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Effects of glucagon-like peptide-1 and sympathetic stimulation on gastric accommodation in humans¹

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Abstract

In humans, glucagon-like peptide-1 (GLP-1) delays gastric emptying by inhibiting vagal activity and also increases gastric volumes, by unclear mechanisms. Because GLP-1 inhibits intestinal motility by stimulating the sympathetic nervous system in rats, we assessed the effects of a GLP-1 agonist and yohimbine, an α_2 -adrenergic antagonist, on gastric volumes in humans. In this double-blind study, 32 healthy volunteers were randomized to placebo, a GLP-1 agonist, yohimbine or GLP-1 and yohimbine. Gastric volumes (fasting predrug and postdrug, and postprandial postdrug) were measured by ^{99m}Tc single photon emission computed tomography imaging. Plasma catecholamines and haemodynamic parameters were assessed. Compared with placebo, GLP-1 increased ($P = 0.03$) but yohimbine did not affect fasting gastric volume. However, GLP-1 plus yohimbine increased ($P < 0.001$) postprandial gastric accommodation vs placebo and vs GLP-1 alone [postprandial volume change = 542 ± 29 mL (mean $\pm$ SEM, placebo), 605 ± 31 mL (GLP-1), 652 ± 54 mL (yohimbine) and 810 ± 37 mL (GLP-1 and yohimbine)]. Plasma noradrenaline and dihydroxyphenylglycol concentrations were higher for yohimbine vs placebo and for GLP-1 and yohimbine vs GLP-1. Yohimbine stimulates central sympathetic activity and in combination with GLP-1, augments postprandial accommodation in humans.

Keywords

accommodation; GLP-1; stomach; stress; sympathetic; yohimbine

INTRODUCTION

Glucagon-like peptide-1 (GLP-1) is an incretin produced by enteroendocrine L cells in the small intestine.¹ The GLP-1 agonist exenatide improves glycaemic control in patients with type II diabetes mellitus inadequately treated with oral hypoglycaemic agents.² In addition to its hormonal effects (i.e. stimulating insulin release and reducing glucagon release) GLP-1 also has powerful effects on gastrointestinal (GI) motility and secretion. In healthy subjects, GLP-1 reduced antral and small bowel motility, increased pyloric tone, delayed gastric emptying for

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solids and liquids, reduced gastric acid secretion and increased gastric volumes.^{3–6} It is necessary to understand the mechanisms of these GI effects because they probably contribute to the therapeutic and side effects of GLP-1. For example, in 1 study, 57% of patients with type II diabetes mellitus (DM) treated with the GLP-1 agonist exenatide reported nausea and 17% reported vomiting;⁷ these side effects prompted discontinuation of therapy in 6% of patients randomized to exenatide.

Considerable evidence suggests that the effects of GLP-1 on antroduodenal motility, pyloric tone and acid secretion are mediated through vagal nerve inhibition. Thus, GLP-1 blocked the gastric acid secretory response to sham feeding, which is indicative of vagal inhibition.⁸ Moreover, GLP-1 did not inhibit acid secretion in vagotomized subjects⁵ and did not inhibit gastric motility after vagal denervation in rats⁹ or pigs.¹⁰ However, the mechanisms responsible for the GLP-1-induced increase in gastric volume are not completely understood.³ Because vagally-mediated release of nitric oxide is at least partly responsible for gastric accommodation,^{11–14} GLP-1-mediated vagal inhibition would be expected to reduce, not augment, accommodation. Thus, it is difficult to understand why GLP-1 did not increase gastric volume in diabetic patients with vagal neuropathy,¹⁵ unless one assumes that these patients also had an enteric neuropathy associated with diabetes mellitus.^{16,17} GLP-1 may also modulate GI motor activity by modulating sympathetic pathways. In addition to modulating vagal activity, a peptidase-resistant analogue of GLP-1 (exendin-4) activated catecholaminergic neurones in the hypothalamus, brainstem and adrenal medulla of rats.¹⁸ Moreover, GLP-1 inhibited GI motor activity in rats by stimulating adrenergic pathways.¹⁹ α_2 -Adrenergic receptors are primarily responsible for the inhibitory effects of sympathetic stimulation on GI motility in humans.²⁰ Based on these data, we hypothesized that α_2 -adrenergic receptors mediated GLP-1-induced increased gastric volumes in humans. Our aims were to compare the effects of GLP-1 and the α_2 -adrenergic antagonist yohimbine alone and in combination on gastric volumes in humans.

