Role of 5-hydroxytryptamine mechanisms in mediating the effects of small intestinal glucose on blood pressure and antropyloroduodenal motility in older subjects

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Submitted 4 May 2007; accepted in final form 23 July 2007

Postprandial hypotension, defined as a fall in systolic blood pressure of ≥20 mmHg within 2 h of a meal that is sustained for at least 30 min, is now recognized as a major cause of morbidity and mortality (7, 16) by predisposing to a number of disorders, including syncope, transient ischemic attacks, stroke, and angina (16). Postprandial hypotension occurs more frequently than orthostatic hypotension (16), and those at greatest risk include the elderly and patients with autonomic dysfunction, the latter usually secondary to diabetes (16). Current treatment options are suboptimal.

Several factors, including impaired regulation of splanchnic blood flow, the release of gastrointestinal hormones, and duodenal sympathetic nerve activity, have been identified as possible pathophysiological mechanisms in postprandial hypotension (16). The magnitude of the fall in blood pressure is dependent on meal composition; ingestion of carbohydrate, particularly glucose, has been reported to have the greatest suppressive effect on blood pressure (17). The onset of the fall in blood pressure after a meal is almost immediate, with a maximum response at 30–60 min (16), suggesting a direct relationship between the magnitude of the hypotensive response and the rate of delivery of carbohydrate to the small intestine. This is the case in both healthy older subjects (18, 32) and patients with type 2 diabetes (19, 36) and has been established by a series of studies by our group. For example, in healthy older subjects when glucose is administered intraduodenally at a rate of 1 kcal/min or 3 kcal/min, the fall in blood pressure and increase in heart rate are substantially greater during the 3 kcal/min infusion (31). The hypotensive response to small intestinal glucose appears to be load dependent rather than concentration dependent (9). Following a meal, there is a substantial increase in splanchnic blood volume (~20% of total blood volume), with an approximate doubling of superior mesenteric arterial flow that is associated with reductions in systemic vascular resistance and skeletal muscle blood flow (16). The magnitude of the postprandial increase in mesenteric blood flow is comparable in healthy young and old individuals, despite the greater fall in blood pressure in the latter group, indicating that there is inadequate cardiovascular adjustment for the shift of blood volume into the splanchnic system (25).

The presence of glucose in the small intestine also inhibits antral and proximal duodenal pressures, stimulates both pyloric tone and phasic pyloric activity (13, 42), and slows gastric emptying (12) [as a result of inhibitory feedback arising from receptors located throughout the small intestine (15, 24)]. In healthy subjects, gastric emptying of glucose is known to be regulated at ~2–3 kcal/min, after an initial emptying phase that may be somewhat faster (14).

5-Hydroxytryptamine (5-HT) is an important neurotransmitter in the gastrointestinal tract, formed predominantly in enterochromaffin cells of the intestinal mucosa (37). Recent studies suggest that enterochromaffin cells (20, 21) act as “glucose sensors” in the small intestine, activating signal-transduction pathways in the presence of D-glucose, galactose, and α-D-glucopyranoside (20, 21) via the release of 5-HT.
There is evidence that 5-HT mechanisms play a role in the regulation of splanchnic blood flow (32, 44). For example, in the dog, both low- (4 μg·kg⁻¹·min⁻¹) and higher- (10 μg·kg⁻¹·min⁻¹) dose 5-HT infusions increase gastrointestinal blood flow (44). The role of 5-HT mechanisms in the regulation of postprandial blood pressure and heart rate has not yet been evaluated in healthy older subjects nor in any other group susceptible to postprandial hypotension.

