Effects of glucagon-like peptide-1, yohimbine, and nitric modulation on sympathetic and parasympathetic activity in humans

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Bharucha AE, Charkoudian N, Andrews CN, Camilleri M, Sletten D, Zinsmeister AR, Low PA. Effects of glucagon-like peptide-1, yohimbine, and nitric modulation on sympathetic and parasympathetic activity in humans. Am J Physiol Regul Integr Comp Physiol 295: R874–R880, 2008. First published July 2, 2008; doi:10.1152/ajpregu.00153.2008.—Glucagon-like peptide-1 (GLP-1), an incretin, which is used to treat diabetes mellitus in humans, inhibited vagal activity and activated nitric pathways. In rats, GLP-1 also increased sympathetic activity, heart rate, and blood pressure (BP). However, the effects of GLP-1 on sympathetic activity in humans are unknown. Our aims were to assess the effects of a GLP-1 agonist with or without α2-adrenergic or -nitrergic blockade on autonomic nervous functions in humans. In this double-blind study, 48 healthy volunteers were randomized to GLP-1-(7-36) amide, the nitric oxide synthase (NOS) inhibitor N(G)-monomethyl-L-arginine acetate (L-NMMA), the α2-adrenergic antagonist yohimbine, or placebo (i.e., saline), alone or in combination. Hemodynamic parameters, plasma catecholamines, and cardiac sympathetic and parasympathetic modulation were measured by spectral analysis of heart rate. Therefore, the effects of GLP-1-(7-36) amide on muscle sympathetic nerve activity (MSNA) were assessed by microneurography in seven subjects. GLP-1 increased (P = 0.02) MSNA but did not affect cardiac sympathetic or parasympathetic indices, as assessed by spectral analysis. Yohimbine increased plasma catecholamines and the low-frequency (LF) component of heart rate power spectrum, suggesting increased cardiac sympathetic activity. L-NMMA increased the BP and reduced the heart rate but did not affect the balance between sympathetic and parasympathetic activity. GLP-1 increases skeletal muscle sympathetic nerve activity but does not appear to affect cardiac sympathetic or parasympathetic activity in humans.

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is an incretin produced by enterocentocrine L cells in the small intestine. Under hyperglycemic conditions, GLP-1 regulates blood glucose by reducing glucagon secretion and increasing insulin secretion by the pancreas, and by delaying gastric emptying by vagal inhibition. Unlike the native peptides [i.e., GLP-1-(7-36) amide and GLP-1-(9-36) amide], the GLP-1 receptor agonist exendin-4 is resistant to inactivation by dipeptidyl peptidase IV and is used as an adjunct to oral hypoglycemic therapy in type II diabetes mellitus (DM).

GLP-1 is also synthesized by neurons in the nucleus of the solitary tract, and GLP-1 receptors are also found in the heart and in areas of the central nervous system that govern autonomic control (25, 49). Peripheral and central GLP-1 agonists induce c-fos expression, suggesting they activate the adrenal medulla and autonomic regulatory neurons in the rat brain (53). GLP-1 receptor agonists have dose-dependent chronotropic and pressor responses in rodents (5, 53), and GLP-1 agonists improve cardiac output and blood pressure (BP) in dogs with pacing-induced cardiomyopathy (28). While GLP-1 did not increase BP in monkeys (55) or during continuous subcutaneous infusion for 6 wk in humans (56), it has beneficial effects on left ventricular function after acute myocardial infarction (29) and on vasodilatation in healthy subjects and patients with type II DM and stable coronary disease (6, 32). GLP-1 has also been implicated to cause tachycardia in patients who have rapid gastric emptying after gastric resection (i.e., postgastrectomy dumping syndrome), because these patients have increased heart rate, plasma GLP-1, and plasma norepinephrine concentrations after an oral glucose challenge (54). However, the effects of GLP-1 on muscle sympathetic nerve activity (MSNA) and cardiac sympathetic and parasympathetic regulation in humans have not been studied. These questions are important because diabetic autonomic neuropathy is associated with significant increases in morbidity and mortality, including an increased risk of sudden death (37, 42, 43).