METHODS

This report is part of a larger study assessing a number of discrete hypotheses on the modulation of gastric volumes by GLP-1, adrenergic and nitrergic pathways in 48 subjects. Observations from 16 subjects on the nitrergic modulation of gastric volumes and the interaction between GLP-1 and the nitrergic pathways will be reported separately. Thirty-two healthy adults aged 18–54 years (mean age, 33 years; 23 women and nine men) participated in this study. None had previous GI surgery (other than appendectomy or cholecystectomy), significant underlying illnesses, or medication use. Functional GI disorders, anxiety and depression were excluded by validated screening questionnaires,²¹ a clinical interview, and a physical examination. Diabetes mellitus was excluded by checking the fasting serum glucose. The studies were approved by the Mayo Clinic Institutional Review Board and conformed to the Declaration of Helsinki guidelines for studies with human subjects.

Experimental design

The hypothesis of this study was that α_2 -adrenergic receptors mediate GLP-1-induced increased gastric volumes in humans. The experimental protocol is illustrated in Fig. 1. Fasting and postprandial gastric volumes, plasma catecholamines and haemodynamic parameters were studied during i.v. infusion of placebo or medication, before and after a meal. Two peripheral venous cannulae were used for drug infusion and a separate peripheral venous cannula was used for blood sampling. Medications were given as a 10-min bolus followed by a continuous infusion lasting for the duration of the study (i.e. 55 min). Subjects were randomized, in a double-blind manner, to one of six arms, i.e. placebo, GLP-1, the nitric oxide synthase (NOS) inhibitor N^G-monomethyl-L-arginine (L-NMMA), the α_2 -adrenoreceptor antagonist

yohimbine, GLP-1 combined with L-NMMA (low dose) [i.e. GLP-1/L-NMMA (low dose)] or GLP-1 combined with yohimbine (i.e. GLP-1/yohimbine). This randomization was balanced on gender and body mass index (BMI; i.e. ≤ 25 vs > 25 kg m⁻²). Due to this stratification the number of subjects randomized to each group was similar but not identical. As control or placebo, we administered 15 mL of 0.9% saline as a 'bolus' followed by an infusion at 42.9 mL h⁻¹ for the entire study. When only one drug was administered, saline was administered through the second i.v. cannula. As noted above, data for subjects randomized to L-NMMA alone or GLP-1 and L-NMMA will be reported separately.

^{99m}Tc-SPECT for measuring gastric volume and accommodation

Gastric volumes were assessed by single photon emission computed tomography (SPECT) as described previously.^{22–24} After i.v. injection of ^{99m}Tc-sodium pertechnetate (10 mCi), dynamic tomographic images of the gastric wall were acquired during fasting and for a total of 32 min after ingestion of a 300 mL nutrient drink (EnsureTM, Abbott Laboratories, Abbott Park, IL, USA; 1 kcal mL⁻¹) through a straw by a dual-head gamma camera (Helix SPECT System, Elscint, Haifa, Israel) in a multiorbit mode system. As the ^{99m}Tc is taken up by gastric parietal and mucus-secreting epithelial cells, the entire gastric mucosa is visualized by this technique. A three-dimensional rendering of the stomach and its volume was obtained using the AVW 3.0 (Core B; Biomedical Imaging Resource, Mayo Foundation, Rochester, MN, USA) image processing libraries.

Medications

Glucagon-like peptide-1 and yohimbine were used under investigator-initiated Investigational New Drug (IND) approvals from the US Food and Drug Administration. All infusions were prepared by standard practice in the Mayo GCRC Research Pharmacy using current ASHP class III procedures for sterile preparation.

