Selective Reversal of Hyperglycemia-Evoked Gastric Myoelectric Dysrhythmias by Nitrergic Stimulation in Healthy Humans

Radoslav Coleski, Sutep Gonlachanvit, Chung Owyang, and William L. Hasler

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

Received June 25, 2004; accepted October 18, 2004

ABSTRACT
Acute hyperglycemia disrupts gastric myoelectric rhythm in healthy humans. Defective nitrergic function is a factor in animal models of diabetic gastropathy. We tested participation of nitrergic pathways in hyperglycemia-evoked myoelectric dysrhythmias and compared their role in preventing dysrhythmic actions of experimental motion sickness. Twelve healthy volunteers underwent electrogastrography (EGG) with and without intravenous 20% dextrose to produce plasma glucoses of 250 mg/dl. EGG continued for 2 h after oral nitroglycerin (9 mg) or the cyclic GMP-specific phosphodiesterase inhibitor sildenafil (100 mg). In separate studies, 12 volunteers underwent circular vection (60°/s) without and 90 min after nitroglycerin (9 mg) or sildenafil (100 mg) with concurrent EGG. Hyperglycemia decreased recording time in normal rhythm, increased tachygastria more than 3-fold, and decreased power of the dominant frequency (P < 0.05). Nitroglycerin and sildenafil reversed effects of hyperglycemia, improving normal rhythm, decreasing tachygastria (both P < 0.05), and blunting power decreases. Neither agent affected EGG rhythm during euglycemia. Vection decreased time in normal rhythm and increased tachygastria (P < 0.05). However, nitroglycerin and sildenafil did not reverse dysrhythmic effects of vection (P = N.S.). In conclusion, administration of a nitric oxide (NO) donor or an inhibitor of cyclic GMP-selective phosphodiesterase reverses the dysrhythmic effects of hyperglycemia on gastric myoelectric activity in healthy humans. These agents have no effect on dysrhythmias during motion sickness. These findings are consistent with selective impairment of nitrergic function in this model of diabetic gastropathy and suggest that NO donors and other agents that increase NO activity may be useful for treating diabetic dysrhythmias.

Several factors are proposed to underlie symptoms in patients with diabetic gastropathy including delayed gastric emptying, impaired antral contractions, pylorospasm, heightened sensitivity to distention, and abnormal gastric myoelectrical activity (Feldman and Schiller, 1983; Mearin et al., 1986; Jebbink et al., 1994a; Samsom et al., 1995). In nauseated diabetics, myoelectric abnormalities including tachygastria and bradygastria are prominent and relate to the degree of glycemic control (Jebbink et al., 1994b). Investigators have utilized hyperglycemic clamping in healthy volunteers to assess the role of blood glucose as a pathogenic factor in diabetic gastropathy. Functional abnormalities evoked by acute hyperglycemia are similar to those observed in diabetic patients (Barnett and Owyang, 1988; Fraser et al., 1991; Takahashi and Owyang, 1995). Gastric dysrhythmias occurring in diabetes and elicited by acute hyperglycemia in healthy volunteers are blunted by eating, suggesting that some meal-related mechanical or neurohumoral factor has myoelectric rhythm-stabilizing effects (Mathur et al., 2001; Defrancisco et al., 2002). Impaired nitrergic function underlies motor abnormalities in animal models of diabetic gastropathy which are correctable by treatments that enhance NO action (Takahashi et al., 1997; Watkins et al., 2000). It is unknown whether measures to enhance the release or action of NO stabilize myoelectric rhythm in human models of diabetic gastropathy.

