous line was inserted into the left antecubital vein for glucose infusion. A 15-minute priming dose of 20% dextrose was given, and the maintenance infusion rates were adjusted as needed by monitoring plasma glucose levels at 5-minute intervals throughout the study, following previously described methods.[10] Using this technique, the plasma glucose level was maintained within ±10% of the desired value. Venous blood was withdrawn for insulin determination before and 15, 30, and 45 minutes after completion of the mixed meal.

Euglycemic, hyperinsulinemic clamp studies were performed according to the method of De Fronzo et al.[10] Insulin infusates were prepared to a concentration of 300 mU/mL (regular human insulin, Novolin R; Novo Nordisk Pharmaceuticals Inc., Princeton, NJ) in 50 mL 0.9% NaCl to which 2 mL of the volunteers' blood was added to prevent adsorption of the insulin to the infusion tubing. A 10-minute priming infusion through a separate intravenous line in the left forearm was followed by a constant insulin infusion for 2 hours before meal ingestion and for 45 minutes thereafter.[10] For this study, it was desired that plasma insulin levels obtained during euglycemic, hyperinsulinemic clamping mimic those observed during hyperglycemic clamping to a plasma glucose of 230 mg/dL. Preliminary studies determined that the appropriate infusion rate was 80 mU/m^2 surface area per minute. Infusion of 20% dextrose was started at 2.0 $mg \cdot kg^{-1} \cdot min^{-1}$ beginning 4 minutes after initiation of the insulin infusion. Plasma glucose level was measured every 5 minutes, and the dextrose infusion rate was adjusted to maintain plasma glucose between 60 and 100 mg/dL. Samples for plasma insulin level determination were withdrawn as described above.

To test the hypothesis that endogenous prostaglandin pathways mediate the EEG and motor abnormalities observed with hyperglycemia, hyperglycemic clamping studies to 230 mg/dL were repeated after treatment with the cyclooxygenase inhibitor indomethacin (Geneva Generics Inc., Broomfield, CO). This medication, administered orally (50 mg three times daily for 3 days before the study and once before placement of the manometry catheter), has been shown to effectively inhibit prostaglandin synthesis in the stomach and elsewhere.[11,12]

Plasma Glucose and Insulin Level Determination

For withdrawal of venous blood samples, an intravenous line was placed in the right forearm in all volunteers. Patency of the line was maintained with periodic infusions of heparin flush-lock solution (100 USP units per milliliter). Plasma glucose levels were determined within 60 seconds using a portable glucose analyzer (One Touch II; Lifescan Inc., Milpitas, CA). Venous blood samples for determining insulin levels were immediately centrifuged, and the plasma was frozen and stored at −20°C. Plasma insulin levels were measured by a specific radioimmunoassay using ^{125}I–porcine insulin (Eli Lilly & Co., Indianapolis, IN) as standard using previously described methods.[13] Guinea pig porcine insulin antiserum was used in a dilution of 1:75,000. One hundred microliters of plasma was added to 100 μL of ^{125}I–porcine insulin (4 μU/100 μL) and vortexed. Then 100 μL of insulin antiserum was added, and the solutions were vortexed and incubated for 24 hours at 4°C. Goat anti–guinea pig serum, 200 μL, was added, and the solutions were incubated for 2 hours at 4°C. After adding 1 mL water, samples were centrifuged at 3000 rpm for 20 minutes, and the pellets were counted in a gamma counter. The sensitivity of the assay was 0.15 μU/tube, and the intra-assay and interassay coefficients of variation were 3.2% and 5%, respectively.[13]

Statistical Analysis

All results are expressed as means ± SEM. Dunnett's test was used to compare hyperglycemic clamping treatment means with control means and to compare euglycemic, hyperinsulinemic clamping and hyperglycemic clamping with indomethacin means with hyperglycemic clamping (230 mg/dL) means. Repeated measures analysis of variance was used to obtain the mean square error used in Dunnett's analysis. Data were transformed as necessary to stabilize the variances. Dunnett's procedure preserves the overall type I error rate at 0.05 within a given analysis. Throughout the text and in the figures, where indicated, a P value of <0.05 indicates a statistically significant difference per Dunnett's test. Because of an expected ordering of the treatment effects, all hypothesis testing was one tailed.