In rodents, the release of 5-HT by d-glucose is also known to inhibit gastric emptying by activation of nervous pathways containing 5-HT₃ receptors (20, 23, 34, 35, 43). The role of 5-HT₃ mechanisms in mediating the antropyloroduodenal (APD) motility responses to intraduodenal glucose and fat has been assessed in two studies in humans (6, 28). Whereas the 5-HT₃ antagonist ondansetron (8 mg po) does not appear to affect the proximal gastric motor responses to intraduodenal infusion of lipid (20%) (6), the 5-HT₃ antagonist granisetron (10 μg/kg iv) has been shown to attenuate the suppression of antral waves induced by intraduodenal lipid, but not intraduodenal glucose, in healthy young subjects (28). A limitation of the latter study was that the amount of glucose infused was small (5 ml of 25% dextrose) and given over a short time interval (60 s) (28). Furthermore, the study design did not allow evaluation of the impact of 5-HT₃ mechanisms on “local” (i.e., near to the site of infusion) duodenal motility to be evaluated.

The aim of our study was to evaluate the role of 5-HT₃ mechanisms in mediating the effects of intraduodenal glucose on blood pressure and APD motility in healthy older subjects.

MATERIALS AND METHODS

Subjects

Ten healthy older subjects (five female and five male), with a median age of 70 yr (range: 65–76 yr) and body mass index of 24.5 kg/m² (range: 21.1–29.1 kg/m²), recruited by advertisement, were studied. All subjects were nonsmokers. None had a history of gastrointestinal disease or surgery; diabetes mellitus; significant respiratory, renal, hepatic, or cardiac disease; chronic alcohol abuse; or epilepsy or was taking medication known to influence blood pressure or gastrointestinal function.

The protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital, and each subject provided written, informed consent before inclusion. All experiments were carried out in accordance with the Declaration of Helsinki.

Protocol

Each subject was studied on two occasions, separated by at least 7 days, in double-blind, randomized order. On each day, the subject attended the University of Adelaide, Discipline of Medicine, Royal Adelaide Hospital, at 0830 following an overnight fast (10.5 h for solids; 8.5 h for liquids) (10). At that time, a 17-channel manometric catheter (Dentsleeve International, Mui Scientific, Mississauga, ON, Canada) was introduced into the stomach via an anaesthetized nostril (26). The catheter was allowed to pass through the stomach and into the duodenum by peristalsis (26) (which took between 20 and 165 min). The catheter included 16 side holes (spaced 1.5 cm apart) and an infusion channel with a port located ~10 cm distal to the pylorus (i.e., in the duodenum, 1.5 cm distal to channel 16). Six side holes (channels 1–6) were positioned in the antrum. A 4.5-cm sleeve sensor (channel 7), with two channels (channels 8 and 9) on the back of the sleeve, was positioned across the pylorus, and seven channels (channels 10–16) were positioned in the duodenum (26) (Fig. 1).

Correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained by measurement of the antroduodenal transmucosal potential difference (TMPD) by using a saline-filled reference electrode (20-gauge intravenous cannula) inserted subcutaneously into the subject’s forearm as a reference (31). The manometric channels were perfused with degassed, distilled water, except the TMPD channels, which were perfused with degassed saline (0.9%), at 0.15 ml/min (12). An intravenous cannula was positioned in a right antecubital vein for blood sampling, and an automated blood pressure cuff was placed around the left arm (31). Once intubated, subjects were studied in the recumbent position. Approximately 25 min after the tube was in position (i.e., 0 min), intravenous granisetron (Kyttril, F. Hoffmann-La Roche, Basel, Switzerland; 10 μg/kg, i.e., 0.58–0.87 ml) was infused at a rate of 5 ml/min (i.e., 3 kcal/min) for 60 min (31). Between t = 60 and 120 min, saline (0.9%) was infused intraduodenally at the same rate. At t = 120 min, the catheter and intravenous cannula were removed and the subject was given a light meal. On one day, cardiovascular autonomic nerve function was evaluated immediately following the study (33).