Glucagon-like peptide-1—GLP-1 (7–36 amide; Bachem, San Diego, CA, USA) was infused at a dose (i.e. 1.2 pmol kg⁻¹ min⁻¹), which results in supraphysiological plasma levels, inhibited antro-duodenal contractility, and increased pyloric tone and gastric volumes in healthy subjects.^{3,4} In an attempt to achieve steady-state levels more rapidly, this infusion was preceded by a 'bolus' dose of 2.4 pmol kg⁻¹ min⁻¹ for 10 min.⁴

Yohimbine—Yohimbine HCl (Spectrum Chemical, Gardena, CA, USA) was administered as a 0.125 mg kg⁻¹ bolus over 10 min followed by an infusion at 0.06 mg kg⁻¹ h⁻¹. This dose is safe for administration to healthy subjects, elicited a two- to threefold increase in plasma noradrenaline concentrations, and stimulated colonic motility in humans.^{20,25}

Plasma catecholamines

A 10 mL venous blood sample was drawn before and after drug infusion during fasting conditions, at 10, and at 20 min after a meal. Blood samples were collected in chilled glass tubes containing 0.1 mL of 15% ethylenediaminetetraacetic acid for measurement of glucose and plasma pancreatic polypeptide (PP) and in tubes containing 0.05 mL of 10% sodium metabisulphite for catecholamines. Plasma was obtained by refrigerated centrifugation and fast-frozen in dry ice and acetone before storage at -70 °C until assayed. Catecholamines were extracted from plasma²⁶ and subjected to HPLC with electrochemical detection.²⁷ In addition to plasma adrenaline and noradrenaline, plasma dihydroxyphenylglycol (DHPG), which is the main metabolite produced by deamination of noradrenaline, was also measured.²⁸

Statistical analysis

The effect of a meal on gastric volumes was analysed by a paired *t*-test. Overall treatment effects on fasting postdrug and postprandial postdrug parameters were assessed by analyses of covariance (ANCOVA) using gender, BMI and the corresponding fasting predrug values as covariates. Thereafter, pairwise comparisons (i.e. between placebo and GLP-1, placebo and yohimbine, and GLP-1 and GLP-1/yohimbine) were performed. The relationship between plasma catecholamines and gastric volumes was assessed by Spearman's rank correlation. A rank transformation was used for parameters which were not normally distributed. Because this was an intent-to-treat (ITT) analysis, missing data were imputed using the corresponding mean value over all non-missing data values. The error degrees of freedom in the respective ANCOVA models were decreased by 1 for each missing value imputed for a given response parameter. The data presented in the text, tables and figures are the 'raw' mean ($\pm$ SE), unadjusted for covariates and do not include any imputed values.

The sample size estimate assumed that the variability in fasting and postprandial gastric volume for this study would be comparable with a previous study.³ With eight subjects per group, there was approximately 80% power, at an α -level of 0.05, to detect a difference of 140 mL in gastric accommodation (i.e. postprandial — fasting gastric volume) values between two treatment groups based on a two-sample *t*-test. This effect size was smaller than the observed effect of GLP-1 vs placebo on postprandial gastric volume change (i.e. postprandial — fasting volume) in healthy subjects.³

RESULTS

None of the subjects experienced significant adverse events during these studies. At baseline, there were no clinically important differences in age, gender, BMI, fasting serum glucose, plasma noradrenaline, plasma DHPG or fasting gastric volume among groups (Table 1).

Effects on fasting and postprandial gastric volume

Overall treatment effects on postdrug fasting gastric volume were borderline significant ($P = 0.06$; Table 2, Fig. 2). Pairwise comparisons suggested that GLP-1 increased ($P = 0.03$) fasting volume compared with placebo. Yohimbine did not affect fasting volume compared with placebo. In contrast, overall treatment effects ($P < 0.0001$) on the postprandial change in gastric volume were significant. Pairwise comparisons revealed that postprandial gastric volume change was higher ($P = 0.0007$) for GLP-1 and yohimbine (i.e. 810 ± 37 mL) than for GLP-1 alone (i.e. 605 ± 31 mL).

