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Beneficial effects of GLP-1 on endothelial function in humans: dampening by glyburide but not by glimepiride

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Basu A, Charkoudian N, Schrage W, Rizza RA, Basu R, Joyner MJ. Beneficial effects of GLP-1 on endothelial function in humans: dampening by glyburide but not by glimepiride. *Am J Physiol Endocrinol Metab* 293: E1289–E1295, 2007. First published August 21, 2007; doi:10.1152/ajpendo.00373.2007.—Sulfonylureas (SU) with glucagon-like peptide-1 (GLP-1)-based therapy are an emerging therapeutic combination for type 2 diabetes. Prior human studies have hinted at endothelial effects of GLP-1 and SU. To study the endothelial effects of GLP-1 per se and to evaluate the modulatory effects, if any, of SU agents on GLP-1-induced changes in endothelial function, healthy, nondiabetic, normotensive, nonsmokers, age 18–50 yr with no family history of diabetes, were studied. Subjects were randomized to either placebo ($n = 10$), 10 mg of glyburide ($n = 11$), or 4 mg of glimepiride ($n = 8$) orally. Euglycemic somatostatin pancreatic clamp with replacement basal insulin, glucagon, and growth hormone was performed for 240 min. Forearm blood flow (FBF) was measured by venous occlusion plethysmography with graded brachial artery infusions of acetylcholine (Ach) and nitroprusside (NTP) before and after intravenous infusion of GLP-1. GLP-1 (preinfusion 3.4 ± 0.2, postinfusion 25.5 ± 2.8 pM) enhanced ($P < 0.03$) Ach-mediated vasodilatation ($\Delta+6.5 \pm 1.1$ vs. $\Delta+9.1 \pm 1.2$ ml·100 ml^{-1}·min^{-1}, change from baseline FBF) in those on placebo. However, in contrast, glyburide abolished GLP-1-induced Ach-mediated vasodilatation ($\Delta+11.7 \pm 2.0$ vs. $\Delta+11.7 \pm 2.5$ ml·100 ml^{-1}·min^{-1}). On the other hand, glimepiride did not alter the ability of GLP-1 to enhance Ach-mediated vasodilatation ($\Delta+7.9 \pm 0.5$ vs. $\Delta+10.2 \pm 1.3$ ml·100 ml^{-1}·min^{-1}, $P < 0.04$). Neither GLP-1 nor SU altered NTP-induced vasodilatation. These data demonstrate that GLP-1 per se has direct beneficial effects on endothelium-dependent vasodilatation in humans that are differentially modulated by SU.

glucagon-like peptide-1; incretin; sulfonylurea; vascular reactivity

GLUCAGON-LIKE PEPTIDE-1 (GLP-1)-BASED THERAPY has recently been approved to treat type 2 diabetes. GLP-1 receptor agonist (e.g., exanetide) and inhibitors of dipeptidyl peptidase IV (e.g., sitagliptin), the enzyme that degrades GLP-1, have been shown to improve glycemic control in several clinical trials and are frequently used in combination with sulfonylureas (SU) to treat subjects with type 2 diabetes. Apart from the effects of GLP-1 on hormonal secretion, gastrointestinal motility, and β-cell function, prior studies have hinted at its beneficial effects on cardiovascular function as well.

Provocative animal and human data suggest a role of GLP-1 on cardiac and endothelial functions. Infusion of GLP-1 into Dahl salt-sensitive rats attenuated the development of hypertension, with a reduction of proteinuria and improvement in endothelial and cardiac functions (35). Mice lacking the GLP-1 receptor exhibit bradycardia, elevated left ventricular end diastolic pressure, increased left ventricular thickness, and impaired systolic and diastolic cardiac functions (15). A recent study in humans (27) has shown beneficial effects on left ventricular function when GLP-1 was infused for 72 h following acute myocardial infarction. Infusion of GLP-1 in subjects with type 2 diabetes and stable coronary disease (28) has shown beneficial effects on flow-mediated vasodilatation. However, the extent to which these effects accrued from hormonal changes induced by GLP-1 (increased insulin and reduced glucagon concentrations) vs. its direct effects on the injured myocardium or endothelium remains unresolved.

