Role of nitric oxide mechanisms in gastric emptying of, and the blood pressure and glycemic responses to, oral glucose in healthy older subjects

Diana Gentilcore,¹ Renuka Visvanathan,¹,² Antonietta Russo,¹ Reawika Chaikomin,¹ Julie E. Stevens,¹ Judith M. Wishart,¹ Anne Tonkin,³ Michael Horowitz,¹ and Karen L. Jones¹

¹Department of Medicine, University of Adelaide, Royal Adelaide Hospital, Adelaide; ²Aged and Extended Care Services, The Queen Elizabeth Hospital, Woodville, and ³Department of Clinical Pharmacology, University of Adelaide, Royal Adelaide Hospital, Adelaide, South Australia, Australia

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Gentilcore, Diana, Renuka Visvanathan, Antonietta Russo, Reawika Chaikomin, Julie E. Stevens, Judith M. Wishart, Anne Tonkin, Michael Horowitz, and Karen L. Jones. Role of nitric oxide mechanisms in gastric emptying of, and the blood pressure and glycemic responses to, oral glucose in healthy older subjects. Am J Physiol Gastrointest Liver Physiol 288: G1227–G1232, 2005. First published February 3, 2005; doi:10.1152/ajpgi.00511.2004.—The primary aims of this study were to evaluate the effects of the nitric oxide (NO) synthase inhibitor N⁴-nitro-L-arginine methyl ester (L-NAME) on gastric emptying (GE) of, and the blood pressure (BP), glycemic, insulin, and incretin responses to, oral glucose in older subjects. Eight healthy subjects (4 males and 4 females, aged 70.9 ± 1.3 yr) were studied on two separate days, in double-blind, randomized order. Subjects received an intravenous infusion of either L-NAME (180 µg·kg⁻¹·h⁻¹) or saline (0.9%) at a rate of 3 ml/min for 150 min. Thirty minutes after the commencement of the infusion (0 min), subjects consumed a 300-ml drink containing 50 g glucose labeled with 20 MBq ⁹⁹ᵐTc-sulfur colloid, while sitting in front of a gamma camera. GE, BP (systolic and diastolic), heart rate (HR), blood glucose, plasma insulin, and incretin hormones, glucose-dependant insulinoptropic-polypeptide (GIP), and glucagon-like peptide-1 (GLP-1), were measured. L-NAME had no effect on GE, GIP, and GLP-1. Between 0 and 150 min L-NAME had no effect on BP or HR. After the drink (0–60 min), systolic and diastolic BP fell (P < 0.05) and HR increased (P < 0.01) during saline; these effects were attenuated (P > 0.001) by L-NAME. Blood glucose levels between 90 and 150 min were higher (P < 0.001) and plasma insulin were between 15 and 150 min less (P < 0.001) after L-NAME. The fall in BP, increase in HR, and stimulation of insulin secretion by oral glucose in older subjects were mediated by NO mechanisms by an effect unrelated to GE or changes in incretin hormones.

N⁴-nitro-L-arginine methyl ester: postprandial; hypotension; elderly

POSTPRANDIAL HYPOTENSION, defined as a fall in systolic blood pressure (SBP) of ≥20 mmHg, occurring within 2 h of a meal (16, 18, 31, 32), is an important clinical problem by predisposing to a number of disorders including syncope, transient ischemic attacks, stroke, and angina (4, 16, 49). Those most at risk include the elderly and patients with autonomic nerve dysfunction; the latter is often secondary to diabetes mellitus (16, 18, 31, 32). Current therapies are suboptimal (16).