We designed studies to test the hypothesis that gastric myoelectric rhythm disturbances occurring during acute hyperglycemia in healthy humans are a consequence of revers-
ible reductions in NO activity. We employed electrogastrography (EGG) to compare gastric myoelectric activity during euglycemia and during hyperglycemic clamping to plasma glucose levels of 250 mg/dl. Studies were repeated under separate conditions in which NO activity was increased either after oral administration of extended release nitroglycerin (a NO donor) or sildenafil (Viagra, Pfizer Laboratories, New York, NY) (a selective inhibitor of cyclic GMP-specific phosphodiesterase type 5). To determine whether effects of nitroglycerin stimulation are generalized or specific for hyperglycemia, the effects of nitroglycerin and sildenafil on gastric dysrhythmias associated with experimental motion sickness evoked by exposure to a rotatory stimulus were tested. Through these investigations, we hoped to gain insight into the role of impaired nitric function in a model of diabetic gastropathy and to determine whether these defects are specific for this model or generalized for other conditions with disturbed gastric myoelectric activity.

Materials and Methods

Study Population. Twelve healthy volunteers (age 19–47 years, eight male and four female) were recruited for participation in hyperglycemia studies. Twelve healthy volunteers (age 18–47 years, seven male and five female) with histories of motion sickness during automobile, boat, or airline travel were recruited for circularvection studies. Twelve healthy volunteers (age 18–47 years, eight male and four female) were recruited for participation in hyperglycemia studies. Twelve healthy volunteers (age 19–47 years, eight male and four female) were recruited for participation in hyperglycemia studies.

Electrogastrography Methodology. EGG was performed according to a modification of previously described methods (Stern et al., 1987b). After gentle skin abrasion to enhance electrical conduc-tion, six Ag-AgCl electrodes (Accutac diaphragmatic electrocardiograph electrodes; New Dimensions in Medicine, 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. Three reference electrodes were affixed in the right upper quadrant below the right costal margin. 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 s and high-frequency cutoffs at 0.3 Hz to minimize interferences 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 exhibiting artifact clearly resulting from body movement or exaggerated respiration activity, such as with a deep sigh or cough, were excluded from analysis. Data were also recorded on a personal computer (4DX2-66V, Gateway 2000; Gateway, North Sioux City, ND) via an analog-to-digital converter (DAS-16; Metrabyte Corp., Taunton, MA). Signals were digitized at 4 Hz and filtered above 15 cycles/min (cpm) and below 0.5 cpm to remove high- and low-frequency noise. After completion of each recording, the three channels were analyzed visually to determine which lead provided the signal most free of noise. The recording from 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.

Hyperglycemia Study Protocol. Each volunteer underwent electrogastrographic studies under four separate test conditions in random order on 4 separate days separated by at least 72 h: two EGG studies under euglycemic conditions (1 study day with nitroglycerin and the second study day with sildenafil) and two EGG studies using hyperglycemic clamping (1 study day with nitroglycerin and the second study day with sildenafil). Prior to each of the 4 study days, subjects fasted for 8 h and abstained from caffeine, alcohol, and tobacco for at least 12 h. An initial 1-h baseline fasting EGG recording was performed. Then, intravenous perfusion of 0.9% saline or 20% dextrose was begun, and EGG recording proceeded for another hour. Subjects then took either one 9-mg extended release tablet of nitroglycerin (Schwarz Pharma, Mequon, WI) or one 100-mg tablet of sildenafil (Pfizer Laboratories), and an additional 2 h of EGG recordings were performed during which time saline or dextrose infusions were continued. Extended release nitroglycerin exhibits an onset of action of 20 to 45 min, a peak response at 90 min, and a duration of effect of 3 to 8 h. Sildenafil reaches maximal plasma concentrations at 30 to 120 min after ingestion (mean 80 min) and has a mean terminal half-life of 3 to 5 h. The sildenafil dose used in this study was the maximum employed for clinical treatment of erectile dys-function. In additional control studies, hyperglycemic clamping with concurrent EGG was performed in selected volunteers for 3 h after 1 h of baseline recording without nitroglycerin or sildenafil adminis-tration to exclude the possibility that gastric myoelectric rhythm stabilizes and dysrhythmias resolve with continued dextrose infusion.