Results

Plasma Glucose and Insulin Levels

Measurements of plasma glucose and insulin levels are shown in Tables 1 and 2. As expected, ingestion of a meal under control conditions evoked increases in both plasma glucose and insulin levels. Hyperglycemic clamp-

Table 1. Plasma Glucose Levels in Healthy Volunteers Under Different Test Conditions

Test condition	Plasma glucose (*mg/dL*)	
	Fasting	Postprandial
Control	85 ± 2	115 ± 8
Hyperglycemic clamping	138 ± 2	136 ± 4
	179 ± 4	175 ± 4
	230 ± 5	229 ± 7
Euglycemic, hyperinsulinemic clamping	83 ± 3	86 ± 6
Hyperglycemic clamping with indomethacin pretreatment	237 ± 6	235 ± 6

ing was performed to within 10% of the desired levels of 140, 175, and 230 mg/dL. This resulted in glucose-dependent increases in plasma insulin concentration. Euglycemic, hyperinsulinemic clamp experiments were designed to reproduce plasma insulin levels observed with hyperglycemic clamping to 230 mg/dL but with maintenance of blood glucose between 60 and 100 mg/dL. Repeat performance of hyperglycemic clamping to 230 mg/dL after 3 days of indomethacin (50 mg three times daily) did not affect plasma insulin responses, indicating that any myoelectric or motor effect of inhibition of prostaglandin synthesis was not caused by alterations in the insulin release pattern.

Hyperglycemic Clamping Studies

Cutaneous EGG. Results of cutaneous EGG were analyzed in the 45-minute postprandial period for the presence of rhythms that deviated from the normal 3-cpm constant frequency. Tachygastria and bradygastria were expressed as the fraction of time in which dominant slow-wave frequencies of >4.5 or <2 cpm, respectively, were detected. A frequency instability index, determined by measuring the standard deviation of the dominant frequencies for all the 4-minute recording segments in the postprandial period, was devised to quantitate the magnitude of slow-wave arrhythmias.

Table 2. Plasma Insulin Levels in Healthy Volunteers Under Different Test Conditions

Test condition	Plasma insulin (*μU/mL*)	
	Fasting	Postprandial
Control	14 ± 2	46 ± 7
Hyperglycemic clamping		
140 mg/dL	20 ± 3	62 ± 10
175 mg/dL	55 ± 5	143 ± 17
230 mg/dL	79 ± 19	228 ± 57
Euglycemic, hyperinsulinemic clamping	133 ± 11	195 ± 29
Hyperglycemic clamping with indomethacin pretreatment	83 ± 17	256 ± 75

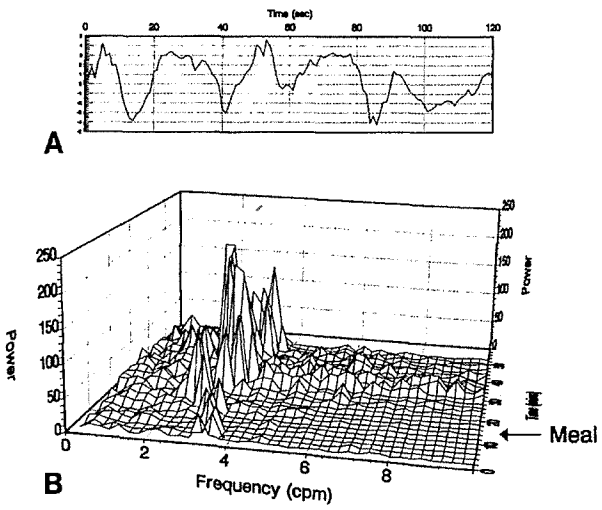

Figure 1. Sample EGG results from a healthy volunteer under control conditions. (*A*) The raw slow-wave signal shows a rhythmic, high-amplitude oscillation with a period of approximately 20 seconds. (*B*) Power spectral analysis shows the frequency distribution of the slow-wave signal as a function of time. Under control conditions, the slow wave exhibits a dominant frequency of approximately 3 cpm throughout the recording, which increases in amplitude after meal ingestion (*arrow*) at 15 minutes.

Under control conditions, the predominant rhythm was that of a 3-cpm waveform. Figure 1 shows a sample raw waveform and power spectral analysis plot of the data from a healthy volunteer before and 45 minutes after a standard test meal. When the results from all volunteers were quantitated, dominant tachygastric frequencies were detected 6% ± 3% of the time during the 45 minutes after a meal (Figure 2). It is likely that this small amount of high-frequency activity represents noise from the cutaneously acquired signal and does not represent true tachygastric activity. Hyperglycemic clamping to 140 and 175 mg/dL did not increase this tachygastric activity (9% ± 5% and 10% ± 4%, respectively), suggesting that moderate increases in plasma glucose levels do not increase slow-wave frequencies. However, hyperglycemic clamping to 230 mg/dL significantly increased the tachygastric activity in all volunteers compared with controls. Figure 3 shows a run of tachygastria on the raw waveform and a predominance of signal frequencies in excess of 4.5 cpm on the power spectral analysis plot. Quantitation of the data from all six volunteers showed tachygastric activity in 24% ± 5% ($P < 0.05$) of the recording time, indicating disruption of normal slow-wave cycling at higher plasma glucose levels.