SU have also been implicated in influencing endothelial and cardiovascular function. These drugs bind to the SU receptor (SUR) subunits in the β-cell, leading to closure of the closely linked ATP-sensitive K^+ (K_{ATP}) channels resulting in insulin secretion. The potassium channels are ubiquitous and are present in the endothelium, cardiac, and vascular smooth muscle cells. In vitro studies (24) have indicated that SU drugs possess varying binding affinities to these channels and to the SUR subunits. This assumes clinical relevance since closure of the K_{ATP} channels in the heart could lead to prevention of ischemic preconditioning, a cardioprotective phenomenon observed in all species, including humans (16, 21). There are reports (12) suggesting early mortality in diabetic patients after angioplasty for acute myocardial infarction with the use of SU drugs. The University Group Diabetes Program study (2) reported that use of the SU tolbutamide increased the number of cardiac events. However, the United Kingdom Prospective Diabetes Study trial (1) failed to demonstrate increased cardiac events in patients treated with either chlorpropamide or glibenclamide compared with those treated with insulin. Studies in subjects with type 2 diabetes (19) undergoing coronary angioplasty have suggested inhibition of cardioprotective effects by glibenclamide (glyburide) but not by glimepiride.

To evaluate the effects of GLP-1 per se, independent of hormonal changes, on endothelial function as measured by forearm blood flow (FBF) with venous occlusion plethysmography, and its modulation, if any, by SU agents commonly used to treat type 2 diabetes, the following study was designed in healthy, nondiabetic subjects.

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Table 1. *Volunteer characteristics*

	Placebo	Glimepiride	Glyburide
Age, yr	29±2	30±3	29±3
Body mass index, kg/m^2	28±2	27±2	26±3
Fasting glucose, mM	4.9±0.2	5.1±0.1	4.9±0.1
Hb A_{1c}, %	5.5±0.2	5.6±0.3	5.3±0.4

$P > 0.2$ between groups for each parameter.

MATERIALS AND METHODS

The subject characteristics are provided in Table 1. Each participant was studied once. All subjects were between and 18 and 50 yr old. The participants had no history of a known systemic illness and were not on any drugs except stable hormone replacement therapy. To ensure that the subjects were healthy, they underwent a history and physical examination at screening; blood was collected for a complete blood count, chemistry group, and lipid profile and urine for routine analysis. Participants did not have a history of diabetes in first-degree relatives, were nonsmokers, and were normotensive. The resting ECG was normal. All women of child-bearing potential had a negative pregnancy test within 24 h of the study. All subjects had a stable weight for ≥1 mo prior to the study and were consuming their customary diet for ≥1 wk prior to study. A brief dietary history was taken at the time of screening to ensure that they were consuming ≥200 g of carbohydrates/day and that their customary diets met American Diabetes Association guidelines for protein, fat, and carbohydrates. Subjects engaging in regular, prolonged, vigorous exercises were excluded from study. No special classes of subjects were enrolled.

Following approval from the Mayo Institutional Review Board, and after informed consent, eligible subjects were admitted to the Mayo Clinic Clinical Research Unit at ~5 PM on the evening prior to study and given a standard meal (10 cal/kg; 55% carbohydrates, 30% fat, and 15% protein). The subjects were then kept nil per oral after midnight except water until completion of the study.

The study design is provided schematically in Fig. 1. At 0600 on the following morning a venous cannula was inserted into the dominant forearm for infusion of hormones and glucose during the study. A cannula was also inserted into one of the hand veins in the dominant hand and the hand placed in a heated box, the temperature of which was raised to 55°C to periodically draw arterialized venous blood for monitoring glucose levels. Blood was not drawn from the arterial catheter to prevent interference with FBF measurements with venous occlusion plethysmography. At 0700, a 20-gauge, 5-cm Teflon arterial catheter was inserted under aseptic precautions and 1% lidocaine as local anesthesia into the brachial artery of the nondominant arm. The catheter was continuously flushed at 3 ml/h with saline containing 2 units/ml of heparin. A three-port connector was placed in series with the catheter tubing and with a pressure transducer to permit drug infusion, blood sampling, and constant measurement of arterial blood pressure. Heart rate was continuously monitored via a five-lead ECG. At 0800, subjects were randomly assigned to ingest either 10 mg of glyburide, 4 mg of glimepiride, or matching placebo. Glucose values were monitored bedside every 10 min throughout the study. At 0900 (*time 0*) an infusion of somatostatin (60 ng·kg^{-1}·min^{-1}), growth hormone (3 ng·kg^{-1}·min^{-1}), insulin (0.15 mU·kg^{-1}·min^{-1}), and glucagon (0.65 ng·kg^{-1}·min^{-1}) was started and continued until the end of the study at 1300 (240 min). Fifty percent dextrose was also infused in amounts sufficient to keep plasma glucose ~5 mM throughout the study.