The aims of this study were to assess the effects of GLP-1, either alone or in combination with the α2-adrenergic antagonist yohimbine, or the nitric oxide synthase (NOS) inhibitor N(G)-monomethyl-L-arginine acetate (L-NMMA) on the hemodynamic parameters, cardiac sympathetic and parasympathetic tone, as assessed by time frequency distribution of heart rate power spectrum and BP, and plasma catecholamines in humans. Because nitric oxide (NO) mediates some effects of GLP-1 (e.g., inhibition of small bowel motility in rats) (48)—protection against ethanol-induced gastric mucosal lesions (20) and relaxation of vascular endothelium (31)—and because NO also augments cardiac vagal control in humans (10), we also evaluated the NOS inhibitor L-NMMA, alone and in combination with GLP-1 in these studies. Yohimbine and placebo were used as positive and negative controls of cardiac sympathetic activity, respectively. After noting that GLP-1 did not significantly affect cardiac autonomic functions as assessed by spectral analysis, we subsequently assessed the effects of GLP-1 on skeletal muscle vasoconstrictor nerve activity measured by microneurography.

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METHODS

Overall Experimental Design

This report incorporates observations on autonomic parameters from two studies. Experiment 1 was designed to assess whether nitrergic and/or α2-adrenergic mechanisms mediate the effects of GLP-1, which is released in response to oral nutrient infusion, on postprandial gastric accommodation. Data on gastric effects, but not autonomic parameters (i.e., hemodynamic effects, spectral analysis of heart rate and BP), have been presented elsewhere (1, 2). Therefore, these studies evaluated the effects of GLP-1 alone or in combination with the NOS inhibitor l-NMMA or the α2-adrenoceptor antagonist yohimbine on autonomic and hemodynamic parameters under fasting and postprandial conditions. Subsequently, the effects of GLP-1 on skeletal muscle vasoconstrictor activity measured by microneurography were evaluated in experiment 2.

Subjects

Fifty-five healthy volunteers, aged 18–54 years (mean age, 31 yr; 42 women) were recruited by public advertisement. None had significant underlying illnesses or medication use, except for oral contraceptives. Functional GI disorders, anxiety, and depression were excluded by validated screening questionnaires (46), 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. Initially, 48 subjects (mean age, 31 yr; 36 women) were studied in experiment 1. Subsequently, seven subjects (mean age, 26 yr; 6 women) were studied in experiment 2.

Experimental Design for Experiment 1

These studies were conducted after an overnight fast. Two peripheral venous canulas inserted for drug infusion and a separate peripheral venous canula inserted used for blood sampling. All studies took place in the morning. Subjects were then placed in the supine position in a quiet room with lights dimmed. At least 30 min was allowed between canula insertion and study commencement. To simulate meal-stimulated GLP-1 release, these studies were conducted under fasting and postprandial conditions. Heart rate, BP, and plasma catecholamines were studied during baseline and during intravenous infusion of placebo or drugs, both before and after a 300-kcal liquid meal (Ensure, Abbott Laboratories, Abbott Park, IL; 1 kcal/ml) (Fig. 1). Drugs were given as a 10-min bolus followed by a continuous infusion lasting for the remainder of the study. Subjects were randomized to each group in a double-blind manner, to one of six arms: placebo, GLP-1, the NOS inhibitor l-NMMA, the α2-adrenoceptor antagonist yohimbine, GLP-1 combined with l-NMMA (i.e., GLP-1/l-NMMA), or GLP-1 combined with yohimbine (i.e., GLP-1/yohimbine). This randomization was balanced on sex and body mass index (BMI) (i.e., <25 vs. >25 kg/m²). Because of 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 for the entire study. When only one drug was administered, saline was administered through the 2nd intravenous cannula.