Under control conditions, dominant bradygastric frequencies were observed 11% ± 2% of the time, again most likely caused by signal noise. In contrast to its

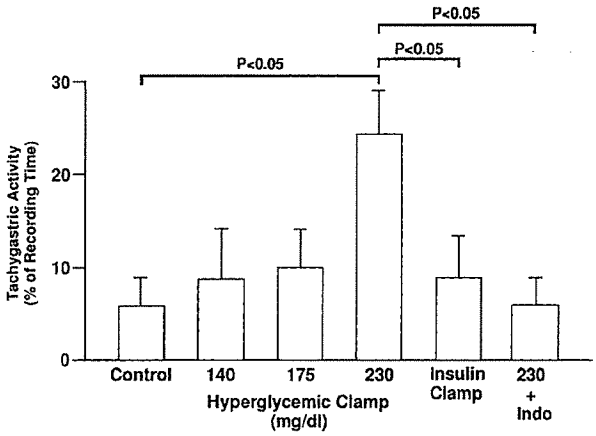

Figure 2. Tachygastric activity as a percent of recording time in the postprandial period. Hyperglycemic clamping leads to a significant increase in tachygastria only with plasma glucose levels of 230 mg/dL. In contrast, euglycemic, hyperinsulinemic clamping to insulin levels that reproduce those observed at the highest plasma glucose levels do not disrupt slow-wave frequencies. Pretreatment with indomethacin (Indo) prevents induction of tachygastria by hyperglycemic clamping to 230 mg/dL. All results are mean ± SEM; n = 6.

effects on high-frequency slow-wave activity, hyperglycemic clamping did not induce appreciable bradygastric activity at plasma glucose levels of 140 mg/dL (14% ± 4%), 175 mg/dL (13% ± 2%), or 230 mg/dL (17% ± 7%), suggesting that acute hyperglycemia does not decelerate the dominant gastric pacemaker (Figure 4).

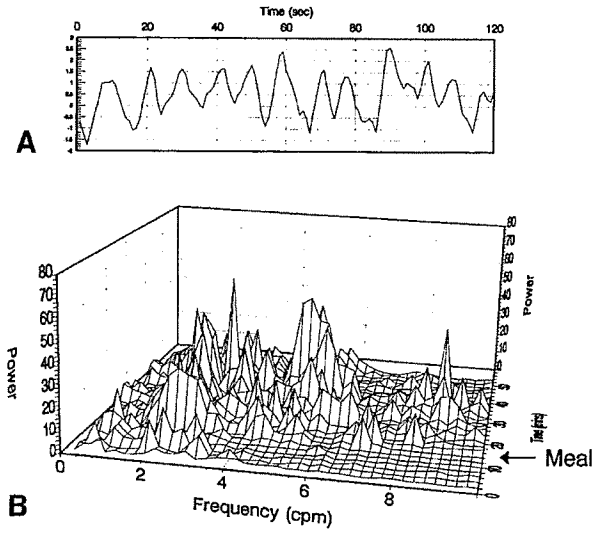

Figure 3. Sample EGG results from a healthy volunteer undergoing hyperglycemic clamping to 230 mg/dL. (*A*) The raw slow-wave signal is rapid, irregular, and of low amplitude with a period of approximately 10 seconds. (*B*) Power spectral analysis shows the absence of a single dominant frequency throughout the fasting and postprandial (*arrow*) periods. A significant increase in signal activity in the tachygastric range (4.5–9 cpm) is observed.

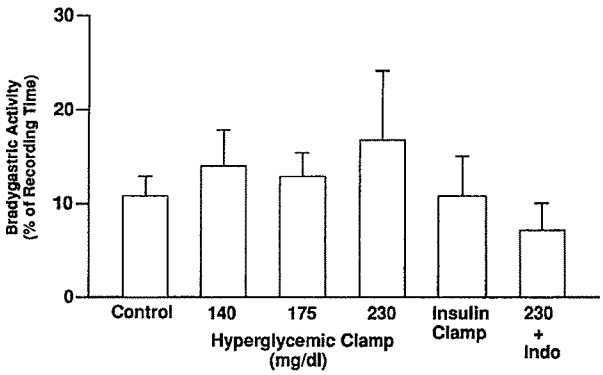

Figure 4. Bradygastric activity as a percentage of recording time in the postprandial period. In contrast to its effects on tachygastric activity, hyperglycemic clamping did not induce bradygastria. Similarly, euglycemic, hyperinsulinemic clamping studies and hyperglycemic clamping studies after indomethacin did not induce bradygastric activity different from control. All results are mean ± SEM; n = 6.