FBF was measured four times each minute using venous occlusion plethysmography with mercury-in-silastic strain gauges as previously described (14). The hand was excluded during FBF measurements by applying a blood pressure cuff at suprasystolic pressures at the wrist. At 90 min (1030), in all groups, FBF responses to acetylcholine (Ach; IOLAB Pharmaceuticals) at each dose were assessed during infusions of 2, 4, and 8 μg·100 ml forearm vol (previously determined by water displacement)$^{-1}$·min^{-1} into the brachial artery. Each dose was infused for 2 min. Ach stimulates muscarinic receptors on the endothelial cells that evoke nitric oxide (NO) release through stimulation of endothelial isoform of the NO synthase enzyme (18). Thereafter, following a 15-min washout period to eliminate any residual effects of Ach, sodium nitroprusside (NTP; Elkin Simms) was infused into the brachial artery as similar infusions of 0.5, 1, and 2 μg·100 ml forearm vol^{-1}·min^{-1} and blood flow determined during each dose. NTP acts as an exogenous NO donor and causes endothelium-independent vasodilatation. After completion of these sets of measurements, at 1100 (120 min) an infusion of GLP-1 (1.2 pmol·kg^{-1}·min^{-1}) was started and continued until the end of the study. The infusion rate of GLP-1 was selected on the basis of prior experiments done by us (32) in individuals with type 1 diabetes. This rate of GLP-1 infusion resulted in normalization of blood glucose in subjects with type 2 diabetes in another study (20). Ninety minutes thereafter (210 min), Ach and NTP were again infused sequentially as described above and FBF responses measured. The study was completed at 1300 (240 min). Venous blood samples were collected at predetermined intervals for glucose and hormone measurements throughout the study.

Thereafter, all arterial and venous infusions were stopped and arterial catheters and cannulae removed, and the subjects were provided lunch. One venous cannula was left in situ until the next morning to monitor glucose levels periodically, since subjects who were administered SU drugs had the potential for hypoglycemia until

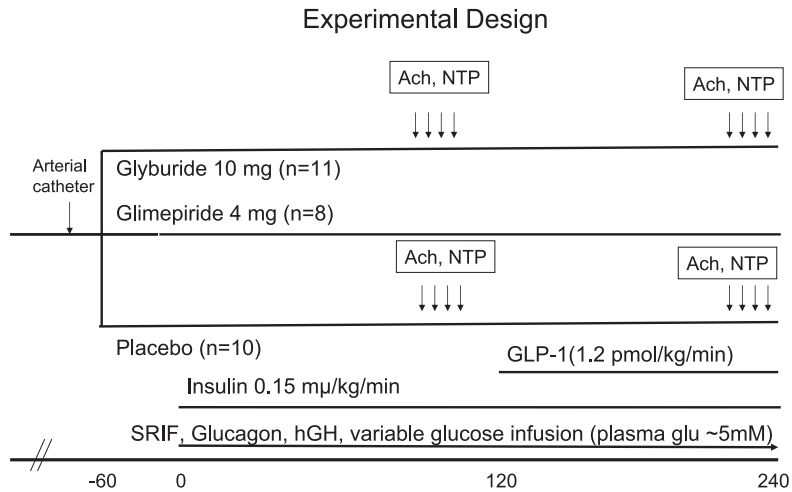

Fig. 1. Experimental design for the study protocol. Ach, acteylcholine; NTP, sodium nitroprusside; GLP-1, glucagon-like peptide-1; SRIF, somatostatin; hGH, human growth hormone.

the next morning. If symptoms of hypoglycemia occurred, appropriate therapy was instituted with either intravenous or oral glucose administration. All subjects were dismissed the following morning. Furthermore, it is important to note that in the first participant in each of the SU groups, we administered 20 mg of glyburide or 8 mg of glimepiride. Both of these individuals had prolonged and recurrent hypoglycemia after completion of the pancreatic clamp, necessitating reduction in the SU doses to one-half the maximal therapeutic dose in the rest of the subjects included. Although we have not included data from these two participants in the final analyses, their FBF responses were similar to the rest of the study participants.