Potential mechanisms mediating postprandial hypotension are poorly defined and include impaired regulation of splanchnic blood flow (40), the release of gastrointestinal peptides (18), and disordered sympathetic nerve activity (29). The magnitude of the fall in blood pressure (BP) is known to be dependent on meal composition; ingestion of carbohydrate, particularly glucose, has the greatest effect on BP, whereas the effects of fat, protein, or water are substantially less (17, 39). We have recently established that the rate of nutrient delivery to the small intestine is an important determinant of the hypotensive response to enterally administered glucose (20, 33, 41). For example, in healthy older subjects, the magnitude of the fall in SBP and rise in heart rate (HR) are substantially greater when glucose is infused intraduodenally at a rate of ~3 kcal/min compared with 1 kcal/min (33). Nitric oxide (NO) is an important neurotransmitter in the gastrointestinal tract (3). The role of NO mechanisms is optimally addressed by the use of specific inhibitors of its production, such as N⁴-monomethyl-L-arginine (L-NMMA) (8), N⁴-nitro-L-arginine methyl ester (L-NAME) (7) or glyceryl trinitrate (3). Studies employing NO synthase blockers have established in animals that NO mechanisms are important in the regulation of splanchnic blood flow (2, 30). The effects of NO synthase inhibition on gastric emptying in humans have been assessed in two studies with inconsistent observations (15, 26). The major aims of our study were to evaluate the role of NO mechanisms in mediating the hypotensive response to oral glucose in healthy older subjects and to determine whether any effect was related to changes in gastric emptying.

The role of NO mechanisms in glucose-induced insulin secretion is controversial (17, 25). In particular, the outcome of animal studies and in vitro studies relating to the role of NO mechanisms on insulin secretion are inconsistent (9, 47). To our knowledge, no human studies have evaluated the role of NO in the insulin response to oral glucose; the latter is known to be dependent on the secretion of the so-called “incretin hormones,” GIP and GLP-1 (44). Our study design allowed evaluation of the effects of NO blockade on the glycemic, insulin, and incretin hormone responses to oral glucose.

MATERIALS AND METHODS

Subjects

Eight healthy older subjects, recruited by advertisement, (4 male and 4 female) with a mean age of 70.9 ± 1.3 yr and median body mass index of 26.5 kg/m² (22.2–29.2 kg/m²), were studied. All subjects were nonsmokers. None had a history of gastrointestinal disease or surgery, diabetes mellitus, significant respiratory, renal, or cardiac disease, chronic alcohol abuse, or epilepsy or was taking medication.

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known to influence BP or gastrointestinal function. In all subjects, resting (sitting) SBP and diastolic BP (DBP) were <160 and 90 mmHg, respectively, and the ECG was normal.

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

Protocol

Each subject underwent two studies in double-blind, randomized order, separated by at least 7 days. On 1 day subjects received an intravenous infusion of L-NAME and on the other an intravenous infusion of saline for 150 min. Subjects attended the Department of Nuclear Medicine, PET and Bone Densitometry at 0830 h following fasting (10.5 h for solids; 8.5 h for liquids). A cannula was placed in a right forearm vein for the infusion and in a left antecubital vein for blood sampling. Subjects were seated with their backs against a gamma camera and a BP cuff around the left arm.

Each subject rested comfortably in the sitting position for ~30 min. At ~30 min, an intravenous infusion of either L-NAME (180 \( \mu \)g\( \cdot \)kg\(^{-1} \cdot \)h\(^{-1} \)) (Clinalfa) (48) or saline (0.9\% w/w) was begun and continued at a rate of 3 ml/min for 150 min. The dose of L-NAME was based on previous studies (48, 51). At 0 min, subjects consumed a drink, which was at room temperature, and comprised 50 g glucose, 30 ml lemon juice, and 20 MBq \(^{99m}\)Tc-sulfur colloid, made up to a total volume of 300 ml with water. At 150 min, the intravenous cannulas were removed; the subject was then given a light meal and allowed to leave the laboratory. Cardiovascular autonomic nerve function was evaluated on one of the study days (11, 38).

Measurements

**BP and HR.** BPs (SBP and DBP) and HR were measured using an automated oscillometric BP monitor (Dinamap; Johnson & Johnson, Tampa, FL) immediately before the infusion (~30 min) at 3-min intervals between ~30 and 60 min and then at 15-min intervals between 60 and 150 min (21). Postprandial hypotension was defined as a fall in SBP ≥ 20 mmHg, after the glucose drink, which was sustained for at least 30 min (16). Baseline BP (i.e., 0 min) was calculated as the mean of measurements taken at 3, 6, and 9 min immediately before consumption of the drink.