The frequency instability index, or standard deviation of the dominant slow-wave frequencies, was 0.5 ± 0.1 cpm under control experiments, indicating that the postprandial slow wave deviated little from a stable 3-cpm pattern (Figure 5). Similarly, hyperglycemic clamping to 140 and 175 mg/dL did not increase arrhythmic slow-wave activity (0.7 ± 0.2 and 0.7 ± 0.1 cpm, respectively). However, hyperglycemic clamping to the highest plasma glucose levels (230 mg/dL) evoked increased arrhythmic activity compared with control (1.3 ± 0.3 cpm; $P < 0.05$). These results indicate that short-term induc-

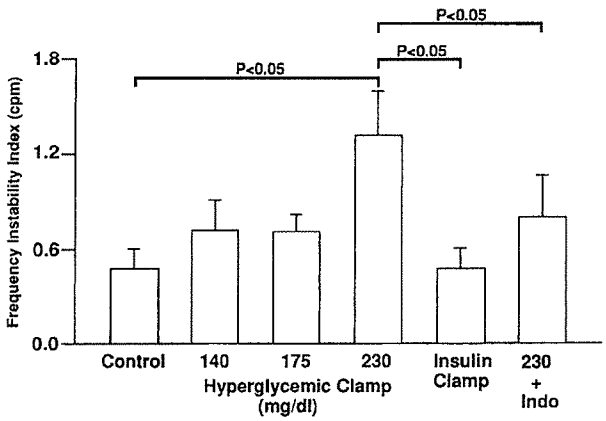

Figure 5. Slow-wave arrhythmic activity was quantitated by the frequency instability index. Hyperglycemic clamping to 230 mg/dL resulted in more than a twofold increase in gastric arrhythmias compared with control. Euglycemic, hyperinsulinemic clamping produced arrhythmic activity that was not different from control and was significantly less than hyperglycemic clamping to 230 mg/dL. Indomethacin pretreatment prevented the induction of slow-wave arrhythmias by hyperglycemic clamping to 230 mg/dL. All results are mean ± SEM; n = 6.

tion of high levels of hyperglycemia evokes gastric slow-wave tachyarrhythmias.

Gastroduodenal manometry. It is known that the gastric slow wave regulates the maximal frequency and direction of propagation of antral contractions under both fasting and fed conditions.[14] Thus, it would be expected that experimental conditions that result in slow-wave disruption also impair normal phasic antral motor activity. We examined whether this was indeed the case and further assessed if slow-wave disturbances were necessary for inhibition of antral motility by measuring the motor response after a meal across a broad range of plasma glucose levels.

Under control conditions, healthy volunteers had a reproducible antral phasic motor response beginning soon after meal ingestion and persisting for the entire postprandial recording period (Figure 6). Measurement of the areas under the pressure curves yielded a motility index of 3.1 ± 0.5 mm Hg × min (Figure 7). Hyperglycemic clamping to 140 mg/dL did not inhibit phasic motility (motility index, 3.7 ± 1.0 mm Hg × min). However, hyperglycemic clamping to 175 mg/dL reduced the antral motility index by 58% to 1.3 ± 0.4 mm Hg × min ($P < 0.05$ compared with control). Hyperglycemic clamping to 230 mg/dL further reduced phasic motor activity to 0.9 ± 0.2 mm Hg × min. Figure 6 shows a sample tracing of postprandial antral motor activity from a hyperglycemic clamp experiment to 230 mg/dL. These results indicate that moderate levels of acute hyperglycemia (<230 mg/dL) are capable of inhibiting the fed antral motor pattern and that this inhibition does not require the presence of gastric slow-wave dysrhythmias. At higher plasma glucose levels (>230 mg/dL), where slow-wave disruption is observed, there is a further reduction of antral motility suggesting a potential further inhibitory effect resulting from the dysrhythmic electrical activity.