Drugs

GLP-1, l-NMMA, and yohimbine were used under investigator-initiated Investigational New Drug approvals from the U.S. Food and Drug Administration. All infusions were prepared by standard practice in the Mayo General Clinical Research Center Research Pharmacy using current American Society of Health-System Pharmacists class III procedures for sterile preparation.

Glucagon-like peptide-1. GLP-1 (7-36 amide) (Bachem, San Diego, CA) was infused at 2.4 pmol·kg⁻¹·min⁻¹ for 10 min followed by 1.2 pmol·kg⁻¹·min⁻¹ for the remainder of the study. This dosing regimen produced supraphysiological plasma levels and normalized blood glucose concentrations in type II DM (23). In healthy subjects, this dose inhibited antro-duodenal contractility, increased pyloric tone, and also increased fasting gastric volumes (1, 40).

Nω-monomethyl-l-arginine acetate. In experiment 1, Nω-monomethyl-l-arginine acetate (t-NMMA) (Clinalfa AG, Laülfingen, Switzerland), a NOS inhibitor, was infused at 4 mg·kg⁻¹·h⁻¹ in healthy subjects, this dose stimulated small intestinal motility (38) and reduced postprandial accommodation (45). In a previous study, a lower dose (i.e., 3 mg·kg⁻¹·h⁻¹) increased BP and induced bradycardia (10).

Yohimbine. Yohimbine HCl (Spectrum Chemical, Gardenia, CA) was administered as a bolus (i.e., 0.125 mg/kg over 10 min) followed by an infusion (i.e., 0.06 mg·kg⁻¹·h⁻¹). This dose is safe for administration to healthy subjects, elicited a two- to three-fold increase in plasma norepinephrine levels, and stimulated colonic motility in humans (7, 14).

Plasma catecholamines. A 10-ml venous blood sample was drawn before and after drug infusion during fasting conditions and at 10 min after a meal (Fig. 1). Blood samples were collected in chilled glass tubes containing 0.05 ml of 10% sodium metabisulfite for catecholamines. Plasma was obtained by centrifugation and fast-frozen in dry ice and acetone before storage at −70°C until assayed. Catecholamines (epinephrine, norepinephrine) were extracted from plasma (12) and subjected to HPLC with electrochemical detection (18). About 70% of norepinephrine released by sympathetic nerves is recycled after release by sympathetic nerves is deaminated to plasma dihydroxyphenylethylglycol (DHPG), which is almost completely derived from metabolism of neuronal norepinephrine (11). Therefore, plasma norepinephrine measurements were complemented by measuring plasma DHPG.

Spectral Analysis

Fig. 1. Design for experiments 1 and 2. Hemodynamic parameters, spectral analysis of heart rate and blood pressure, and plasma catecholamines were assessed as shown.
GLP-1 AND SYMPATHETIC ACTIVITY

Analysis software (BMDV32, Mayo Clinic, Rochester, NY). High resolution was achieved by independent time and frequency smoothing (30).

For heart rate variability (HRV), power was calculated in the low frequency (LF; 0.03–0.14 Hz) and in high frequency (HF; 0.14–0.30 Hz) components. HF power of R–R variability (HRV_{HF}) is a measure of cardiac parasympathetic activity (34). The LF component of R–R variability (HRV_{LF}) primarily responds to variations in cardiac sympathetic activity (3, 33). Sympathetic modulation of BP variability was also assessed by analyzing power in the LF component (0.03–0.14 Hz, SBPLF) for beat-to-beat systolic BP. The LF-to-HF power ratio of R–R variability (LFR_{R}/HFR_{R}) is a representative index of sympathetic to parasympathetic balance in physiological and pathophysiological conditions (3, 26, 33).

Experimental Design and Procedure in Experiment 2

In this study, MSNA was directly measured by peroneal nerve microneurography for 10 min before and 60 min after GLP-1, administered at the same dose as in experiment 1. Burst activity in sympathetic nerves could be recorded in 7 of 9 healthy volunteers who were recruited for this part of the study. Cardiac rhythm and BP were also monitored by ECG and Finapres sphygmonanometry (Ohmeda, Madison, WI), respectively.