Analyses

Blood flow responses. Data were digitized at 200 Hz and stored on a computer for subsequent offline analyses. Data were then analyzed with signal-processing software (Powerlab 8/30; AdInstruments). FBF was calculated from the first derivative of the plethysmogram (slope = change in volume/time) during the last minute of each drug dose. Heart rate was obtained from the ECG waveform and mean arterial pressure from the integral of arterial pressure waveform. FBF values were expressed per unit forearm volume (ml·100 ml^{-1}·min^{-1}). Forearm volume for each subject was measured by water displacement prior to the blood flow protocol as previously described (10, 18).

Glucose and hormone concentrations. All blood samples were immediately placed on ice, centrifuged at 4°C, separated, and stored at −20°C until analysis. Plasma glucose was measured using a Beckman's (Beckman Instruments, Chaska, MN) glucose analyzer. Insulin was measured using a chemiluminescence method (Access Ultrasensitive Immunoenzymatic assay), C-peptide, and glucagon concentrations measured as previously described by radioimmunoassay (Linco Research, St. Louis, MO) (6). GLP-1-(7–36) amide level was measured by enzyme linked immunosorbent assay (Linco Research), with the lowest levels of detection 3 pM with no cross-reactivity to GLP-1-(9–36) amide, GLP-2, or glucagon (intra-assay precision 3% at 8.4 pM) (32). Glucagon was measured by a direct, double-antibody radioimmunoassay (Linco Research). Growth hormone was measured by a simultaneous one-step immunoenzymatic ("sandwich") assay performed on the Beckman Coulter UniCel DxI 800 (Beckman Instruments).

SU assay. Glyburide and glimepiride were detected qualitatively in samples obtained during FBF measurements between 90–120 and 210–240 min by liquid chromatography tandem mass spectrometry. The assay available does not permit quantitative estimation of drug levels. It detects the presence or absence of specific SU in the plasma.

Statistical Analyses

Within-group analyses were performed by paired two-tailed student's *t*-test or Wilcoxon's signed rank test, whereas between-group analyses were performed by unpaired two-tailed *t*-test or rank sum test. Repeated-measures analysis of variance was used to assess differences in baseline and stimulated FBF between groups. A P value of <0.05 was deemed statistically significant.

RESULTS

Volunteer Characteristics

There were no differences in age, body mass index, fasting plasma glucose, or glycated hemoglobin concentrations between groups, as shown in Table 1.

Glucose, Insulin, C-Peptide, and GLP-1 Concentrations

Glucose concentrations did not differ between groups either at baseline at *time 0* (5.1 ± 0.1 vs. 5.2 ± 0.2 vs. 4.9 ± 0.2 mM, placebo vs. glimepiride vs. glyburide), before GLP-1 infusion between 90 and 120 min (7.2 ± 0.6 vs. 7.4 ± 0.4 vs. 6.7 ± 0.3 mM, placebo vs. glimepiride vs. glyburide), or during GLP-1 infusion between 210 and 240 min (5.5 ± 0.1 vs. 5.5 ± 0.1 vs. 5.6 ± 0.1 mM, placebo vs. glimepiride vs. glyburide), when FBF was recorded (Fig. 2). Glucose concentrations rose gently and comparably during the clamp in all three groups, achieving a broad peak between 90 and 120 min, and then returned to baseline and equal levels by 210 min in all groups. Although glucose concentrations were lower ($P < 0.01$) in each of the three groups during 210–240 than during 90–120 min, they did not differ among groups at each of these time intervals when FBF responses were measured. The integrated area under the curve (AUC) glucose did not differ between groups (272.0 ± 66.6 vs. 272.2 ± 57.3 vs. 247.0 ± 34.8 mM/240 min, placebo vs. glimepiride vs. glyburide). Glucose solution was infused in a few of the subjects, as necessary, to maintain euglycemia.