Euglycemic, Hyperinsulinemic Clamping Studies

Cutaneous EGG. Induction of acute hyperglycemia in healthy volunteers evokes a significant release of insulin into the bloodstream.[10] It is possible that the disruptive effects of hyperglycemic clamping to 230 mg/dL on slow-wave activity result either from the elevated blood glucose level itself or from the secondary release of insulin. To rule out the possibility that gastric dysrhythmias are evoked by hyperinsulinemia, euglycemic, hyperinsulinemic clamping studies were performed. Insulin was infused to reproduce levels observed with hyperinsulinemic clamping to 230 mg/dL (Table 1). Intra-

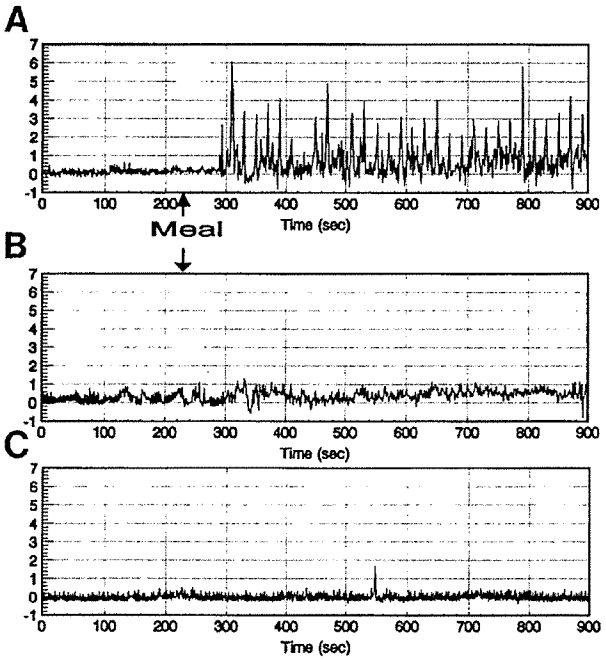

Figure 6. Sample antral manometry tracings from a healthy volunteer are plotted under (*A*) control conditions and during hyperglycemic clamping to 230 mg/dL (*B*) without and (*C*) with indomethacin pretreatment. (*A*) Under control conditions, meal ingestion results in a prompt induction of phasic contractions of irregular amplitude with a maximal frequency of 3 cpm, which persists for the 45-minute postprandial recording period. (*B*) With hyperglycemic clamping, phasic postprandial antral motor activity is markedly reduced. (*C*) In contrast to the EGG results, indomethacin pretreatment did not prevent the inhibition of fed antral motility by acute hyperglycemia.

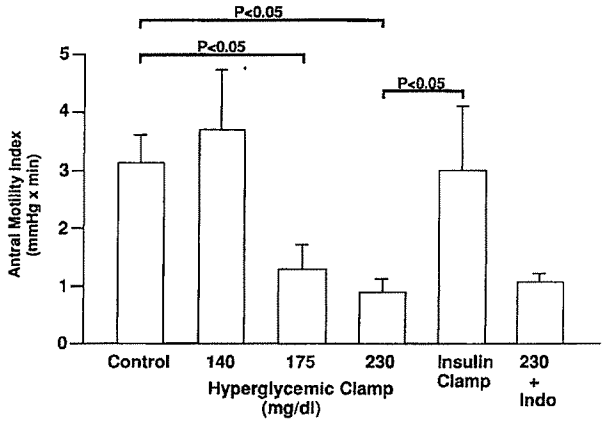

Figure 7. Antral motility indices were calculated by measuring the areas under the pressure curves. Hyperglycemic clamping to 140 mg/dL did not inhibit postprandial antral motor activity. However, the threshold for motor inhibition was lower than for slow-wave disruption with significant decreases in antral motility index noted at plasma glucoses of both 175 and 230 mg/dL. Euglycemic, hyperinsulinemic clamping did not inhibit antral motor activity. In contrast to the EGG results, indomethacin pretreatment had no effect on the postprandial antral hypomotility evoked by acute hyperglycemia to 230 mg/dL. All results are mean ± SEM; n = 6.

venous 20% dextrose was simultaneously administered to maintain plasma glucose level between 60 and 100 mg/dL. Under these test conditions, tachygastric activity was observed 9% ± 4% and bradygastric activity was observed 11% ± 4% of the recording time (Figures 2 and 4). The frequency instability index with euglycemic, hyperinsulinemic clamping was 0.5 ± 0.1 cpm (Figure 5). These values are not different from control conditions and are significantly less than observed with hyperglycemic clamping to 230 mg/dL ($P < 0.05$). Thus, the gastric slow-wave rhythm disturbances observed with marked hyperglycemia are not secondary to endogenous release of insulin and are most likely caused by the elevated plasma glucose level itself.