Hyperglycemic clamping was performed according to previously described methods (DeFronzo et al., 1979). Intravenous catheters were inserted into antecubital veins in each arm. For euglycemic studies, one line was used for intravenous infusion of normal saline and the second for obtaining blood glucose samples at 30-min intervals. For hyperglycemic clamping studies, one venous line was used for infusion of 20% dextrose and one for monitoring blood glucose levels. After a 15-min priming dose of 20% dextrose, the maintenance infusion rates were adjusted as needed by monitoring plasma glucose levels at 5-min intervals throughout the study to maintain the blood glucose concentration level at 250 mg/dl. Patency of the lines was maintained with periodic infusions of heparin flush-lock solution (100 USP units/ml). Plasma glucose level was determined using a portable glucose analyzer (One Touch II; LifeScan Inc., Milpitas, CA). Using these methods, plasma glucose levels were maintained within ±10% of the desired concentrations.

Power spectral analysis was performed on digitized EGG signals across the frequency range from 1 to 9 cpm on 256-s segments of recording with a 76% overlap using commercially available software (MatLab; The Mathworks, Inc., Natick, MA). From this analysis, the dominant frequency was measured using a portable glucose analyzer (One Touch II; LifeScan Inc., Milpitas, CA). Using these methods, plasma glucose levels were maintained within ±10% of the desired concentrations.

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Circular Vection Study Protocol. Circularvection was performed on each volunteer using previously described methods on three separate occasions separated by at least 1 week (one control study, one study after oral nitroglycerin, and one study after oral sildenafil) (Stern et al., 1987a). Prior to each study day, subjects fasted for 8 h and abstained from caffeine, alcohol, and tobacco for at
least 12 h. Volunteers were seated vertically in the center of a drum (76-cm diameter, 92 cm in height) with the use of a chin rest to maintain head position. The drum interior was painted with alternating black and white 3.8-cm vertical stripes and was illuminated by a stationary light above the volunteer. After an initial 15-min basal EGG recording period, clockwise drum rotation was begun at 60°/s and continued for 15 min or until the level of symptomatology precluded further stimulation. Subjects were instructed to report if they experienced severe nausea with impending vomiting. If severe nausea was reported, drum rotation was immediately discontinued. Times to maximal EGG rhythm disruption from the onset of circular vection were recorded. For nitroglycerin studies, basal EGG recording was begun 90 min after ingestion of one 9-mg extended release tablet of nitroglycerin (Schwarz Pharma). For sildenafil studies, basal EGG recording was begun 90 min after ingestion of one 100-mg sildenafil tablet (Pfizer Laboratories).

Power spectral analysis was performed on digitized EGG signals from the circular vection studies across the frequency range from 1 to 9 cpm on 128-s segments of recording with a 76% overlap using commercially available software (Fourier Perspective III; Alligator Technologies, Fountain Valley, CA). As with the hyperglycemia studies, the frequency range ≥2 and ≤4 cpm was defined to represent normal, whereas the frequency range >4 and ≤9 cpm represented tachygastria, and the range ≥1 and <2 cpm represented bradygastria. The summed signal powers in the bradygastric, normal, and tachygastriac frequency ranges were quantified as a percentage of a total signal power from 1 to 9 cpm. This signal analysis protocol has been specifically employed for circular vection studies because dysrhythmias may develop quickly in association with severe nausea that necessitates cessation of drum rotation (Hasler et al., 1995a). In such instances, it is not possible to calculate the percent recording times with dominant frequencies in normal rhythm, bradygastria, and tachygastria due to the brief nature of the recordings. The effects of circular vection on the summed power of the dominant frequency band were expressed as a fraction of the power of the dominant frequency band prior to initiation of drum rotation.

Statistical Analysis. All results are expressed as means ± S.E.M. Longitudinal regression analysis of repeated measures was performed to compare EGG parameters in the hyperglycemic clamping studies including percentages of recording time in the different frequency ranges and powers of the dominant frequencies. For circular vection studies, paired two-tailed Student’s t testing was performed to compare percentages of signal power in different frequency ranges before and during vection within each given test condition. One-way analysis of variance with Student-Newman-Keuls post testing compared latencies to maximal dysrhythmias and changes in power in the normal and tachygastriac frequency ranges between the different test conditions before and during vection. A P value of <0.05 was defined as statistically significant.