Measurements

Blood pressure and heart rate. Systolic and diastolic blood pressure and heart rate were measured by using an automated oscillometric blood pressure monitor (DINAMAP ProCare 100; GE Medical Systems, Milwaukee, WI) immediately before intravenous administration of granisetron (i.e., t = 25 min), at the commencement of the intraduodenal infusion (i.e., “baseline,” at t = 0 min), and then every 3 min until t = 120 min (31). Postprandial hypotension was defined as a fall in systolic blood pressure ≥20 mmHg after commencement of intraduodenal glucose that was sustained for at least 30 min (16). Blood pressure and heart rate before the administration of granisetron were calculated as the mean of measurements taken at t = −34, −31, and −28 min.

APD pressures. Manometric pressures were digitized and recorded on a computer-based system running commercially available software [Flexisoft, v. 3; G. S. Hebbard, Royal Melbourne Hospital, Melbourne, Australia, written in Labview 3.1.1 (National Instruments)] and were stored for subsequent analysis. APD pressures pertaining to channels 1–16 were analyzed for 1) number and amplitude of antral pressure waves (PWs), 2) basal pyloric pressure (tone), 3) number and
amplitude of isolated pyloric PWs (IPPWs), 4) number and amplitude of duodenal PWs, and 5) number and length of PW sequences (PWSSs) involving the antrum, pylorus, and duodenum, by using custom-written software (Gastrointestinal Motility Unit, University Hospital Utrecht, Utrecht, The Netherlands (26)). In addition, pressures recorded from the channel closest to the duodenal infusion port (i.e., channel 16) were analyzed separately for the number and amplitude of duodenal PWs to evaluate potential local effects of intraduodenal glucose. Basal pyloric pressure was determined by subtracting the mean basal pressure recorded at the most distal antral side from the mean basal pressure recorded at the sleeve by using custom-written software (MAD; Professor Charles Malbert, Institut National de la Recherche Agronomique, Rennes, France) (26). Phasic PWs in the antrum and pylorus were defined by pressure increases that lasted 1–20 s and that had an amplitude of >10 mmHg, with a minimum interval of 15 s between peaks. Phasic PWs in the duodenum were defined as those having an amplitude of >10 mmHg, with a minimal interval of 3 s between peaks. APD PWs were defined as two or more temporally related PWs with onsets within ±5 s (in the antrum) or ±3 s (in the duodenum) of each other (26).

Blood glucose concentrations. Venous blood samples (5 ml) were obtained both before the intravenous dose of granisetron (t = −27 min) and at commencement of the intraduodenal infusion (i.e., t = −2 min) and then at 15 min intervals between t = 0 and 120 min. Blood glucose concentrations were determined immediately by using a portable blood glucose meter (Medisense Precision Q·I·D System; Abbott Laboratories, Bedford, MA) (19).

Autonomic function. On one of the study days, after completion of the intraduodenal infusions, autonomic nerve function was evaluated by using standardized cardiovascular reflex tests (33). Parasympathetic function was evaluated by the variation (R-R interval) of the heart rate during deep breathing and the response to standing (“30:15” ratio). Sympathetic function was assessed by the fall in systolic blood pressure in response to standing. Each of the test results was scored according to age-adjusted predefined criteria as 0 = normal, 1 = borderline, and 2 = abnormal for a total maximum score of 6. A score ≥3 was considered to indicate autonomic dysfunction (33).

Statistical analysis. Data were evaluated by using repeated-measures two-way ANOVA, with “treatment” and “time” as within-subject factors. Systolic and diastolic blood pressure and heart rate were analyzed as changes from baseline. Differences in blood glucose were analyzed as absolute values. The maximum fall in blood pressure was defined as the greatest mean change from baseline for a treatment at any given time point. All APD pressure data, with the exception of the number of APD PWSSs, are expressed as change from baseline. For the number and amplitude of antral PWs, duodenal PWs and IPPWs, basal pyloric pressures, and number of APD PWSSs, baseline values were calculated as the mean of values obtained between t = −15 and 0 min (26).