Gastroduodenal manometry. Postprandial antral motility indexes were also measured under conditions of euglycemic, hyperinsulinemic clamping to assess if the hypomotility observed with hyperglycemia is caused by secondary hyperinsulinemia. Euglycemic, hyperinsulinemic clamping did not inhibit the phasic antral motor response after a meal, and the fed antral motility index was not significantly different from control conditions (3.0 ± 1.1 mm Hg × min) (Figure 7). However, the fed motility index was significantly greater during euglycemic, hyperinsulinemic clamping than during hyperglycemic clamping to 230 mg/dL ($P < 0.05$). Thus, as with the electrogastrographic results, inhibition of fed antral motor activity by acute hyperglycemia is not mediated by secondary insulin release.

Role of Endogenous Prostaglandins

Cutaneous EGG. To test the hypothesis that hyperglycemia-evoked slow-wave dysrhythmias are mediated by endogenous prostaglandin pathways, hyperglycemic clamp experiments to 230 mg/dL were repeated in healthy volunteers after 3-day pretreatment with the cyclooxygenase inhibitor indomethacin, 50 mg three times daily. In contrast to the results observed with hyperglycemic clamping alone, hyperglycemic clamping after indomethacin did not disrupt the normal 3-cpm slow-wave rhythm, as shown in the EGG tracing and spectal analysis plot in Figure 8. Quantitative analysis of the data showed no increase in tachygastric (6% ± 3%) or bradygastric (7% ± 4%) activity compared with control conditions (Figures 2 and 4). Similarly, there was no increase in the frequency instability index (0.8 ± 0.3 cpm) (Figure 5). These values are significantly less than observed with hyperglycemic clamping to 230 mg/dL ($P < 0.05$), indicating that inhibition of prostaglandin synthesis prevents hyperglycemic induction of gastric slow-wave dysrhythmias.

Gastroduodenal manometry. Because it was

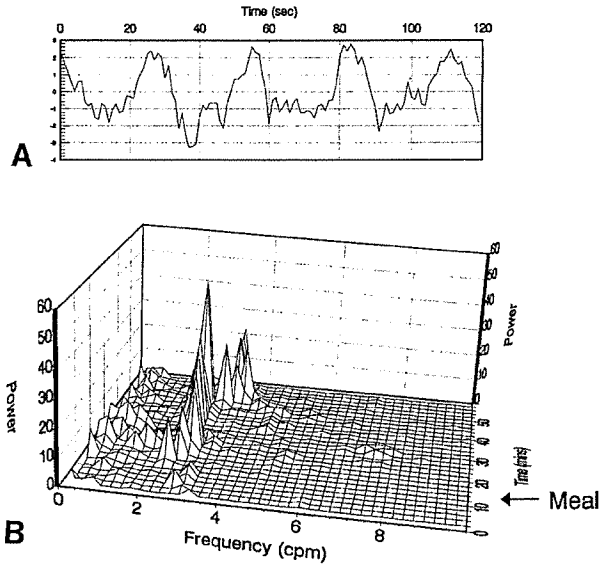

Figure 8. Sample EGG results from a healthy volunteer undergoing hyperglycemic clamping to 230 mg/dL after indomethacin pretreatment. (*A*) As under control conditions, the raw slow wave is intense and rhythmic with a period of approximately 20 seconds. (*B*) Power spectral analysis shows a stable dominant slow-wave frequency between 2 and 4 cpm that increases appropriately after meal ingestion (*arrow*) at 15 minutes.

shown that endogenous prostaglandins mediate gastric electrical disruption induced by hyperglycemia, we questioned if prostaglandin pathways also regulate the hypomotility observed with intravenous infusion of glucose. Hyperglycemic clamping to 230 mg/dL was repeated after 3 days of indomethacin, and antral motility indexes were calculated after a standard test meal. In contrast to the results observed with cutaneous EGG, indomethacin did not reverse the postprandial reduction in antral motor activity evoked by marked hyperglycemia, as shown in a representative tracing (Figure 6). After indomethacin, the fed antral motility index remained suppressed to 35% of control (1.1 ± 0.1 mm Hg × min) (Figure 7) and was not different from hyperglycemic clamping to 230 mg/dL, suggesting that, in contrast to the prostaglandin dependence of hyperglycemia-evoked dysrhythmias, antral hypomotility during marked hyperglycemia does not require production of endogenous prostaglandins.