Results

Hyperglycemia Studies

Plasma Glucose Levels. Results of plasma glucose measurement are shown in Table 1. Fasting glucose levels during euglycemic studies and before hyperglycemic clamping showed no differences between the different test conditions. Initiation of hyperglycemic clamping produced prompt achievement of the desired plasma glucose level of 250 mg/dl. This concentration was maintained for the duration of the study after administration of both nitroglycerin and sildenafil.

Effects of Nitroglycerin on EGG Parameters. Hyperglycemic clamping studies. Sample EGG tracings and spectral analyses from a hyperglycemic clamping study before and after nitroglycerin are shown in Fig. 1. During the initial baseline recording, the raw signal exhibits a regular sinusoidal morphology with a period of 20 s. Frequency analysis shows a regular 3-cpm peak throughout the recording. Hyperglycemia elicited a chaotic low-amplitude wave form with more rapid cycling. Spectral analysis of this signal showed a dominant frequency of nearly 8 cpm. After nitroglycerin administration, the raw signal showed normalization to a sinusoidal wave form with a period of 20 s and a dominant frequency of 3 cpm on frequency analysis.

The effects of hyperglycemic clamping on EGG rhythm and power before and after nitroglycerin were compared for all volunteers. Hyperglycemia to 250 mg/dl significantly decreased the percentage of recording time in normal rhythm from 84 ± 4 to 69 ± 7% (P < 0.05) and increased the percent
Percentage of recording time in tachygastria from 7 ± 3 to 23 ± 7% (P < 0.01) (Fig. 2, A and B). There were no significant effects of hyperglycemia on bradygastric activity. Hyperglycemia also significantly reduced powers of the dominant frequency to 0.33 ± 0.22 of baseline values (P < 0.05) (Fig. 2C). Although hyperglycemia was ongoing, nitroglycerin reversed the decrease in the percentage of recording time in normal rhythm (83 ± 5%) (P < 0.05) and the increase in tachygastria (11 ± 5%) (P < 0.01) (Fig. 2, A and B). The mean latency for nitroglycerin action to reverse tachygastric activity was 61 ± 5 min. Similarly, nitroglycerin significantly reversed the blunting effects of hyperglycemia on power of the dominant frequency to 0.84 ± 0.30 of control values (P < 0.05 compared with hyperglycemia alone) (Fig. 2C).

Euglycemic studies. Control studies of 0.9% saline infusion were performed. In contrast to hyperglycemic clamping studies, saline infusion had no effect on percentages of recording time in normal rhythm (86 ± 4 versus 83 ± 5%) or tachygastria (2 ± 1 versus 6 ± 3%) (P = N.S.). Similarly, saline had no effect on EGG power (0.80 ± 0.35 of baseline) (P = N.S.). Nitroglycerin did not change percentages of time in normal rhythm (82 ± 6%) or tachygastria (9 ± 4%) (P = N.S.). Nitroglycerin did not significantly affect EGG power during saline infusion studies (0.81 ± 0.40 of baseline levels) (P = N.S.).

Effects of Sildenafil on EGG Parameters. Hyperglycemic clamping studies. Sample EGG tracings and spectral analyses from a hyperglycemic clamping study before and after sildenafil are shown in Fig. 1. As with the nitroglycerin studies, sildenafil converted the chaotic, rapid, low-amplitude wave form to a more regular sinusoidal wave form with a period of 20 s. Spectral analysis confirmed that sildenafil reversed a prolonged tachygastria at a frequency of 4 to 7 cpm to a more normal pattern with a dominant frequency of 3 cpm.