Studies were performed in a temperature-controlled room (23°C) with subjects in the supine position. An invravenous cannula was placed in an antecubital vein, a minimum of 45 min before baseline recordings. Upon arrival in the laboratory, subjects were instrumented for recording of ECG, arterial pressure, respiration, and MSNA. Finapres monitor readings were verified with manual sphygmonanometry to ensure accuracy. After instrumentation, 10 min of baseline data were recorded.

Efferent multunit, postganglionic MSNA was measured directly using standard microneurographic techniques, as previously described (44). Briefly, two sterile tungsten microelectrodes (tip diameter, 5–10 μm; Frederick Haer; Bowdoinham, ME) were inserted, one into the peroneal nerve for recording of MSNA and the other (not in a nerve) to serve as a reference. A signal processing system (662C-3 Nerve Traffic Analysis System; University of Iowa Bioengineering, Iowa City, IA) amplified (8 × 10^4 times), band-pass filtered (700–2,000 Hz), rectified, and integrated (time constant, 0.1 s) the nerve signal.

MSNA was quantified as burst frequency (bursts/min). Sympathetic bursts in the integrated neurogram were identified by a custom-manufactured analysis program (8, 9); burst identification was then confirmed by visual inspection by a single investigator. MSNA data were averaged over 4 min at the end of the baseline period and over 4 min at the end of the GLP-1 period.

Statistical Analysis

Consistent with the experimental design, the statistical analysis was designed to assess the effects of GLP-1 with or without L-NMMA and GLP-1 with or without yohimbine on summary parameters during the fasting postdrug and postprandial postdrug periods. Overall treatment effects were assessed by analyses of covariance (ANCOVA) using sex, BMI, and the corresponding fasting predrug values as covariates. Thereafter, appropriate pairwise comparisons were also analyzed, that is, between individual agents (i.e., GLP-1 and separately L-NMMA vs. placebo) and between the combination (i.e., GLP-1 + L-NMMA) vs. GLP-1 alone were assessed. Similar comparisons were performed for the GLP-1 + yohimbine data. Pairwise comparisons were only considered significant if overall treatment effects were also significant. Because this was an intent-to-treat analysis, missing data were imputed using the corresponding mean value over all nonmissing data values. The error degrees of freedom in the respective ANCOVA models were decreased by one for each missing value imputed for a given response parameter. The data presented in the text, tables, and figures are the “raw” means ± SE, unadjusted for covariates and do not include any imputed values. In experiment 2, the effects of GLP-1 on MSNA and hemodynamic parameters were assessed by a signed rank test using the seven subjects with available data.

RESULTS

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

Effects of GLP-1 and L-NMMA on Cardiovascular Parameters

GLP-1 did not significantly affect heart rate or BP compared with placebo during the fasting or postprandial periods in experiment 1 (Table 2). Although GLP-1 increased systolic BP (P < 0.05, signed rank test) and diastolic BP (P = 0.06) during experiment 2, these effects were rather modest. GLP-1 did not significantly increase the heart rate during experiment 2. During the fasting period, L-NMMA had overall treatment effects (P < 0.01) on diastolic BP and heart rate. L-NMMA alone, and in combination with GLP-1, increased (P ≤ 0.02) the diastolic BP and reduced (P ≤ 0.02) the heart rate, compared with placebo and GLP-1, respectively. During the postprandial period, GLP-1 + L-NMMA increased the diastolic BP compared with GLP-1 alone (P < 0.04 for overall treatment effect, P < 0.05 for pairwise comparison). During the postprandial period, L-NMMA reduced the heart rate compared with placebo (P < 0.01 for overall treatment effect, P < 0.01 for pairwise comparison). Moreover, GLP-1 and L-NMMA reduced the heart rate compared with GLP-1 alone (P < 0.01 for overall treatment effect, P < 0.01 for pairwise comparison).