Discussion

Gastroparesis is a troubling complication that occurs in patients with long-standing diabetes mellitus. Although the relationships of motor abnormalities in these individuals to symptoms and underlying consequences of diabetes, including retinopathy, nephropathy, and peripheral neuropathy, are not always clear-cut, most investigators believe that abnormal gastric emptying

complicates diabetes (usually type I) of more than 10 years duration with superimposed peripheral and/or autonomic neuropathy.[15,16] The pathogenesis of gastroparesis remains obscure. Abnormalities of vagal function have been proposed; however, the patterns of liquid emptying in diabetic gastroparesis are different from those observed after vagotomy.[17] Furthermore, a substantial fraction of patients with diabetic gastroparesis have no demonstrable anatomic abnormalities on histological examination of the vagus.[18] Using animal models of diabetes, investigators have shown alterations in staining patterns for various neuropeptides and defective neurotransmitter release profiles from myenteric plexus preparations, indicating possible enteric neural dysfunction.[19] Finally, although the available evidence is not as strong, studies of rats and mice suggest that there may be impaired contractility at the smooth muscle cells level and impaired neural function.[20]

In addition to neural and myogenic factors, elevated plasma glucose levels can contribute to gastric motor defects in healthy volunteers and in patients with diabetic gastroparesis. Gastric emptying is delayed in healthy volunteers undergoing hyperglycemic clamping experiments.[5,6] A similar finding was noted by Horowitz in type I and type II diabetics, who observed a strong correlation of delays in gastric emptying to plasma glucose levels above 270 mg/dL.[21,22] In correlative manometric studies, Barnett and Owyang have further shown loss of fasting migrating motor complex activity in healthy volunteers with plasma glucose levels as low as 140 mg/dL.[7] Although fasting motor complexes are responsible for the clearance of undigested debris from the stomach, phasic antral contractions, which initiate soon after nutrient ingestion, are responsible for the emptying of a solid meal.[14] The effects of acute hyperglycemia on postprandial antral motor activity had not been previously evaluated.

The phasic motility of the distal stomach is regulated by a rhythmic electrical depolarization, known as the pacesetter potential or gastric slow wave, which is generated in the proximal gastric body.[14] Contractions occur only during the plateau phase of the slow wave; thus, the slow wave controls the maximal frequency and direction of antral motor activity. Under normal conditions, the slow wave oscillates at 3 cpm; however, certain rhythm disturbances have been described in clinical disease in which the rhythm is too fast (tachygastria), too slow (bradygastria), or too irregular (arrhythmia).[9] With bradygastria, the contractile efficiency of the stomach is reduced because of a decrease in the maximal number of antral contractions. Tachygastria develops when an ectopic pacemaker, usually in the antrum, generates a high-frequency depolarization that overdrives the rest of the distal stomach. Although retrograde depolarizations propagate at a high frequency with tachygastria, retrograde motor activity rarely develops because the electrical activity is of insufficient amplitude to induce contraction. Thus, in most instances, tachygastria is associated with gastric atony. A number of clinical conditions have associated gastric dysrhythmias, including the nausea of pregnancy, motion sickness, and anorexia nervosa.[23–25] Tachygastria and bradygastria are found in many patients with severe diabetic gastroparesis. In one study, 9 of 10 diabetic patients with gastroparesis had runs of tachygastria compared with only 1 individual from a comparable control population.[3] In another study of 6 diabetic patients with delayed gastric emptying, tachygastria with a dominant frequency of 4–9 cpm was observed in 1 patient, whereas the other 5 had either bradygastria or no detectable slow-wave activity at all.[4] The pathogenesis of these dysrhythmias in diabetics with gastroparesis is not well understood.

The present study shows gastric motor and electrical abnormalities in healthy volunteers in response to elevated plasma glucose levels that mimic those observed in diabetic gastroparesis. Using hyperglycemic clamping to sequentially increasing plasma glucose levels, we documented a threshold of approximately 175 mg/dL for inhibition of postprandial phasic antral motor activity, the manometric finding that correlates most strongly with emptying of solid foods. With additional increases in plasma glucose levels to 230 mg/dL, impressive disruption of gastric slow-wave cycling was observed, with marked increases in both tachygastric and arrhythmic activity. The importance of these observations is twofold. Firstly, acute hyperglycemia in healthy volunteers produces postcibal motor dysfunction in the healthy stomach mimicking that seen in diabetic gastroparesis, indicating that underlying neuropathy or myopathy is not necessary for impairment of gastric function. Secondly, although more impressive hypomotility was observed at 230 mg/dL, the presence of significant reductions in phasic motor activity at 175 mg/dL in the absence of slow-wave disruption indicates that motor dysfunction does not require simultaneous disturbance of the electrical pacemaker activity of the stomach. This suggests that lower levels of hyperglycemia may have inhibitory effects on the spike potentials that induce antral contractions, whereas higher glucose concentrations disrupt the pacemaker that controls their frequency and directionality, additionally exacerbating the hyperglycemia-evoked antral hypomotility. This possibility cannot be addressed using the technique of EGG but requires use of serosal electrodes that can distinguish slow wave electrical activity from