Insulin concentrations did not differ among groups either at baseline at *time 0* (33.1 ± 4.1 vs. 41.1 ± 8.6 vs. 65.2 ± 12.9

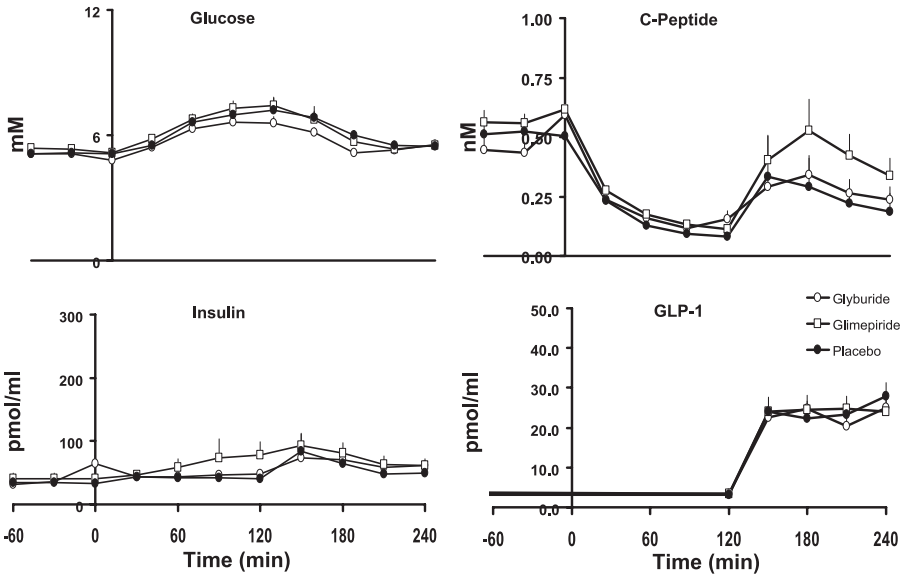

Fig. 2. Plasma glucose, insulin, C-peptide, and GLP-1 concentrations obtained during the study protocol in the placebo (●), glimepiride (□), and glyburide (○) arms.

pM, placebo vs. glimepiride vs. glyburide), before GLP-1 infusion between 90 and 120 min (40.4 ± 2.6 vs. 77.4 ± 22.1 vs. 47.6 ± 4.7 pM, placebo vs. glimepiride vs. glyburide), or during GLP-1 infusion between 210 and 240 min (49.4 ± 8.6 vs. 61.6 ± 9.9 vs. 50.9 ± 5.9 pM, placebo vs. glimepiride vs. glyburide), when FBF was recorded. Likewise, there were no differences within groups in insulin concentrations between 90 to 120 min and 210 to 240 min. The integrated AUC insulin did not differ between groups (700.3 ± 209.1 vs. 1,082.4 ± 420.3 vs. 466.20 ± 377.7 pM/240 min, placebo vs. glimepiride vs. glyburide).

C-peptide concentrations did not differ between groups either at baseline at *time 0* (0.50 ± 0.06 vs. 0.62 ± 0.04 vs. 0.59 ± 0.08 nM, placebo vs. glimepiride vs. glyburide) or before GLP-1 infusion between 90 and 120 min (0.08 ± 0.01 vs. 0.11 ± 0.01 vs. 0.15 ± 0.04 nM, placebo vs. glimepiride vs. glyburide). Despite somatostatin, GLP-1 infusion began at 120 min and resulted in an increase in insulin secretion. However, this increase did not differ among groups, especially between 210 and 240 min (0.19 ± 0.08 vs. 0.34 ± 0.07 vs. 0.24 ± 0.05 nM, placebo vs. glimepiride vs. glyburide), when FBF was recorded. The integrated AUC C-peptide did not differ between groups (−68.7 ± 6.9 vs. −72.0 ± 13.1 vs. −82.9 ± 17.9 nM/240 min, placebo vs. glimepiride vs. glyburide).

GLP-1 concentrations did not differ between groups either before GLP-1 infusion between 90 and 120 min (3.1 ± 0.07 vs. 3.5 ± 0.27 vs. 3.5 ± 0.29 pM, placebo vs. glimepiride vs. glyburide) or during GLP-1 infusion between 210 and 240 min (27.9 ± 3.5 vs. 24.0 ± 3.2 vs. 25.0 ± 2.7 pM, placebo vs. glimepiride vs. glyburide).

Glucagon and growth hormone concentrations also did not differ between groups either at baseline at *time 0* or during the two time periods 90–120 and 210–240 min, when FBF was measured (data not shown).

