Renal extraction and acute effects of glucagon-like peptide-1 on central and renal hemodynamics in healthy men

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1Department of Clinical Physiology and Nuclear Medicine, Bispebjerg University Hospital, Copenhagen, Denmark; 2Department of Endocrinology, Hvidovre University Hospital, Copenhagen, Denmark; 3NKF Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark; 4Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; 5Department of Diagnostics, Clinical Physiology and Nuclear Medicine, Glostrup University Hospital, Copenhagen, Denmark; and 6Institut National de la Santé et de la Recherche Médicale, UMR 1048, Institute of Metabolic and Cardiovascular Diseases, Paul Sabatier University, Toulouse, France

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Asmar A, Simonsen L, Asmar M, Madshad S, Holst JJ, Frandsen E, Moro C, Jonassen T, Bülow J. Renal extraction and acute effects of glucagon-like peptide-1 on central and renal hemodynamics in healthy men. Am J Physiol Endocrinol Metab 308: E641–E649, 2015. First published February 10, 2015; doi:10.1152/ajpendo.00429.2014.—The present experiments were performed to elucidate the acute effects of intravenous infusion of glucagon-like peptide (GLP)-1 on central and renal hemodynamics in healthy men. Seven healthy middle-aged men were examined on two different occasions in random order. During a 3-h infusion of either GLP-1 (1.5 pmol·kg−1·min−1) or saline, cardiac output was estimated noninvasively, and intraarterial blood pressure and heart rate were measured continuously. Renal plasma flow, glomerular filtration rate, and uptake/release of hormones and ions were measured by Fick’s Principle after catheterization of a renal vein. Subjects remained supine during the experiments. During GLP-1 infusion, both systolic blood pressure and arterial pulse pressure increased by 5 ± 1 mmHg (P = 0.015 and P = 0.002, respectively), Heart rate increased by 5 ± 1 beats/min (P = 0.005), and cardiac output increased by 18% (P = 0.016). Renal plasma flow and glomerular filtration rate as well as the clearance of Na+ and Li+ were not affected by GLP-1. However, plasma renin activity decreased (P = 0.037), whereas plasma levels of atrial natriuretic peptide were unaffected. Renal extraction of intact GLP-1 was 43% (P < 0.001), whereas 60% of the primary metabolite GLP-1 9-36amide was extracted (P = 0.017). In humans, an acute intravenous administration of GLP-1 leads to increased cardiac output due to a simultaneous increase in stroke volume and heart rate, whereas no effect on renal hemodynamics could be demonstrated despite significant extraction of both the intact hormone and its primary metabolite.

Glucagon-like peptide-1; blood pressure; heart rate; cardiac output; renal plasma flow

GLUCAGON-LIKE PEPTIDE (GLP)-1 is a 30-amino acid peptide hormone primarily synthesized by enteroendocrine L cells distributed in the small and large intestines and secreted in a nutrient-dependent manner. GLP-1 stimulates insulin secretion and inhibits glucagon secretion and gastric emptying, resulting in reduced postprandial glycaemia (14). The GLP-1 receptor is a G protein-coupled receptor and a member of the glucagon receptor family (18). The GLP-1 receptor was originally identified in islet β-cells in the pancreas, but it is also widely expressed in extrapancreatic tissues in humans (17, 25, 33).

GLP-1 receptor agonists have been approved for the treatment of hyperglycemia in subjects with diabetes, and, in addition, they may have significant cardiovascular effects (6, 28). However, results regarding the effects on arterial blood pressure are conflicting (2, 9, 11, 20–22, 29, 31). The reasons for the apparent discrepancies are not clear, although differences between species, doses applied, and durations of treatment may contribute.

Human studies have reported a natriuretic effect of native GLP-1, possibly due to reduced Na+ reabsorption in the proximal tubule (12, 29). However, in a recent study (25) validating a new, monoclonal GLP-1 receptor antibody, GLP-1 receptors could not be identified in the proximal tubule, whereas they were expressed in renin-secreting cells of the juxtaglomerular apparatus. It has not yet been clarified whether GLP-1 influences central and renal hemodynamics in men. The present study was designed with the purpose of investigating native GLP-1’s central and renal hemodynamic effects.

METHODS

Subjects. Seven male subjects of Caucasian origin (age: 47 ± 2 yr, height: 1.78 ± 0.01 m, and weight: 76.0 ± 2.4 kg) participated in the present study, which involved two experiments performed in random order separated by ~4 wk. All subjects were healthy and none took medication at the time of the study (Table 1). Consent to participate was obtained after subjects had read a description of the experimental protocol, which was approved by the Scientific Ethics Committee of the Capital Region of Copenhagen (H-2-2012-139).

Protocol. All subjects consumed a controlled diet for 4 days before testing (16% protein, 55% carbohydrate, and 29% fat). The energy content was 2,822 kcal/day. The basal NaCl content of the diet, as measured at Eurofins Stein’s Laboratory in Denmark, was 55–75 mmol/day. This was supplemented with additional 2 mmol NaCl/kg body wt−1·day−1. To assess the compliance of the subjects with the dietary regimen, 24-h urine collections were performed on the last day, and electrolyte concentrations were analyzed. Water intake was ad libitum, and strenuous excess physical activity was not allowed. Subjects were given 600 mg Li+ orally at 09:00 PM the day before each experiment. Under Na+-standardized conditions as in the present study, it is generally accepted that the renal clearance and renal extraction of Li+ are in accord with fractional Na+ and thereby fluid reabsorption in proximal tubules.