The effects of hyperglycemic clamping on EGG rhythm and power before and after sildenafil were compared for all volunteers. As observed during nitroglycerin studies, hyperglycemia to 250 mg/dl significantly decreased the percentage of recording time in normal rhythm from 87 ± 4 to 73 ± 5% (P < 0.05) and increased the percentage of recording time in tachygastria from 5 ± 3 to 18 ± 4% (P < 0.01) (Fig. 3, A and B). There were no significant effects of hyperglycemia on bradygastric activity. In the sildenafil studies, hyperglycemia showed a trend to reducing powers of the dominant frequency to 0.58 ± 0.28 of baseline levels (P = 0.06) (Fig. 3C). Although hyperglycemia was ongoing, sildenafil administration reversed the decrease in percentage of recording time in normal rhythm (86 ± 3%) (P < 0.05) and the increase in tachygastria (5 ± 2%) (P < 0.01) (Fig. 3, A and B).
mean latency for sildenafil action to reverse tachygastri
activity was 72±8 min. Sildenafil showed a trend to reversal
of the blunting effects of hyperglycemia on power of the
dominant frequency to 1.14±0.40 of baseline values (P =
0.08 compared with hyperglycemia alone) (Fig. 3C).

Euglycemic studies. As with the nitroglycerin studies, con-
trol experiments of 0.9% saline infusion were performed to
exclude significant independent effects of sildenafil on EGG
parameters. Saline infusion had no effect on percentages of
recording time in normal rhythm (94±2 versus 90±3%) or
tachygastria (2±1 versus 5±2%). Similarly, saline had no
effect on EGG power (61±44% of baseline) (P = N.S.).
Sildenafil did not change percentages of time in normal
rhythm (91±3%) or tachygastria (5±2%), but it did show
a trend to reducing power of the dominant frequency to
0.32±0.31 of baseline levels (P = 0.08).

Control Hyperglycemic Clamping Studies. To confirm
that this stabilization of EGG rhythm by nitroglycerin and
sildenafil was not due to intrinsic adaptation to prolonged
hyperglycemia, experiments were performed in four healthy
volunteers in which hyperglycemia was performed for 3 h
without nitroglycerin or sildenafil administration. For each
hour of EGG recording, significant increases in tachygastic
activity were observed (Fig. 4). There were no differences in
the magnitude of tachygastriac activity between the second,
third, and fourth hours of recording (P = N.S.).

Circular Vection Studies

Circular vection disrupted EGG rhythm in all 12 volun-
teers. Representative responses to circular vection without
and after nitroglycerin and sildenafil are shown in Fig. 5.
Prior to initiation of drum rotation, the raw signal exhibits a
regular wave form at a frequency of 3 cpm. Soon after initi-
ation of circular vection, EGG rhythmicity deteriorated and
was replaced with intense high-frequency EGG signal oscil-
lations. In contrast to the hyperglycemia studies, nitroglyc-
erin and sildenafil pretreatment had no effect on EGG dys-
rhythmias in this individual.

EGG responses to circular vection without and after nitro-
glycerin were compared in all volunteers. In control studies,
circular vection evoked maximal dysrhythmic activity with a
latency of 355±57 s (Fig. 6). Drum rotation evoked maximal
decreases in percentages of signal power in the normal fre-
quency range from 64±8 to 32±6% (P < 0.01) and
increases in tachygastriac activity from 16±4 to 56±5% (P <
0.01) (Fig. 7, A and B). Power of the dominant frequency

![Fig. 3. The cumulative results of EGG anal-
yses for the hyperglycemic clamping studies
before and after sildenafil are shown. The
percentage of time in normal rhythm de-
creased with initiation of hyperglycemic clamping, but sildenafil reversed this de-
crease (A). The percentage of time in tachy-
gastria increased during hyperglycemia but
decreased to near normal values after silde-
nafil (B). Hyperglycemia decreased EGG power, an effect partially reversed with silde-
nafil treatment (C). All results are mean ±
S.E.M., n = 12.](image-url)
slightly increased during circular vection to $1.57 \pm 0.44$ of baseline ($P = N.S.$) in contrast to the effects of hyperglycemia (Fig. 7C). After nitroglycerin, latencies to maximal dysrhythmia after initiation of drum rotation were similar to control studies ($402 \pm 68$ s, $P = N.S.$) (Fig. 6). Circular vection after nitroglycerin elicited maximal decreases in normal rhythm from $62 \pm 6$ to $27 \pm 3$% ($P < 0.01$) and increases in tachygastria from $11 \pm 2$ to $52 \pm 4$% ($P < 0.01$) (Fig. 7, A and B). Similarly, circular vection after sildenafil evoked maximal decreases in normal rhythm from $70 \pm 9$ to $42 \pm 5$% ($P < 0.01$) and increases in tachygastria from $11 \pm 3$ to $48 \pm 7$% ($P < 0.01$) with latencies of $330 \pm 76$ s (Fig. 6; Fig. 7, A and B). When differences in power distribution in each of the frequency ranges before and during circular vection were compared, these effects were not different from control studies ($P = N.S.$). Likewise, nitroglycerin and sildenafil did not affect the increase in power of the dominant frequency in response to circular vection compared with control studies ($P = N.S.$) (Fig. 7C).