**Gastric emptying.** Subjects consumed the drink within 2 min. Radioisotopic data were acquired for 120 min (60-s frames for the first 60 min and 3-min frames thereafter) (21). Time zero was defined as the time of completion of the drink. Data were corrected for subject movement, radionuclide decay, and gamma-ray attenuation (10). Regions-of-interest were drawn around the total stomach and gastric emptying curves (expressed as %retention over time) were derived for the total stomach at 0, 15, 30, 45, 60, 75, 90, 105, and 120 min. The 50% emptying time (T\(_{50}\)) was also determined (10).

**Blood glucose, plasma insulin, GIP, and GLP-1 concentrations.** Venous blood samples (~20 ml) were obtained immediately before the infusion (~30 min) at 15-min intervals between ~30 and 60 min and at 30-min intervals between 60 and 150 min. Blood glucose concentrations were determined immediately using a portable blood glucose meter (Medisense Companion 2 meter; Medisense, Waltham, MA) (21). Plasma was stored at −70°C until analysis of insulin concentrations by ELISA immunoassay (Diagnostic System Laboratories, Webster, TX) ( intra-assay coefficients of variation 2.6% at 8.48 \( \mu \)U/ml, 2.2% at 21.73 \( \mu \)U/ml, and 1.3% at 44.23 \( \mu \)U/ml) (41). Plasma GIP was measured by radioimmunoassay (35); the minimum detectable limit was 2 pmol/l and both intra-assay and interassay coefficients of variation were 15%. Plasma GLP-1 was determined by radioimmunoassay; the intra-assay coefficient of variation was 17% and interassay coefficient of variation 18% (35).

Cardiovascular autonomic function. Cardiovascular autonomic nerve function was evaluated using standardized cardiovascular reflex tests (11, 38). Parasympathetic function was evaluated by the variation (R-R interval) of the HR during deep breathing and the response to standing (“30:15”). Sympathetic function was assessed by the fall in SBP 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 (11, 38).

Statistical Analysis

Data are presented as means ± SE, unless stated otherwise. Contrasts were used to examine point-by-point comparisons to test preplanned hypotheses of interest. Repeated-measures ANOVA was used to examine the overall effects of treatment and time on the change in SBP, DBP, HR, blood glucose, plasma insulin, GIP, and GLP-1 concentrations. Where relevant, post hoc analysis was performed using Bonferroni/Dunn adjustment of the significant level. One-way repeated-measures ANOVA was conducted to evaluate the effects of treatment on SBP, DBP, HR, gastric emptying, blood glucose, plasma insulin, GIP, and GLP-1 concentrations [i.e., for the 30-min period immediately preceding the drink (i.e., ~30–0 min)] and 0–60 min, because the maximum postprandial fall in BP is known to occur during this time (16). Data were also evaluated between 0 and 120 min (i.e., after ingestion of the drink and while either L-NAME or saline was infused). All analyses were performed using StatView (version 5.0; Abacus Concepts, Berkeley, CA) and SuperANOVA (version 1.11, Abacus Concepts). A P value <0.05 was considered significant in all analyses.

RESULTS

The studies were well tolerated and there were no untoward events. No subject had definite autonomic neuropathy; the median score for autonomic nerve dysfunction was 1.0 (range: 0–2).

**BP and HR.**

There was no difference in SBP, DBP, or HR at ~30 min between the two groups (Fig. 1). Furthermore, there was no significant change in any of these parameters between ~30 and 0 min; nor any difference between the saline and L-NAME infusions (Fig. 1).

Between 0 and 60 min, SBP fell (P < 0.005) during the saline, but did not change (P = 0.17) during the L-NAME infusion (Fig. 1A). During saline infusion, the maximum fall in SBP was 9.5 ± 3.0 mmHg at 46.5 ± 4.4 min; in one subject, the magnitude of the fall in SBP was >20 mmHg. SBP had returned to baseline by ~75 min on the saline day. SBP was less during the saline, compared with the L-NAME, infusion between 0 and 60 min (P < 0.001) and between 0 and 120 min (P < 0.01).