spike potentials. In addition, as shown by Koch et al., it is possible that dysrhythmias are symptomatically important in diabetic gastroparesis because correction of the slow wave disturbance, but not the gastric emptying defect, correlates best with reduction in nausea in these patients.[4]

The mechanisms by which acute hyperglycemia inhibits gastric motor activity and disrupts slow-wave cycling were explored in the present study. In healthy volunteers, short-term intravenous infusion of glucose solutions evokes a significant release of insulin into the bloodstream.[10] A number of investigators have shown alterations in both fasting and fed antral motor activity with induction of acute hyperinsulinemia; however, most of these studies were performed under conditions of hypoglycemia.[26–28] In the present investigation, we infused insulin to levels that reproduced those in the highest hyperglycemic clamp studies, but with maintenance of euglycemia, to rule out the possibility that hyperglycemic effects on gastric electrical and motor activity are secondary to supraphysiological insulin release. We showed that euglycemic, hyperinsulinemic clamping has no effect on either postcibal antral motility or slow wave rhythmicity, indicating that secondary hyperinsulinemia is not involved. In diabetics with gastroparesis, it is unlikely that antral hypomotility and tachygastria result from hyperinsulinemia because most patients with diabetes have subnormal insulin release. Thus, the findings of the present study are consistent with what is observed with diabetes.

Endogenous prostaglandins have been proposed as mediators of gastric slow-wave dysrhythmias. In dogs, infusion of prostaglandin E_2 evokes significant slow-wave disruption.[8] Furthermore, also in dogs, gastric dysrhythmias pharmacologically induced by epinephrine and metenkephalin are prevented by pretreatment with the cyclooxygenase inhibitor indomethacin.[8] Finally, in vitro studies of antral smooth muscle tissue from a woman with severe idiopathic gastroparesis and tachygastria documented an abnormally rapid spontaneous electrical oscillation that was converted into the normal range with incubation in a concentrated indomethacin solution, suggesting that overproduction of prostaglandins by this patient's gastric smooth muscle was responsible for the underlying slow-wave disturbance.[29] We hypothesized that endogenous prostaglandin pathways were responsible for the antral hypomotility and slow-wave tachyarrhythmias observed with induction of acute hyperglycemia. Our studies showed that pretreatment with indomethacin prevented the slow-wave disruption induced by hyperglycemic clamping to 230 mg/dL, indicating mediation of the dysrhythmia by endogenous prostaglandin production. In contrast, the reduction in postprandial antral motor activity observed during hyperglycemic clamping was not inhibited by prior administration of indomethacin. This suggests that motor inhibition is mediated by prostaglandin-independent pathways and that the electrical and motor effects of acute hyperglycemia are regulated by distinct mechanisms. The clinical implication of this investigation is that prostaglandin synthesis inhibitors may be useful for the slow-wave disturbances observed with diabetic gastroparesis but may not be useful for the motor defects. The present study, in conjunction with the previously described studies, also indicates that endogenous prostaglandin production may be required for development of tachygastria in a broad range of clinical conditions.[8,29] These issues are worthy of investigation in a controlled fashion.

In conclusion, acute hyperglycemia to 175 mg/dL inhibits postprandial antral motor activity in healthy volunteers, whereas higher levels of plasma glucose are associated with slow-wave rhythm disruption. These effects are not mediated by secondary supraphysiological insulin release. Induction of gastric dysrhythmias, but not postcibal antral hypomotility, is dependent on synthesis of endogenous prostaglandins. These observations provide pathophysiological insight into the motor and myoelectric disturbances in diabetic gastroparesis and suggest a possible use for prostaglandin synthesis inhibitors in treatment of gastric dysrhythmias in this condition.

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Address requests for reprints to: Chung Owyang, M.D., 3912 Taubman Center, Box 0362, University of Michigan Medical Center, Ann Arbor, Michigan 48109-0362. Fax: (313) 936-7392.

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