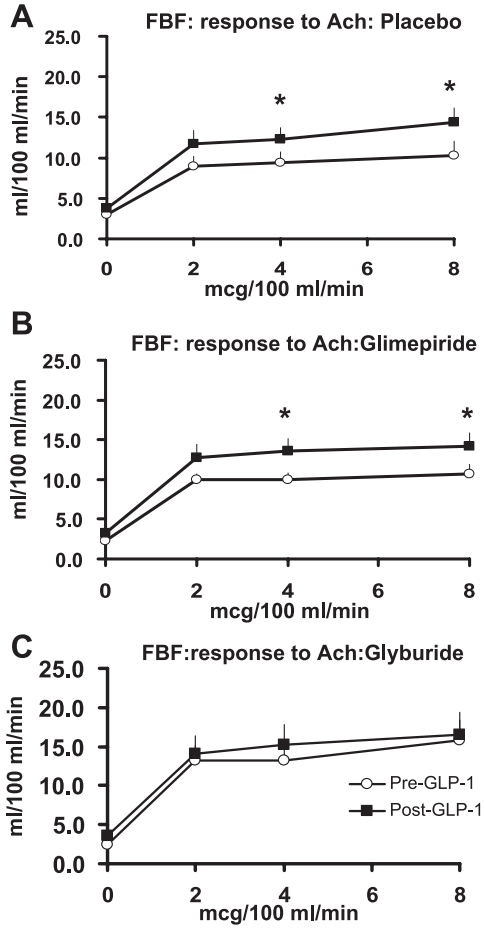

Fig. 3. Forearm blood flow (FBF) responses to graded infusions of Ach at 2, 4, and 8 μg·100 ml forearm vol^{-1}·min^{-1} before (○) and after (■) GLP-1 infusion in the placebo (*A*), glimepiride (*B*), and glyburide (*C*) arms of the study. *$P < 0.05$ in FBF responses pre- vs. post-GLP-1 infusion.

FBF Responses to Ach

There were no alterations in heart rate or blood pressure in response to intra-arterial infusions of Ach and NTP during the study protocol in any participant (Fig. 3). Following administration of placebo, GLP-1 infusion increased FBF slightly, but significantly ($P = 0.049$), at baseline (3.0 ± 0.3 vs. 3.9 ± 0.2 ml·100 ml^{-1}·min^{-1}). GLP-1 increased FBF responses to Ach, resulting in an increase ($P < 0.03$) in both the peak incremental response (Δ+6.5 ± 1.1 vs. Δ+9.1 ± 1.2 ml·100 ml^{-1}·min^{-1}) and the AUC (15.8 ± 2.9 vs. 21.9 ± 3.4 ml·100 ml^{-1}·min^{-1}) following GLP-1 infusion.

In contrast, glyburide abolished changes in baseline FBF (2.6 ± 0.9 vs. 3.4 ± 1.0 ml·100 ml^{-1}·min^{-1}), the incremental vasodilatory response (Δ+11.7 ± 2.0 vs. Δ+11.7 ± 2.5 ml·100 ml^{-1}·min^{-1}), and the AUC (28.2 ± 5.1 vs. 28.6 ± 6.2 ml·100 ml^{-1}·min^{-1}) to Ach in the presence of GLP-1, whereas glimepiride had no effect on changes in baseline FBF (2.3 ± 0.2 vs. 3.3 ± 0.4 ml·100 ml^{-1}·min^{-1}, $P < 0.03$), the incremental response (Δ+7.9 ± 0.5 vs. Δ+10.2 ± 1.3 ml·100 ml^{-1}·min^{-1}, $P < 0.04$), or AUC (19.5 ± 1.9 vs. 25.2 ± 3.5 ml·100 ml^{-1}·min^{-1}, $P < 0.05$) in the presence of GLP-1. This augmented FBF response with glimepiride was not different from that seen during GLP-1 infusion with placebo.

FBF Responses to NTP

Following administration of placebo, GLP-1 infusion did not alter FBF responses either at baseline (3.9 ± 0.5 vs. 4.4 ± 0.8 ml·100 ml^{-1}·min^{-1}) or during graded infusions of NTP (Fig. 4). This resulted in similar peak incremental response of FBF to NTP following GLP-1 infusion (Δ+8.4 ± 0.8 vs. Δ+8.8 ± 1.3 ml·100 ml^{-1}·min^{-1}, pre vs. post). Furthermore, neither glyburide (Δ+10.1 ± 1.0 vs. Δ+9.8 ± 1.0 ml·100 ml^{-1}·min^{-1}, pre vs. post) nor glimepiride (Δ+11.1 ± 1.3 vs. Δ+12.1 ± 1.3 ml·100 ml^{-1}·min^{-1}, pre vs. post) altered FBF responses to NTP following GLP-1 infusion.