Table 1. Demographic and baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>GLP-1</th>
<th>Yohimbine</th>
<th>GLP-1 + Yohimbine</th>
<th>L-NMMA</th>
<th>GLP-1 + L-NMMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>10</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Age, yr</td>
<td>34 ± 5</td>
<td>34 ± 3</td>
<td>37 ± 3</td>
<td>29 ± 3</td>
<td>33 ± 4</td>
<td>28 ± 3</td>
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<td>Sex, % female</td>
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<td>86</td>
<td>57</td>
<td>70</td>
<td>86</td>
<td>67</td>
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<tr>
<td>BMI, kg/m²</td>
<td>26 ± 2</td>
<td>27 ± 2</td>
<td>26 ± 2</td>
<td>27 ± 2</td>
<td>27 ± 2</td>
<td>27 ± 1</td>
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<td>Serum glucose, mg/dl</td>
<td>87 ± 2</td>
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<td>88 ± 2</td>
<td>89 ± 2</td>
<td>90 ± 5</td>
<td>88 ± 2</td>
</tr>
<tr>
<td>NE level, pg/ml</td>
<td>214 ± 31</td>
<td>177 ± 33</td>
<td>266 ± 77</td>
<td>176 ± 35</td>
<td>167 ± 17</td>
<td>171 ± 25</td>
</tr>
<tr>
<td>DHPG level, pg/ml</td>
<td>1578 ± 266</td>
<td>1514 ± 206</td>
<td>1732 ± 168</td>
<td>1430 ± 79</td>
<td>1857 ± 128</td>
<td>1830 ± 231</td>
</tr>
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</table>

All values except sex are actual means ± SE. BMI, body mass index; GLP-1, glucagon-like peptide; L-NMMA, N⁵-monomethyl-L-arginine acetate; NE, norepinephrine; DHPG, dihydroxyphenylglycol.

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SBP, which is also a measure of cardiac sympathetic activity. During the fasting period, yohimbine also increased power in the HRVLF component (Fig. 2). Similarly, power in the HRVLF component was higher (P < 0.05) for GLP-1 and yohimbine compared with GLP-1 alone.

Effects of GLP-1, L-NMMA, and Yohimbine on Plasma Catecholamines

L-NMMA did not significantly affect fasting plasma norepinephrine concentrations (Fig. 3). Yohimbine had overall treatment effects (P = 0.02) on fasting plasma norepinephrine concentrations; pairwise comparisons revealed that plasma norepinephrine was higher for yohimbine than placebo (P = 0.06) and for GLP-1 and yohimbine than GLP-1 alone (P = 0.01) (Fig. 3). Yohimbine also increased postprandial plasma norepinephrine concentrations (P = 0.003 for overall treatment effects). Pairwise comparisons demonstrated higher plasma norepinephrine concentrations for yohimbine compared with placebo (P ≤ 0.04) and for GLP-1/yohimbine compared with GLP-1 alone (P < 0.005). In contrast, fasting norepinephrine concentrations were lower (P = 0.02) for GLP-1/L-NMMA compared with GLP-1 alone.

Drug effects on fasting DHPG levels were not significant (Fig. 4). However, yohimbine increased postprandial DHPG levels compared with placebo, and GLP-1/yohimbine increased