Between 0 and 60 min, there was a fall in DBP during both saline (P = 0.05), and L-NAME (P = 0.02) infusions. DBP was less during the saline than the L-NAME infusion between 0 and 60 min (P < 0.001) and 0 and 120 min (P = 0.05) (Fig. 1B).

Between 0 and 60 min there was a rise in HR during the saline (P = 0.01) and a fall in HR (P < 0.005) during the L-NAME, infusion (Fig. 1C); between 0 and 60 min and 0 and 120 min HR was higher (P < 0.001 for both) during the saline than the L-NAME infusion.
GASTRIC EMPTYING

Gastric emptying (Fig. 2) approximated an overall linear pattern on both days; L-NAME had no effect on gastric emptying (e.g., T50; saline: 55.8 ± 3.8 min vs. L-NAME: 61.8 ± 3.8 min; P = 0.28) (Fig. 2).

Blood Glucose, Plasma Insulin, GIP, and GLP-1 Concentrations

There were no initial differences in blood glucose concentrations between the two days; however, between 90 and 150 min blood glucose levels were higher (P < 0.007) during the L-NAME infusion (Fig. 3A). Between 15 and 150 min, plasma insulin levels were lower after L-NAME (P < 0.001) (Fig. 3B). There was no difference in plasma GLP-1 (P = 0.55) or GIP (P = 0.39) concentrations between the 2 days. There was a significant “time” effect for GIP (P < 0.001 for both) on both days. Between -2 and 150 min, there was a rise in plasma GLP-1 (P < 0.02) over time during the saline infusion but not after L-NAME (P = 0.87).

DISCUSSION

The major observations in this study are that the NO synthase blocker, L-NAME, when administered acutely to healthy older subjects in a dose of 180 μg·kg⁻¹·h⁻¹, 1) had no effect on either BP or HR in the 30-min period before a glucose drink, 2) attenuated the falls in SBP and DBP and increase in HR after oral glucose, 3) had no effect on gastric emptying of glucose, 4) attenuated the glucose-induced rise in plasma insulin, and 5) had no effect on the incretin hormone (i.e., GIP and GLP-1) response to oral glucose. The study, accordingly, indicates that the magnitude of the fall in BP and increase in HR and stimulation of insulin secretion induced by oral glucose in healthy older subjects are mediated by NO mechanisms by an effect unrelated to changes in gastric emptying or the secretion of GIP and GLP-1.

Our recent studies (20, 34, 41) have established that gastric emptying is a determinant of the hypotensive response to oral glucose in both healthy older subjects and type 2 diabetes. For example, the slowing of gastric emptying and glucose absorption induced by guar is associated with a reduction in the magnitude of the fall in BP after a glucose drink (20, 41). Hence, in interpreting our observations, it was important to determine the effects of NO blockade on gastric emptying, particularly because the outcome of two previous studies is
conflicting (15, 26). Whereas Konturek et al. (26), reported that the NO synthase inhibitor L-NMMA administered in a dose of 1 mg·kg⁻¹·h⁻¹ accelerated gastric emptying of a nutrient liquid as assessed by a carbon breath test, Hirsch et al. (15) found that L-NMMA, given in a dose of 3.75 mg/kg for 5 min and 7.1 mg·kg⁻¹·h⁻¹, thereafter, had no effect on either gastric emptying of a pancake meal as assessed by the more sensitive scintigraphic method. Both studies involved healthy, young adult males. Our observations concur with those of Hirsch et al. (15) in that a dose of L-NAME, which had biological effects, did not affect gastric emptying and discount a role for gastric emptying in the observed effects of L-NAME on the BP and HR responses to oral glucose. Hence, these effects must be mediated by other mechanism(s).