**Discussion**

Disturbances of gastric myoelectric activity are prominent in diabetics with nausea and vomiting (Jebbink et al., 1994a). The gastric slow wave regulates the direction of propagation and the maximal frequency of phasic contractions in the distal stomach, thus abnormal slow wave patterns are postulated to underlie many gastric dysmotility syndromes (Stern et al., 1987b). Slow wave activity is measured using EGG, the findings of which show close correlations with recordings from serosal electrodes (Chen et al., 1994). Rhythm disturbances such as tachygastria and bradygastria are observed in up to 75% of patients with diabetic gastroparesis (Koch et al., 1989; Rothstein et al., 1993; Jebbink et al., 1994a). These dysrhythmias have been reported to improve, but not resolve, with eating suggestive of mediation by meal-related mechanical or neurochemical factors (Mathur et al., 2001). An additional abnormality, a reduced EGG signal amplitude after meal ingestion, correlates with delayed gastric emptying of solids (Chen et al., 1996).

In diabetic patients, the magnitude of gastric myoelectric rhythm disruption correlates with the degree of glycemic
control. During hyperglycemia, dysrhythmias are observed 41% of the time, whereas clamping of the plasma glucose to the euglycemic range decreases rhythm disruption to 6% of the recording (Jebbink et al., 1994b). These findings support the postulate that plasma glucose is an important cofactor in the regulation of gastric function and the clinical manifestations of diabetic gastropathy. Indeed, the effects of hyperglycemic clamping in healthy volunteers mimic those observed in patients with diabetes including slowing of solid gastric emptying, inhibition of antral motor function, and induction of increased pyloric motility (Barnett and Owyang, 1988; Fraser et al., 1991; Schwarez et al., 1997). Increases in gastric tachyarrhythmic activity in healthy volunteers were evoked by hyperglycemic clamping to plasma glucose levels of 230 mg/dl in prior studies from our laboratory (Hasler et al., 1995b). We further showed that these dysrhythmias could be prevented by pretreatment with the prostaglandin synthesis inhibitor indomethacin, suggesting that hyperglycemia-induced myoelectric disturbances are mediated by endogenous prostaglandins (Hasler et al., 1995b). As observed in diabetic patients, we also have noted greater degrees of hyperglycemia-evoked dysrhythmic activity during fasting than after ingestion of a mixed meal (Defrancesco et al., 2002).

NO released by nonadrenergic, noncholinergic inhibitory myenteric neurons is a key inhibitory neurotransmitter in the regulation of gastric motor activity, through production of cyclic GMP (Bult et al., 1990). Defects in NO pathways are postulated to underlie gastric motor dysfunction in animal models of gastroparesis. In animals and healthy volunteers, neuronal NO synthase (nNOS) inhibitors increase fundus tone and blunts fundic relaxation to pharmacologic stimulation and meal ingestion, whereas NO donors such as nitroglycerin evoke fundic relaxation and enhance meal-induced accommodation (Desai et al., 1991; Coulie et al., 1999). In diabetic rats, reduced nNOS activity in gastric and duodenal myenteric plexus is associated with impaired nonadrenergic, noncholinergic relaxation in gastric and duodenal muscle strips (Takahashi et al., 1997; Watkins et al., 2000). Delayed gastric emptying and increases in pyloric tone are observed in diabetic mice in association with relative reductions in pyloric nNOS levels, similar to findings in mice lacking the nNOS gene (Huang et al., 1993). Sildenafil, a selective inhibitor of cyclic GMP-specific phosphodiesterase type 5, reversed...
many of the functional defects noted in those diabetic mice. This agent enhances the effectiveness of endogenously released NO by preventing breakdown of cyclic GMP generated by nitrergic activation of guanylate cyclase (Corbin and Francis, 1999).