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Fasting</th>
<th>Postprandial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n = 8)</td>
<td></td>
<td></td>
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<tr>
<td>SBP</td>
<td>122±6</td>
<td>121±7</td>
<td>123±9</td>
</tr>
<tr>
<td>DBP</td>
<td>66±3</td>
<td>64±4</td>
<td>63±5</td>
</tr>
<tr>
<td>HR</td>
<td>64±2</td>
<td>67±4</td>
<td>71±3</td>
</tr>
<tr>
<td>GLP-1 (n = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>120±6</td>
<td>122±5</td>
<td>130±9</td>
</tr>
<tr>
<td>DBP</td>
<td>65±3</td>
<td>64±3</td>
<td>66±7</td>
</tr>
<tr>
<td>HR</td>
<td>65±3</td>
<td>68±3</td>
<td>69±2</td>
</tr>
<tr>
<td>Yohimbine (n = 7)</td>
<td></td>
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<td></td>
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<tr>
<td>SBP</td>
<td>119±7</td>
<td>126±9</td>
<td>114±11</td>
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<tr>
<td>DBP</td>
<td>68±6</td>
<td>76±7</td>
<td>74±9</td>
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<tr>
<td>HR</td>
<td>61±3</td>
<td>64±3</td>
<td>66±4</td>
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<td>GLP-1 + Yohimbine (n = 10)</td>
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<tr>
<td>SBP</td>
<td>125±7</td>
<td>135±9</td>
<td>127±2</td>
</tr>
<tr>
<td>DBP</td>
<td>69±4</td>
<td>73±6</td>
<td>76±3</td>
</tr>
<tr>
<td>HR</td>
<td>63±3</td>
<td>64±3</td>
<td>69±3</td>
</tr>
<tr>
<td>L-NMMA (n = 7)</td>
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<td></td>
</tr>
<tr>
<td>SBP</td>
<td>125±8</td>
<td>132±8</td>
<td>119±8</td>
</tr>
<tr>
<td>DBP</td>
<td>68±3</td>
<td>76±6</td>
<td>70±5</td>
</tr>
<tr>
<td>HR</td>
<td>66±6</td>
<td>57±2</td>
<td>61±3*</td>
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<tr>
<td>GLP-1 + L-NMMA (n = 9)</td>
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<tr>
<td>SBP</td>
<td>116±5</td>
<td>127±6</td>
<td>129±6</td>
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<tr>
<td>DBP</td>
<td>61±5</td>
<td>73±5†</td>
<td>75±4†</td>
</tr>
<tr>
<td>HR</td>
<td>59±3</td>
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<td>57±3†</td>
</tr>
<tr>
<td>GLP-1 (n = 7)</td>
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<tr>
<td>SBP</td>
<td>138±8</td>
<td>145±9†</td>
<td>NA</td>
</tr>
<tr>
<td>DBP</td>
<td>74±6</td>
<td>77±6</td>
<td>NA</td>
</tr>
<tr>
<td>HR</td>
<td>67±5</td>
<td>70±4</td>
<td>NA</td>
</tr>
</tbody>
</table>

All values are actual means ± SE in mmHg. SBP, systolic blood pressure, given in mmHg; DBP, diastolic blood pressure, given in mmHg; HR, heart rate, given in beats per minute, bpm. NA, not available. *P ≤ 0.05 vs. placebo. †P ≤ 0.05 vs. GLP-1 alone. ‡0.02 < P ≤ 0.05, pre vs. post (signed rank test).

Neither GLP-1 nor L-NMMA alone or in combination had significant effects on HRV (Fig. 2).

Effects of Yohimbine on Cardiovascular Parameters

Yohimbine did not significantly affect heart rate or BP compared with placebo during the fasting or postprandial periods. However, yohimbine had significant and mostly similar effects on HRV during fasting and postprandial periods. Thus, yohimbine increased power in the HRVLF component (P < 0.05 for overall treatment effects, P < 0.05 vs. placebo), suggestive of increased cardiac sympathetic activity (Fig. 2). Similarly, power in the HRVLF component was higher (P < 0.05) for GLP-1 and yohimbine compared with GLP-1 alone. During the fasting period, yohimbine also increased power in SBP_LF, which is also a measure of cardiac sympathetic activity (P = 0.057 for overall treatment effect, P < 0.01 vs. placebo) (Table 3). In contrast to the HRVLF component, these medications did not have significant effects on HRVHF or the HRVLF/HRVHF ratio.