SU Assay

The presence of glimepiride or glyburide was detected appropriately in each of the subjects in the respective study arms during both FBF measurements between 90 to 120 min and 210 to 240 min. The placebo group did not show presence of SU.

DISCUSSION

The current studies indicate that GLP-1 per se increases baseline and Ach-induced vasodilatation. This effect is independent of alterations in glucose or insulin concentrations and

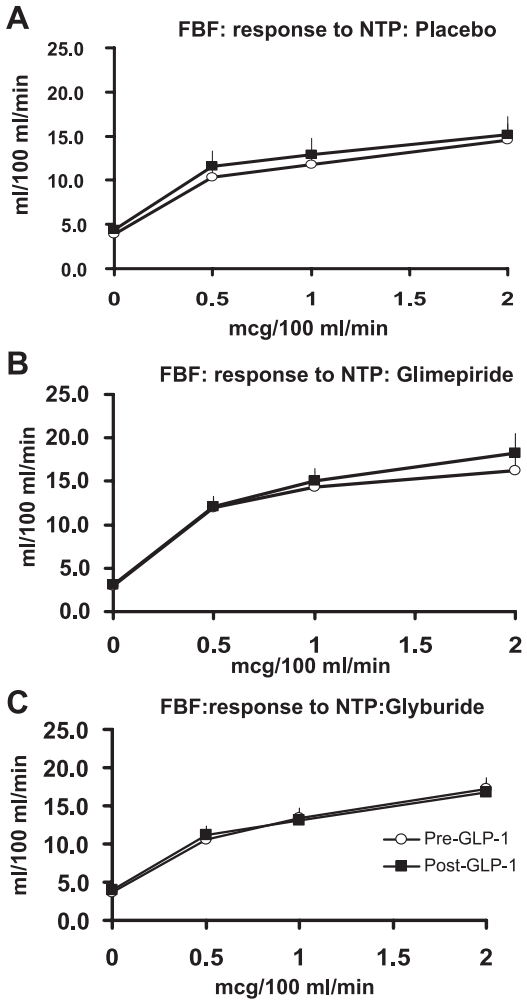

Fig. 4. FBF responses to graded infusions of NTP at 0.5, 1, and 2 μg·100 ml forearm volume^{-1}·min^{-1} before (○) and after (■) GLP-1 infusion in the placebo (*A*), glimepiride (*B*), and glyburide (*C*) arms of the study.

is abolished by glyburide but not by glimepiride. There were no alterations in endothelium-independent FBF responses. These data suggest but do not confirm that GLP-1 causes vasodilatation, at least in part, via the nitric oxide pathway and that this effect is modulated by K_{ATP} channels. These data also have important clinical implications. Our results suggest that that the combination of glyburide with GLP-1 may increase the risk of endothelial dysfunction and cardiovascular disease.

With regard to physiological mechanisms of circulatory control, the specifics of our blood flow measurement deserve particular attention. We measured total FBF using venous occlusion plethysmography. Therefore, our data provide information about endothelium-dependent vasodilatation in the vessels that control blood flow to the forearm, i.e., the resistance vessels (arterioles and small arteries). This is in contrast to more common flow-mediated dilation studies of endothelial function in which responses of the brachial artery (a conducting vessel) are assessed. This is important because mechanisms of vasodilatation in conducting vessels such as the brachial artery are often different from those in the resistance vessels where blood flow is regulated (11). Thus, our study demonstrates influences of GLP-1 on control of blood flow as it occurs in the microcirculation and similarly shows the differential effects of two SU on this influence of GLP-1 to promote vasodilatation.