Effects on catecholamines

Due to technical difficulties with drawing blood, two subjects had one or more missing values for DHPG or NE. Overall treatment effects on fasting postdrug ($P = 0.02$), early postprandial ($P = 0.003$) and late postprandial ($P = 0.0003$) plasma noradrenaline concentrations were significant. Yohimbine increased ($P \leq 0.04$) plasma noradrenaline concentrations compared with placebo during the postprandial period (Fig. 3). The combination of GLP-1 and yohimbine increased ($P \leq 0.01$) plasma noradrenaline concentrations compared with GLP-1 alone during the fasting and postprandial periods. The relationship between the change in fasting plasma noradrenaline (i.e. postdrug—predrug) and the change in fasting gastric volume (i.e. fasting postdrug—predrug) was not significant. In contrast, the relationship between the postprandial change in plasma noradrenaline (i.e. postprandial postdrug—fasting predrug) and the postprandial change in gastric volume was significant ($r = 0.40$, $P = 0.03$).

Yohimbine increased ($P \leq 0.03$) plasma DHPG concentrations compared with placebo at during the early and late postprandial epochs while GLP-1 and yohimbine increased ($P = 0.03$) plasma

DHPG concentrations compared with GLP-1 alone during the early postprandial epoch (Fig. 4).

Plasma adrenaline concentrations were below the threshold of detection for the assay (i.e. <20 pg mL⁻¹) in most subjects (data not shown).

Effects on haemodynamic parameters

Overall treatment effects on blood pressure ($P = 0.3$) heart rate and ($P = 0.06$) were not significant. Pairwise comparisons revealed that the combination of GLP-1 and yohimbine increased ($P = 0.05$) the heart rate compared with GLP-1 alone (Table 3).

DISCUSSION

The incretin GLP-1 regulates postprandial glycaemia by modulating glucoregulatory hormones and by delaying gastric emptying.² Previous studies suggest that GLP-1 agonists inhibit gastric motility and acid secretion by vagal blockade.^{4,29} However, vagal inhibition would be anticipated to reduce, not increase gastric volumes. Based on a previous study in rats,³⁰ we hypothesized that the sympathetic nervous system was responsible for increased gastric volumes during administration of GLP-1. Contrary to our hypothesis, we observed that GLP-1 and the α_2 -adrenergic antagonist yohimbine acted synergistically to augment postprandial gastric accommodation in humans.

Yohimbine and clonidine can affect sympathetic outflow and GI motility by interacting with central and peripheral α_2 -adrenergic receptors.^{31,32} We previously showed that the α_2 -agonist clonidine relaxed the stomach in humans,³³ probably because stimulation of α_2 -adrenergic receptors reduced acetylcholine release in the myenteric plexus and at the neuromuscular junction. Clonidine also reduces colonic tone in humans.^{20,34,35} In contrast, yohimbine increased colonic tone in humans, presumably by blocking peripheral α_2 -receptors in the myenteric plexus and at the neuromuscular junction.^{20,34} Therefore, we anticipated that yohimbine, administered at the same dose here as in our previous colonic studies, would reduce gastric accommodation via peripheral effects.

To our surprise, yohimbine and GLP-1 given concurrently augmented postprandial accommodation. Because yohimbine increased plasma noradrenaline and DHPG, indicating activation of sympathetic neurones,²⁸ these observations, including the relationship between plasma noradrenaline and postprandial gastric accommodation, suggest that the central effect of yohimbine (i.e. increased gastric volumes) overwhelmed its peripheral effect (i.e. reduced gastric volumes). In contrast, the previously demonstrated effects of yohimbine on colonic motor activity were more likely due to peripheral actions.^{20,34}

Yohimbine did not increase fasting gastric volume. The effects of yohimbine on plasma catecholamines and DHPG as also on gastric volume were more pronounced after a meal, consistent with previous studies demonstrating that ingestion of carbohydrate-rich or mixed meals in healthy young subjects is associated with reduced splanchnic and skeletal muscle vascular resistance as also increased sympathetic nervous outflow, heart rate, cardiac output and plasma noradrenaline concentrations.^{36,37}

The observed effects of yohimbine on gastric volumes are consistent with the concept that the sympathetic nervous system inhibits gastric motility. For example, experimental stress by the cold pressor test or labyrinthine stimulation reduced gastric antral motor activity and delayed gastric emptying in humans.^{38,39} Yohimbine provides a robust pharmacological model of anxiety,⁴⁰ yet yohimbine's effects on postprandial accommodation are contrary to the observation that experimentally induced anxiety reduced gastric accommodation in humans.