The number and amplitude of antral PWs, duodenal PWs (channels 10–16) and IPPWs, and basal pyloric pressures are expressed as mean values for the 60-min intraduodenal glucose infusion period (i.e., t = 0–60 min). The number and amplitude of duodenal PWs (channel 16) are expressed as mean values for 15-min periods during the entire 120-min duodenal infusion period (i.e., 0–15, 15–30, . . . , 105–120 min) (26). The number and amplitude of antral PWs, duodenal PWs (channels 10–16) and IPPWs, and basal pyloric pressures were analyzed with treatment as a factor and the number and amplitude of duodenal PWs (channel 16) with time (t = −15–0, 0–15, 15–30, 105–120 min) and treatment as factors (26). APD PWSSs are expressed as the total number of PWs traveling over 2 (i.e., 1.5 cm), 3 (i.e., 3 cm), . . . , 15 (i.e., 21 cm) channels during the 120-min infusion period (26). The number of APD PWSSs was analyzed with length of propagation (1.5, 3, 4.5, . . . , 21 cm) and treatment as factors (26). Phase III activity was excluded from the data analysis.

Systolic and diastolic blood pressure, heart rate, blood glucose concentration, and the number and amplitude of duodenal PWs (channel 16) were analyzed separately from t = 0–60 min and t = 60–120 min. The number and amplitude of antral PWs, duodenal PWs (channels 10–16) and IPPWs, and basal pyloric pressures were analyzed between t = 0–60 min. One-way ANOVAs were used to analyze the effects of time from t = 0–60 min and t = 60–120 min on systolic blood pressure, diastolic blood pressure, heart rate, blood glucose concentrations, the number and amplitude of duodenal PWs (channel 16), and the effects of “length of propagation” of the number of APD PWSSs.

In all analyses, post hoc comparisons of adjusted means were performed using Student’s t-tests. All analyses were performed by using Statview (v. 5.0; Abacus Concepts, Berkeley, CA) and SuperANOVA (version 1.11; Abacus Concepts). Data are presented as means ± SE, and a P value <0.05 was considered significant in all analyses.

RESULTS

The studies were well tolerated. Three of the ten subjects reported constipation after granisetron, which, in all cases, had resolved within 48 h of completion of the experiment. The median score for autonomic nerve dysfunction was 1.2 (range 0–3); 1 of the 10 subjects had definite autonomic dysfunction. Two subjects (not including the one with autonomic neuropathy) had postprandial hypotension that was evident on both study days. In one subject, basal pyloric pressure data were not available because of technical difficulties.

Blood Pressure and Heart Rate

There was no difference in blood pressure or heart rate at t = −25 min (i.e., before intravenous granisetron) between the two study days (control vs. granisetron) (systolic blood pressure, 133.0 ± 5.6 vs. 131.9 ± 4.5 mmHg, P = 0.64; diastolic blood pressure, 69.9 ± 1.9 vs. 70.5 ± 1.8 mmHg, P = 0.57; and heart rate, 57.1 ± 2.4 vs. 58.3 ± 2.7 bpm, P = 0.43). Nor was there any difference in baseline (i.e., t = 0 min) blood pressure or heart rate between the two days (control vs. granisetron) (systolic blood pressure, 133.2 ± 5.3 vs. 130.8 ± 4.3 mmHg, P = 0.37; diastolic blood pressure, 69.3 ± 2.8 vs. 66.6 ± 2.2 mmHg, P = 0.27; and heart rate, 57.3 ± 2.3 vs. 57.1 ± 2.7 bpm, P = 0.88). There was no effect of granisetron on systolic blood pressure (P = 0.46), diastolic blood pressure (P = 0.48), or heart rate (P = 0.68) before the commencement of intraduodenal glucose infusion (i.e., between t = −25 and t = 0 min) (Fig. 2).

Between t = 0 and 60 min, there were falls in systolic and diastolic blood pressure on both the control day and after granisetron (P < 0.0001 for both) with no difference (systolic blood pressure, P = 0.65; diastolic blood pressure, P = 0.16) between the two days. Similarly, between t = 60 and 120 min, there was no difference in systolic or diastolic blood pressure between the two days (systolic blood pressure, P = 0.69; diastolic blood pressure, P = 0.87) (Fig. 2).