Effects of GLP-1, L-NMMA, and Yohimbine on Plasma Catecholamines

L-NMMA did not significantly affect fasting plasma norepinephrine concentrations (Fig. 3). Yohimbine had overall treatment effects (P = 0.02) on fasting plasma norepinephrine concentrations; pairwise comparisons revealed that plasma norepinephrine was higher for yohimbine than placebo (P = 0.06) and for GLP-1 and yohimbine than GLP-1 alone (P = 0.01) (Fig. 3). Yohimbine also increased postprandial plasma norepinephrine concentrations (P = 0.003 for overall treatment effects). Pairwise comparisons demonstrated higher plasma norepinephrine concentrations for yohimbine compared with placebo (P ≤ 0.04) and for GLP-1/yohimbine compared with GLP-1 alone (P < 0.005). In contrast, fasting norepinephrine concentrations were lower (P = 0.02) for GLP-1/L-NMMA compared with GLP-1 alone.

Drug effects on fasting DHPG levels were not significant (Fig. 4). However, yohimbine increased postprandial DHPG levels compared with placebo, and GLP-1/yohimbine increased

Table 3. Effect of drugs on variability in systolic blood pressure

<table>
<thead>
<tr>
<th>Group</th>
<th>Variability in Low-Frequency Component of Systolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Placebo (n = 8)</td>
<td>0.93±0.28</td>
</tr>
<tr>
<td>GLP-1 (n = 7)</td>
<td>1.67±0.73</td>
</tr>
<tr>
<td>L-NMMA (n = 7)</td>
<td>0.78±0.33</td>
</tr>
<tr>
<td>GLP-1 + L-NMMA (n = 9)</td>
<td>1.36±0.64</td>
</tr>
<tr>
<td>Yohimbine (n = 7)</td>
<td>0.80±0.31</td>
</tr>
<tr>
<td>GLP-1 + yohimbine (n = 10)</td>
<td>1.05±0.26</td>
</tr>
</tbody>
</table>

All values are actual means ± SE.
Effects of GLP-1, L-NMMA, and Yohimbine on Plasma Glucose

Drug effects on fasting and postprandial plasma glucose concentrations were significant. Compared with placebo, GLP-1 reduced \( P < 0.05 \) fasting (i.e., \( 77 \pm 3 \text{ mg/dL} \) vs. \( 87 \pm 3 \text{ mg/dL} \)) and postprandial (i.e., \( 74 \pm 5 \text{ mg/dL} \) vs. \( 88 \pm 4 \text{ mg/dL} \)) plasma glucose concentrations. Similar effects were observed for GLP-1 and L-NMMA combined compared with GLP-1 alone (data not shown). However, yohimbine and L-NMMA did not have significant effects on plasma glucose concentrations (data not shown).

Effects of GLP-1 on Muscle Sympathetic Nerve Activity

GLP-1 increased muscle sympathetic nerve activity from \( 17 \pm 2 \) to \( 23 \pm 2 \) bursts/min (0.02 < \( P \leq 0.05 \), signed rank test) (Fig. 5).

DISCUSSION

In addition to regulating glycemia, there is evidence that the incretin GLP-1, administered peripherally or centrally, inhibits vagal activity in animals and humans (19, 50, 51) and increases sympathetic activity in rats (52, 53). In this study, GLP-1 increased muscle sympathetic nerve traffic but did not affect heart rate, BP, plasma catecholamines, or cardiovascular sympathetic activity. GLP-1 increased MSNA by 25%, which is comparable to the rise in MSNA upon 30° head-upright tilt in healthy subjects (27) but smaller than the increase (i.e., \( 73 \pm 13\% \)) induced by yohimbine in a previous study (16). The lack of a control arm (i.e., sham infusion) in these studies is a potential limitation. However, in a controlled environment, MSNA is very stable within subjects, even over several weeks to months (13, 44). No blood was drawn while recording MSNA. Increased MSNA cannot be attributed to a small volume of fluid administered during GLP-1 infusion, which, if anything, would reduce, not increase MSNA. It is unclear whether GLP increased MSNA directly or by stimulating insulin secretion (24). Infusion of GLP-1 (7-36 amide) at a rate comparable to our studies increased plasma insulin concentrations on average from \( 8 \) to \( 15 \) mU/l (24), which is comparable to the insulin concentrations (i.e., \( 10 \) to \( 25 \) mU/l), which increased MSNA (i.e., from \( 16 \) to \( 25 \) bursts/min) during insulin euglycemic clamp infusion (17).