⁴¹ Perhaps these differences reflect the fact that the effects of stress on GI motility are mediated by more than one mechanism (i.e. reduced vagal tone, increased sympathetic tone).⁴² The effect of yohimbine on symptoms, heart rate, blood pressure and plasma catecholamines is exaggerated in patients with panic disorder and post-traumatic stress disorder,⁴³ partly reflecting stress-related increased noradrenaline turnover and increased neuronal responsiveness to excitatory stimulation in the locus ceruleus.⁴³ Along the same lines, further studies are required to compare the effect of yohimbine on gastric volumes in health and dyspepsia, particularly in patients with increased anxiety.

In rats, central or peripheral GLP-1 agonists (i.e. exendin-4) increase blood pressure and heart rate and stimulate sympathetic activity by stimulating brainstem nuclei regulating sympathetic activity (i.e. area post-trema).^{18,44} Moreover, GLP-1 inhibits intestinal motility by stimulating sympathetic pathways in rats.³⁰ However, previous studies suggest that acute administration of GLP-1 does not significantly affect haemodynamic parameters in other species, including monkeys⁴⁵ or when infused continuously for 6 weeks in type II diabetic subjects.⁴⁶ Our data confirm that in healthy subjects GLP-1 did not affect haemodynamic parameters or plasma catecholamines alone or act synergistically with yohimbine to augment sympathetic activity.

In contrast to a previous study,³ GLP-1 did not increase postprandial gastric volumes in this study. Using the observed variation in postprandial gastric volume change (pooled SD = 109 mL), we had 80% power to detect a difference of 165 mL between placebo and GLP-1 with $n = 8$ per group based on a two-sample t -test (and two-sided α -level of 0.05). The ANCOVA would have provided similar power for somewhat smaller pairwise differences between groups by incorporating relevant covariates. The observed differences in mean postprandial volume change were not that large (i.e. 542 mL for placebo vs 605 mL for GLP-1). Thus, this does not appear to be a type II error. Rather, we suspect that differences between placebo and GLP-1 were not significant because the postprandial volume change for subjects randomized to placebo was higher (i.e. 542 mL) for this compared with our previous study (i.e. 435 mL).

We recently showed that nitric oxide mediated the effect of GLP-1 on postprandial gastric volume (data under review). However, it is unclear how GLP-1 increases fasting gastric volume. A recent abstract suggested that peripherally administered GLP-1 at physiological plasma levels stimulated vagal afferents.⁴⁷ Moreover, intraportal administration of a truncated form of GLP-1 stimulated afferents in the hepatic vagus, thereby activating efferents in pancreatic branches of the vagus nerve.⁴⁸ These observations suggest that GLP-1 may modulate GI functions via GLP-1 receptors on vagal afferents, which in turn activate vago-vagal pathways. Similar to enteropancreatic and other vago-vagal reflexes, these pathways probably entail sensory vagal afferents that travel to brainstem vagal nuclei and project to excitatory cholinergic or inhibitory non-adrenergic non-cholinergic postganglionic neurones in the stomach to complete the loop.^{49,50}

Conceivably, this effect is mediated by stimulation of vagal afferents⁴⁷ and activation of peripheral vagovagal pathways.⁴⁸ GLP-1 also releases somatostatin,⁵¹ which can increase fasting gastric volume.⁵² However, the inhibitory effect of GLP-1 on fasting small bowel motility is not mediated by somatostatin.⁵³ Because direct arterial infusion of GLP-1 did not inhibit antral or pyloric motor activity in dogs, a direct effect of GLP-1 on gastric smooth muscle seems unlikely.⁵⁴ Although it is conceivable that GLP-1 may increase postprandial gastric volume by slowing gastric emptying (in addition to gastric relaxation), it appears unlikely that delayed gastric emptying contributes to the effects of GLP-1 on fasting gastric volume. GLP-1 reduces gastric acid secretion,^{5,8} which would be anticipated to reduce and not increase gastric volumes.

In conclusion, we confirmed that GLP-1 augments fasting gastric volume and postprandial gastric accommodation in healthy subjects. Yohimbine also augmented GLP-1-mediated postprandial gastric accommodation in healthy subjects, in part by centrally mediated increased sympathetic tone.

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Figure 1.