Between t = 0 and 60 min, there was a rise in heart rate both on the control day and after granisetron (P < 0.0001 for both) with no difference (P = 0.24) between the two days. Similarly, there was no difference (P = 0.53) in heart rate between t = 60 and 120 min between the two days.

APD Pressures

There was no difference in the baseline (i.e., t = −15–0 min) number of antral PWs (control, 31.7 ± 9.0 vs. grani-
There was no difference in the number of duodenal PWs in channel 16 between baseline and \(t = 0–15\) min on the control day \((P = 0.03)\) but no change in the number of duodenal PWs in channel 16 after granisetron \((P = 0.19)\) and no difference between the two days \((P = 0.89)\). Between \(t = 0\) and 60 min, there was a decrease in the number of duodenal PWs in channel 16 from baseline on both the control day \((P < 0.05)\) and after granisetron \((P < 0.001)\), and the magnitude of the reduction in the number of duodenal PWs in channel 16 from baseline was greater \((P < 0.05)\) after granisetron when compared with control (Fig. 3A). Between \(t = 60\) and 120 min, there was no difference in the number of duodenal PWs in channel 16 between the two study days \((P = 0.12)\). Between \(t = 0\) and 60 min, there was no overall change in the amplitude of duodenal PWs in channel 16 on the control day \((P = 0.11)\); however, the amplitude of duodenal PWs was decreased after granisetron \((P < 0.001)\); the reduction in the amplitude of duodenal PWs in channel 16 from baseline between \(t = 0\) and 60 min was greater \((P < 0.05)\) after granisetron when compared with control (Fig. 3B). Between \(t = 60\) and 120 min, there was no difference \((P = 0.37)\) in the amplitude of duodenal PWs in channel 16 between the two days.

**APD Sequences**

The number of PW sequences decreased with increasing length of propagation on both the control day and after granisetron \((P < 0.0001\) for both) without any significant difference \((P = 0.17)\) between the two study days (data not shown).

**Table 1. Effects of control and granisetron on change in number and amplitude of antropyloroduodenal pressure waves from baseline between \(t = 0–60\) min during intraduodenal glucose infusion**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Granisetron</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antral pressure waves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>-85.0±41.5</td>
<td>-108.8±29.8</td>
<td>0.70</td>
</tr>
<tr>
<td>Amplitude, mmHg</td>
<td>-161.0±67.5</td>
<td>-114.0±35.8</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>Pyloric pressure waves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal Amplitude, mmHg</td>
<td>4.4±4.6</td>
<td>-5.8±20.0</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>Phasic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>22.8±11.4</td>
<td>12.5±15.9</td>
<td>0.52</td>
</tr>
<tr>
<td>Amplitude, mmHg</td>
<td>70.3±61.0</td>
<td>68.6±73.7</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Duodenal pressure waves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal Amplitude, mmHg</td>
<td>-113.0±143.7</td>
<td>-285.0±112.3</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>Phasic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>-16.3±18.2</td>
<td>-19.3±11.8</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Data are presented as control vs. granisetron and are means ± SE \((n = 10)\). Changes in number and amplitude of antral, pyloric phasic, and duodenal pressure waves and change in amplitude of basal pyloric pressure waves from baseline during intraduodenal glucose at a rate of 3 kcal/min after administration of control and granisetron \((10 \mu g/kg)\) at \(t = -25\) min. Intraduodenal glucose was given between \(t = 0\) and 60 min.
from the stomach to the small intestine in healthy older subjects; these effects were evident within 60 min of ingestion and infusion of glucose. The latter studies excluded the potential effects of gastric emptying/distension on blood pressure; hence, in the current study, we infused glucose directly into the small intestine for a period of 60 min and at a rate (i.e., 3 kcal/min) that has been shown to decrease blood pressure in healthy older subjects (31) and approximates the normal rate of gastric emptying (14). The dose of granisetron (i.e., 10 μg/kg iv) employed was comparable with that given in previous studies (2, 22, 28) and is the recommended dose for clinical use (2). Enteral glucose induced a gradual fall in systolic and diastolic blood pressure and a rapid increase in heart rate, all of which were comparable with those observed previously (9, 31, 32). Furthermore, in accordance with previous studies, on the control day, there was a transient increase in duodenal motility within the first 15 min of the commencement of the glucose infusion (42) as well as reductions in the number of antral, and proximal, duodenal, PWs and an increase in the frequency of IPPWs (13).