In the present study, we tested whether enhanced nitrergic function reverses gastric myoelectric rhythm disturbances elicited by acute hyperglycemia in healthy volunteers. Two interventions were employed: administration of the NO donor nitroglycerin and administration of the phosphodiesterase inhibitor sildenafil. Each of these agents produced significant reductions in dysrhythmic activity during hyperglycemic clamping to 250 mg/dl and further, partly reversed the decrease in EGG power elicited by hyperglycemic clamping. In contrast, neither treatment affected EGG rhythm during euglycemia, although sildenafil evoked modest reductions in signal power. These findings agree with those of a previous investigation in which NO pathways did not influence gastric pacemaker activity during euglycemia (Hou et al., 2001). Our results are consistent with the hypothesis that hyperglycemia-elicited gastric myoelectric rhythm disruption in healthy humans is mediated by reduced gastric NO activity. These observations also raise the possibility that the observed incomplete myoelectric rhythm stabilization with eating in diabetics and healthy subjects during hyperglycemia may be secondary to meal-evoked NO release. This hypothesis is speculative and should be confirmed in animal models.

The mechanisms by which nitrergic stimulation stabilizes gastric dysrhythmias evoked by hyperglycemia are unknown. In cultured murine intestinal interstitial cells of Cajal, NO donors and cyclic GMP analogs slow pacemaker frequencies (Koh et al., 2000). Responses of muscle strips containing interstitial cells are similar suggesting that NO-activated cyclic GMP-dependent pathways regulate slow wave frequency at the level of the cells that generate pacemaker activity. The relation of the stabilizing effects of nitrergic stimulation on gastric myoelectric activity to the mediation of hyperglycemia-evoked dysrhythmias by endogenous prostaglandins is unexplored. In rats, the delay in gastric emptying evoked by endotoxin may be mediated by nNOS down-regulation and increased prostaglandin synthesis (Calatayud et al., 2002). This model would fit well with observations of our studies investigating nitrergic and prostaglandin pathways in the hyperglycemic induction of myoelectric rhythm disturbances. However, in rat ileum, NO activates cyclooxygenase with production of prostaglandin \( E_2 \) which then acts synergistically with NO to elicit smooth muscle relaxation (Españo and Sales, 2003). These divergent findings likely are a function of the different models that were studied. Further studies addressing the coinvolvement of prostaglandin and nitrergic pathways in control of gastric slow wave frequency should be performed.

The effects of nitrergic stimulation on hyperglycemia-evoked gastric myoelectric disruption were contrasted to its actions in experimental motion sickness. Circular vection was chosen because gastric myoelectric rhythm disturbances elicited by this technique are mediated by distinct pathways from those activated by acute hyperglycemia. Specifically, indomethacin does not prevent induction of tachygastria by circular vection indicating the lack of participation by endogenous prostaglandins (Hasler et al., 1995a). Rather, gastric myoelectric rhythm disturbances in motion sickness may be mediated in part by cholinergic pathways and vasopressin release (Kim et al., 1997). In the present investigation, nitroglycerin and sildenafil had no prophylactic effect on circular vection-induced dysrhythmias. The findings of these studies are consistent with mediation of motion sickness-associated slow wave disruptions by NO- and prostaglandin-independent pathways in contrast to the NO- and prostaglandin-dependent mechanisms with acute hyperglycemia.