We used a relatively low dose of L-NAME shown to affect cardiovascular function in young, healthy subjects (48, 51); much higher doses have been used by others (6, 12, 42, 50). Given that we observed highly significant effects on BP and HR, it appears that the degree of NO inhibition was adequate. Moreover, effects have also been observed in humans using lower doses of L-NAME (9). Hence, although ageing may be associated with defective NO release (23, 28), and it would certainly be of interest to evaluate the effects of a dose of L-NAME higher than we used, this may pose ethical issues. It is also appropriate to note that we cannot determine whether the cardiovascular effects induced by the infusion of L-NAME reflect the accumulation of the long-acting metabolite N⁵-nitro-L-arginine which may play a role in BP regulation (5, 12). This may have relevance to the observation that L-NAME had no effect on BP or HR in the 30-min period before consumption of the drink. Changes in sympathetic activity may also contribute to the cardiovascular effects of L-NAME (37, 42). Although only one of our subjects had postprandial hypotension, the mean fall in SBP during the saline infusion was substantial at 9.5 mmHg; this fall was abolished by L-NAME. Our observations may accordingly have implications for the treatment of postprandial hypotension, and the effects of NO synthase inhibition warrant evaluation in this group.

There are a number of potential pathways by which NO mechanisms could influence the cardiovascular response to oral glucose. Changes in splanchnic blood flow are likely to be important. Following a meal, there is an increase in splanchnic blood flow (31), associated with reductions in total systemic vascular resistance and skeletal muscle blood flow (36). The magnitude of the increase in mesenteric blood flow is comparable in normal young and older individuals, despite the greater

Fig. 3. Effects of intravenous administration of 0.9% saline and L-NAME (180 μg·kg⁻¹·h⁻¹) on blood glucose concentrations (A), plasma insulin concentrations (B), plasma glucose-dependent insulino-tropic-polypeptide (GIP) concentrations (C), and plasma glucagon-like peptide (GLP-1) concentrations (D). The intravenous infusions were given between −30 and 120 min. Data are means ± SE.
fall in BP in the elderly (27). There is compelling data from animal studies (2, 30) that NO mechanisms are important in the regulation of splanchnic blood flow. In pigs, L-NMMA attenuates the increase in mesenteric blood flow after a meal (2), and in rats, intestinal arteriolar distension induced by topical application of glucose is blocked by L-NAME (30). Further studies are also indicated to determine whether the effects of L-NAME on BP reflect changes in systemic and/or splanchnic blood flow. The effects of L-NAME on SBP and HR were sustained until at least 120 min (the time of completion of the L-NAME infusion); at this time gastric emptying was not completed and we are, accordingly, unable to determine whether the effects were dependent on ongoing nutrient entry into the small intestine, or not.

Various peptides released by food, including insulin, vasoactive intestinal polypeptide, substance P, neurotensin, and calcitonin gene-related peptide have been implicated in the etiology of postprandial hypotension (16, 18, 31). While insulin has vasodilatory properties (43), the observations that postprandial hypotension occurs in patients with type 1 diabetes, who are by definition insulin deficient (17), and that intravenous glucose does not affect BP in the elderly (19), argue against a significant role.

We observed that blood glucose levels between 90 and 150 min after the drink were higher and plasma insulin between 15 and 150 min less after L-NAME, indicating that NO mechanisms play a role in mediating insulin secretion induced by oral glucose. The involvement of NO mechanisms in insulin secretion remains controversial (1, 9, 13, 22, 45–47). Our observations concur with recent in vitro studies (24, 47) that support the concept that endogenous NO is involved in the regulation of insulin release; the effect of NO on insulin secretion may be concentration dependent (24). There is little information about the role of NO in insulin release in humans. Coiro et al. (9) reported that L-NAME inhibited the stimulation of insulin secretion induced by intravenous L-arginine (the substrate for NO) but not intravenous glucose in healthy young subjects. It should however, be noted that in this study (9), after intravenous glucose, plasma insulin levels were less with L-NAME. Furthermore, the dose of L-NAME used (i.e., 90 μg·kg⁻¹·h⁻¹) was much less than in our study (9), and the negative observations may, accordingly, reflect a lesser degree of NO blockade. It is also possible that the effects of NO blockade on glucose-induced insulin release are dependent on the route of glucose administration (i.e., enteral or parenteral), sympathetic nerve activity and/or blood flow. The relative increase in plasma insulin in response to oral, compared with intravenous, glucose is accounted for by the secretion of the incretin hormones, GIP and GLP-1 (14, 44). L-NAME had no effect on plasma GIP or GLP-1, discounting the possibility that the reduction in insulin secretion was attributable to a diminished incretin response.

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