Our findings shed light on the possible mechanism of action of GLP-1 on endothelial function. GLP-1 preferentially enhanced endothelium-dependent (and not endothelium-independent) vasodilatation, implying upregulation of endothelial nitric oxide synthase enzyme system as a possible mediator of its effects. However, this remains to be proven. It was also intriguing to note that GLP-1 infusion slightly but significantly increased FBF at baseline (i.e., prior to infusion of Ach). Although this effect could also have been mediated via enhanced nitric oxide availability, other potential mechanisms of action via alterations in autonomic nervous system activities could have also played a role and remain to be determined. GLP-1 receptors have been identified in the heart (9) and in human coronary artery endothelial cells (28), confirming the basis of their actions on human endothelium. Animal studies have demonstrated direct effects of GLP-1 on pulmonary vasculature, leading to relaxation of pulmonary arterial rings in vitro (13, 30). GLP-1 receptor agonists administered centrally and peripherally increased blood pressure and heart rate in the rat (5, 34). Although we are unaware of any previous studies investigating the role of GLP-1 per se on vascular function in humans, a recent study reporting beneficial effects of GLP-1 infusion on regional and global left ventricular dysfunction in patients with acute myocardial infarction is of considerable interest (27). However, since control experiments were not performed in which circulating insulin concentrations were matched, it was intriguing whether these effects were due to a direct effect of GLP-1 on vascular function or were due to differences in insulin and/or glucose concentrations between groups. However, as has been shown in our study, since GLP-1 per se appears to modulate vascular function, this could have potentially important implications since people with type 2 diabetes mellitus have both impaired vasodilatory response to insulin that is believed to be due to reduced nitric oxide availability and/or response (4, 7) as well as reduced postprandial GLP-1 concentrations (33). It remains to be seen in future studies whether GLP-1 could improve endothelial function in subjects with type 2 diabetes.

The K_{ATP} channels comprising the SUR subunits and the Kir components play a significant role in endothelial and vascular smooth muscle functions. Kir6.1 knockout mice have been shown to develop coronary spasms (23), leading to sudden death. SUR2$^{-/-}$ mice develop hypertension and die suddenly (8). Our current data imply that SU modulate GLP-1 induced alterations in endothelial function. However, the situation is obviously complex given the differential effects of SUR agonists observed in our study. This differential effect of glyburide vs. glimepiride on the cardiovascular system has been recognized for some time, especially with regard to cardiac ischemic preconditioning in both animals and humans (16, 19, 26). The selectivity of K_{ATP} channel inhibition differs amongst SU agents. However, a double-blind randomized crossover study in individuals with type 2 diabetes did not show any difference on basal and stimulated FBF after 8 wk of therapy with glyburide, metformin, or glimepiride (3). Then again, FBF measurements were undertaken in the presence of differing insulin and C-peptide concentrations among groups and in the presence of considerable hyperglycemia that could have con-

founded results. The current study attempts to circumvent these potential confounders and examines the effects of these agents in the presence of comparable hormone and substrate levels.

In the pancreatic β-cells, a role for the modulatory effects of SUR subunits on GLP-1 receptor-dependent K_{ATP} channel closure has been described, allowing for cross-talk between GLP-1 receptors and SUR. $SUR1^{-/-}$ mice have significantly attenuated insulinotropic responses to GLP-1 (25, 31). In contrast, $Kir6.2^{-/-}$ mice retain insulinotropic effects of GLP-1 (22), thus demonstrating complex interactions between SUR and GLP-1 receptor signaling within the islets. Thus far, we are not aware of any prior reports demonstrating cross-talk between SUR and GLP-1 receptors in the endothelium. Our findings provide proof of concept of modulatory effects of SU on GLP-1-induced endothelial cell functions.

Our study has a few limitations. Somatostatin was infused to inhibit endogenous insulin and C-peptide secretion. We did so since both insulin and C-peptide have effects on endothelial function, but at about a 10-fold higher concentration than was allowed in our study. Although it is noteworthy that C-peptide concentrations increased in all groups following GLP-1 infusion, the levels between groups at the time of FBF measurements did not differ. Furthermore, the levels of C-peptide concentrations observed after GLP-1 infusion were far below prior reports (17) that have been shown to influence endothelial function in humans. The modest rise in insulin concentrations in one of the participants given glimepiride did not influence our results even when this subject was excluded from analysis. Somatostatin per se has effects on blood flow. However, since somatostatin was infused in all participants, such confounding would have been minimized. Furthermore, hyperglycemia also plays a modulatory role in endothelial function and FBF. Glucose concentrations, however, were comparable in all groups. The gentle and modest rise in plasma glucose in our study was very unlikely to have had a major impact on affecting FBF measurements. A previous study (29) did not demonstrate modulatory effects of acute hyperglycemia on stimulated FBF in healthy nondiabetic subjects.

In summary, the present study shows that GLP-1 per se augments endothelium-dependent vasodilatation in nondiabetic humans and that this effect is differentially modulated by SU agents. On the basis of the results of our study, the endothelial benefits of GLP-1-based therapy may be mitigated largely by concomitant utilization of the SU glyburide. Future investigations need to be directed to determine the effects of GLP-1 with or without SU in people with endothelial dysfunction, i.e., type 2 diabetes mellitus.

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