In a recent study in healthy adult volunteers (2), granisetron (10 μg/kg iv) had no effect on systolic blood pressure or heart rate. However, it is unclear whether granisetron was administered in the postprandial or fasted state. We observed that granisetron had no effect on the systolic and diastolic blood pressure or heart rate responses, suggesting that the stimulation of 5-HT3 receptors is not involved in modulating the effects of enteral glucose in healthy older subjects. It should be recognized that 5-HT is released with increasing intraluminal pressures in the small intestine (3, 4). Hence, there is a potential for 5-HT3 receptors to modulate distension-induced postprandial changes in blood pressure. In rodents, the decreases in diastolic blood pressure resulting from duodenal distension with intraluminal pressures of 10–75 cmH2O are attenuated by intravenous granisetron in doses of 1–100 μg/kg (27).

Although the release of 5-HT is known to slow gastric emptying in rodents by activation of pathways containing 5-HT3 receptors (20, 23, 34, 35, 43), in humans, the reported effects of 5-HT3 antagonists on gastric emptying are unclear. 5-HT3 antagonists have been shown to have no effect on (11, 27).
29, 30, 39, 41), accelerate (1), or delay (40) gastric emptying. A recent study (28) suggests that the suppression of antral waves by intraduodenal lipid infusion is mediated via a 5-HT pathway; intravenous granisetron (10 μg/kg) attenuated the suppression of antral waves after intraduodenal lipid but not intraduodenal glucose in healthy young subjects. However, glucose (5 ml of 25% dextrose) also failed to induce a significant duodenal motor response (28). In the current study, although there were no apparent effects of granisetron in the motor response across all duodenal channels (i.e., channels 10–16), granisetron suppressed the increase in the number and amplitude of duodenal PWs from baseline, which were evident within the first 15 min of the commencement of intraduodenal glucose infusion, in the channel closest to the infusion port (i.e., channel 16). These observations suggest that the local duodenal motor response to glucose is mediated via 5-HT3 receptors and that enterochromaffin cells may potentially play a role by releasing 5-HT in response to the presence of glucose. Although it would be of interest to determine whether topical anesthesia modifies the response to granisetron, the local anesthetic benzocaine does not appear to affect the effects of intraduodenal glucose on APD motility in healthy young subjects (5).

Acute changes in the blood glucose concentration, including deviations that are within the normal postprandial range, have been shown to have an impact on gastric motility and gastric emptying (8, 38). For example, in healthy young adults, hyperglycemia is associated with the stimulation of localized pyloric, and inhibition of antral, contractions (8). Hence, it was important to monitor blood glucose levels in this study. That granisetron had no effect on the glycemic response to intraduodenal glucose tends to discount a role for glycemia in the observed effects of granisetron on duodenal motility, but this issue warrants formal evaluation.

In conclusion, in healthy older subjects, the falls in systolic and diastolic blood pressure and rise in heart rate induced by intraduodenal glucose infusion are unaffected by granisetron, suggesting that the cardiovascular response to enteral glucose is mediated by mechanisms unrelated to the stimulation of 5-HT3 receptors. In contrast, granisetron suppressed the local duodenal motor response to intraduodenal glucose, suggesting that 5-HT3 receptors are involved in modulating the effects of glucose on small intestinal motility.

GRANTS

This study was supported by the National Health and Medical Research Council (NHMRC) of Australia. K. L. Jones’ salary is funded jointly by Diabetes Australia and the NHMRC of Australia. C. Feinle-Bisset is supported by a NHMRC of Australia Career Development Award.

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