GLP-1 did not affect the heart rate and BP in experiment 1. Although GLP-1 increased systolic BP and had borderline significant effects on diastolic BP in experiment 2, these effects were numerically small. Overall, our observations are generally consistent with previous studies demonstrating that GLP-1 did not increase BP in monkeys (55) or during continuous subcutaneous infusion for 6 wk in humans (56). The demographic characteristics of subjects in experiments 1 and 2 were similar. Although GLP-1 increased MSNA in this study, our spectral analysis did not reveal changes consistent with an influence on cardiac sympathetic or parasympathetic activity. This is in contrast to data in rodents (55, 56). Perhaps we had insufficient statistical power to identify GLP-1-induced alterations in cardiac sympathetic or parasympathetic activity. Alternatively, the hemodynamic effects of increased sympathetic activity may have been masked by activation of baroreceptor reflexes or by the direct vascular effects of GLP-1 (6). In addition, GLP-1-induced insulin release might have interfered with sympathetically mediated vasoconstriction (22, 39, 41). It is also conceivable that differences in the distribution of GLP-1 receptors on catecholaminergic neurons in the area postrema or.
central neural circuitry, may explain why GLP-1 had different effects on hemodynamic parameters in rats and humans.

The α2-adrenergic antagonist yohimbine increased power in the low-frequency component of HRV and systolic BP power spectra, indicating that despite their limitations (35, 47), these indices can identify increased cardiac sympathetic activity. In addition to augmenting sympathetic activity via central effects, it is also conceivable that the peripheral effects of yohimbine (i.e., blockade of α2-adrenoceptors on sympathetic nerve endings) increase the amount of norepinephrine released from sympathetic nerve endings for a given amount of sympathetic nerve traffic (16). Moreover, yohimbine increased plasma norepinephrine and DHPG compared with placebo, and the combination of GLP-1 and yohimbine augmented plasma norepinephrine and DHPG compared with GLP-1 alone. The combined increase in plasma norepinephrine and DHPG concentrations indicates this was attributable to increased release rather than reuptake of norepinephrine (15). As anticipated, the NOS inhibitor L-NMMA increased the diastolic BP and reduced the heart rate by reflex mechanisms (10) but did not affect the power spectral analysis of heart rate or BP in humans.

**Perspectives and Significance**

These observations provide direct evidence that GLP-1 increases sympathetic vasoconstrictor neural activity in humans. From a biological perspective, these observations are plausible since GLP-1 is produced in the brain and passively diffuses across the blood-brain barrier (21). Moreover, GLP-1 receptors are abundantly distributed throughout the central nervous system, including the brain stem. In addition to direct effects on GLP-1 receptors in the brain, the central effects of GLP-1 and/or exendin-4 may be mediated via peripheral effects (e.g., GLP-1 receptors in the brain, the central effects of GLP-1 are abundantly distributed throughout the central nervous system). Diabetes mellitus is associated with hypertension and autonomic neuropathy. Moreover, diabetic autonomic neuropathy is associated with significant increases in morbidity and mortality, including an increased risk of sudden death. (37, 42, 43) The effects of GLP-1 on sympathetic neural activity in diabetes mellitus, controlling for autonomic neuropathy and hypertension, are necessary.

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**REFERENCES**

23. Meier JJ, Gallwitz B, Salmen S, Goetze O, Holst JJ, Schmidt WE, Nauck MA. Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like pep-