This investigation has potential clinical implications for patients with diabetic gastropathy. Until now, nitroglycerin has been investigated primarily for its fundic relaxant properties. In this capacity, the drug has shown benefits in small studies of patients with functional dyspepsia (Gilja et al., 1997). The current study suggests NO donors and other agents that increase NO activity may have other actions which may be useful in diabetics with nausea and vomiting and associated gastric myoelectric dysrhythmias; however, it is uncertain if the findings of the present study can be extended to diabetic patients. Indeed, the use of nitrergic agents that inhibit motor function might conceptually be expected to worsen delays in gastric emptying. However, if symptoms stem from slow wave disruptions rather than delays in emptying, such treatments might prove to be beneficial. Further studies will define any clinical utility of nitroglycerin or sildenafil in diabetic gastropathy and other dyspeptic disorders.

Some issues can be raised about the findings of the present investigation. First, it can be questioned whether the EGG abnormalities seen reflect changes in gastric myoelectric activity versus electrical signals from other organs. We have performed preliminary studies employing endoscopically directed mucosal recordings of gastric slow wave frequency which demonstrate that there are specific disruptive effects of hyperglycemia on electrical activity in the stomach (Coleski et al., 2004). We also addressed the question of whether the improvements seen with nitroglycerin and sildenafil resulted from adaptation to the dysrhythmic effects of hyperglycemia. Control studies were performed which showed persistence of slow wave rhythm disruption for the full 3 h of hyperglycemic clamping, making this less likely. One could question if the selective benefits of nitrergic stimulation with hyperglycemic clamping stems from enrollment of different subjects in the two phases of the study. However, 10 individuals participated in both hyperglycemic clamping and circular vection studies making this possibility unlikely. Additionally, no placebo arm was included in this investigation. However, subjects did not perceive the onset of dysrhythmias and did not observe real-time recordings of dysrhythmic activity. Additionally, subjects showed impressive responses to drugs with one stimulus (hyperglycemia) but not the other (vection). Thus, we do not believe the responses observed are placebo effects. Finally, it is uncertain if the antidysrhythmic effects of nitroglycerin and sildenafil are accompanied by reductions in nausea and vomiting. Hyperglycemia in healthy volunteers elicits postprandial fullness and early satiety but little nausea (Hasler et al., 1995b; Defrancisco et al., 2002). Possible explanations include: 1) the dysrhythmias are only one cofactor for symptom development, 2) hyperglycemia has concurrent effects on gastric perception which blunt any nausea-inducing effects of the dysrhythmias, or 3) the dysrhythmias are markers of gastric
Another concern relates to the benefits observed with sildenafil administration. Animal studies have localized the type 5 phosphodiesterase to pyloric tissues in the proximal gut (Kotera et al., 1998). It is uncertain if the antidysrhythmic effects of sildenafil in the current model might be a consequence of selective action on the most distal gastric regions, or if the drug has more generalized actions on the human stomach. In humans, sildenafil decreases lower esophageal sphincter tone in patients with achalasia, nutcracker esophagus, and hypertensive lower esophageal sphincter indicating effects in regions other than the pylorus showing species-related differences in the actions of the drug (Bortolotti et al., 2000; Eherer et al., 2002). It also is not defined if the myoelectric rhythm stabilizing effects of the two agents used in this study might be secondary to NO-mediated fundic relaxation. In preliminary studies, we reported increases in EGG power with fundic distention suggesting that distal gastric myoelectric activity may be modulated by more proximal stimulation (Koshy et al., 1996). These and other areas are worthy of future investigation.

In conclusion, nitroglycerin, a NO donor, and sildenafil, a selective inhibitor of cyclic GMP-specific phosphodiesterase type 5, reverse the dysrhythmic effects of hyperglycemic clamping to plasma glucose levels of 250 mg/dl on gastric myoelectric activity in healthy humans. These agents also blunt the effects of hyperglycemia on electrogastrographic power. In contrast, nitroglycerin and sildenafil administration have no effect on gastric dysrhythmias associated with endotoxin-induced delay in gastric emptying. (Abstract).  

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