Experimental design. In addition to fasting and postprandial gastric volumes, haemodynamic parameters and plasma catecholamines were assessed during the study. The asterisks indicate time points when blood samples were obtained.

Figure 2.

Effects of drugs on gastric volumes. Glucagon-like peptide (GLP-1) increased fasting gastric volume. GLP-1 and yohimbine combined increased postprandial gastric accommodation. * $P = 0.03$ vs placebo; † $P = 0.0007$ for GLP-1 and yohimbine vs GLP-1 alone.

Figure 3.

Effect of drugs on plasma noradrenaline. During the fasting and postprandial periods, plasma noradrenaline concentrations were higher among subjects who received yohimbine alone or in combination with glucagon-like peptide (GLP-1), compared with placebo or to GLP-1 alone, respectively. * $P < 0.05$ for yohimbine vs placebo; † $P < 0.05$ for GLP-1 and yohimbine vs GLP-1 alone.

Figure 4.

Effect of drugs on plasma dihydroxyphenylglycol (DHPG). During the postprandial period, plasma DHPG concentrations were higher among subjects who received yohimbine alone or in combination with glucagon-like peptide (GLP-1), compared with placebo or to GLP-1 alone, respectively. * $P < 0.05$ for yohimbine vs placebo; † $P < 0.05$ for GLP-1 and yohimbine vs GLP-1 alone.

Table 1

Demographic and baseline characteristics

	Placebo	GLP-1	Yohimbine	GLP-1 + yohimbine
N	8	7	7	10
Age (years)	34 ± 5	34 ± 3	37 ± 3	29 ± 3
Gender (% female)	75			70
BMI (kg m ⁻²)	26 ± 2	27 ± 2	26 ± 2	27 ± 2
Fasting serum glucose (mg dL ⁻¹)	87 ± 2	88 ± 2	88 ± 2	89 ± 2
Postprandial serum glucose (mg dL ⁻¹)	95 ± 4	79 ± 5	90 ± 6	90 ± 8
Plasma noradrenaline (pg mL ⁻¹)	214 ± 31	177 ± 33	266 ± 77	176 ± 35
Plasma DHPG (pg mL ⁻¹)	1578 ± 266	1514 ± 206	1732 ± 168	1430 ± 79

BMI, body mass index; DHPG, dihydroxyphenylglycol; GLP, glucagon-like peptide.
All values except gender are actual mean ± SEM.
Postprandial serum glucose values were measured 20 min after Ensure (300 mL).

Table 2
Effects of drugs on fasting and postprandial gastric volume

	Fasting volume (mL)			Postdrug	Change	Postprandial volume (mL)	Difference (postprandial — fasting volume)
	Predrug						
Placebo	210 ± 18		227 ± 14		17 ± 11	774 ± 18	542 ± 29
GLP-1	230 ± 31		291 ± 35*		61 ± 13	896 ± 39	605 ± 31
Yohimbine	271 ± 16		308 ± 14		37 ± 18	959 ± 65	652 ± 54
GLP-1 + yohimbine	210 ± 18		273 ± 18		62 ± 10	1082 ± 46	810 ± 37†

All values are actual mean ± SEM.

* $P = 0.03$ vs placebo.

† $P < 0.01$ vs glucagon-like peptide (GLP)-1 alone.

Table 3

Effect of drugs on haemodynamic parameters

Group	Baseline		Postdrug fasting		Postdrug postprandial	
	MAP (mmHg)	Heart rate (min ⁻¹)	MAP (mmHg)	Heart rate (min ⁻¹)	MAP (mmHg)	Heart rate (min ⁻¹)
Placebo	84 ± 4	64 ± 7	83 ± 4	67 ± 4	83 ± 6	71 ± 3
GLP-1	82 ± 4	65 ± 3	84 ± 3	68 ± 3	87 ± 7	69 ± 2
Yohimbine	85 ± 6	61 ± 3	93 ± 8	64 ± 3	87 ± 10	66 ± 4
GLP-1 + yohimbine	87 ± 5	63 ± 3	94 ± 6	70 ± 4*	93 ± 2	69 ± 3

MAP, mean arterial pressure; GLP, glucagon-like peptide.

All values are actual mean ± SEM.

* $P = 0.05$ vs GLP-1 alone.