Subjects fasted for 12 h before the beginning of the experiment (Fig. 1). After emptying the bladder, confirmed by ultrasound, the subject remained supine throughout the experiment. A forearm vein was catheterized with a 18-gauge catheter (BD Venflon: 1.2-mm outer
Fig. 1. Glucagon-like peptide-1 (GLP-1; 1.5 pmol kg⁻¹ min⁻¹) or saline (0.9 %NaCl)
Blood pressure, heart rate, and cardiac output

GLP-1 (1.5 pmol kg⁻¹ min⁻¹) or saline (0.9 %NaCl)

<table>
<thead>
<tr>
<th>$^{51}$Cr EDTA (1.6 MBq hour⁻¹)</th>
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<tr>
<td>Urine collection (at voluntary voiding)</td>
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<td>Blood samples</td>
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Table 1. Subject characteristics

<table>
<thead>
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<th>Variable</th>
<th>Value</th>
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<tr>
<td>Number of subjects</td>
<td>7</td>
</tr>
<tr>
<td>Age, yr</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.78 ± 0.01</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76.0 ± 2.4</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/l</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>Fasting insulin, pmol/l</td>
<td>41.5 ± 7.5</td>
</tr>
<tr>
<td>Fasting C-peptide, ng/ml</td>
<td>2.8 ± 0.4</td>
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Data are presented as means ± SE.
Plasma Li$^+$ concentrations were measured by atomic absorption spectrophotometry (model 2380, Perkin-Elmer, Norwalk, CT).

Plasma and urine were analyzed for gamma radiation activity on a WIZARD$^3$ 1480 automatic gamma counter (Perkin-Elmer, Waltham, MA).

Plasma epinephrine and norepinephrine concentrations were measured by radioimmunoassay using a commercial kit (2-CAT RIA, Labor Diagnostika Nord, Nordhorn, Germany).

Plasma renin concentrations were determined using Liaison Direct Renin measurements (DiaSorin, Saluggia, Italy).

Plasma levels of proANP were measured by radioimmunoassay using antiserum and proANP(1–30) calibrator from Peninsula Laboratories. The tracer was prepared by in-house iodination and HPLC purification. Plasma samples were diluted 11-fold with radioimmunoassay buffer before assay.

Plasma levels of ANP were measured by enzyme immunoassay using a commercial kit (RayBio Human ANP Enzyme, RayBiotech, Norcross, GA).

Urine electrolytes and urine Li$^+$ were measured by atomic spectrophotometry (model 2380, Perkin-Elmer).

Urine creatinine was measured by an enzymatic method (Cobas 8000 System, Indianapolis, IN).

Urine pH was measured by a XC161 Combination pH electrode (Radiometer Medical Aps).

Renal plasma flow was calculated as follows: $^{51}$Cr-EDTA infusion rate/(arterial $^{51}$Cr-EDTA – venous $^{51}$Cr-EDTA) at steady state.

Glomerular filtration rate was calculated as follows: renal plasma flow $\times$ (arterial $^{51}$Cr-EDTA – venous $^{51}$Cr-EDTA)/arterial $^{51}$Cr-EDTA.

Renal extraction fraction of GLP-1 was calculated as follows: (arterial GLP-1 – venous GLP-1)/arterial GLP-1.

Renal electrolyte clearance was calculated as follows: electrolyte clearance = $U_x \times V/P_x$, where $U_x$ and $P_x$ are the concentrations of substance $X$ in urine and plasma, respectively, and $V$ is the urine excretion rate.

Statistical analysis. Data were analyzed using SigmaPlot 12 (Sysstat Software, Chicago, IL) and GraphPad Prism 5 (GraphPad Software, La Jolla, CA). Results are presented as means ± SE. The area under the curve (AUC) was calculated using the trapezoidal rule, and a t-test for paired data was used to compare changes in the AUC during GLP-1 infusion with changes in the AUC during saline infusion. $P$ values of < 0.05 were considered statistically significant.

RESULTS

Standardized NaCl intake. On the last day of the 4-day period with standardized NaCl intake before GLP-1 or saline infusion, 24-h urine volumes (2,469 ± 337 and 2,498 ± 559 ml/24 h, $P = 0.961$), renal Na$^+$ excretions (215 ± 30 and 209 ± 12 mmol/24 h, $P = 0.842$), and renal Li$^+$ excretions (6.4 ± 1.9 and 6.4 ± 0.6 mmol/24 h, $P = 0.977$) were similar.

Renal extraction of GLP-1. Arterial and renal venous plasma concentrations of total GLP-1, intact GLP-1, and GLP-1 metabolite (GLP-1 9-36amide) are shown in Fig. 2. During saline infusion, arterial and renal venous plasma concentrations of total GLP-1 remained constant throughout the experiments (6.1 ± 0.5 and 5.0 ± 0.3 pmol/l; Fig. 2A). During GLP-1 infusion, arterial and venous plasma concentrations of total GLP-1 increased significantly (128.4 ± 36.6 and 64.5 ± 5.0 pmol/l), and steady state was obtained within 40 min (Fig. 2A). Analysis of the contributions of intact GLP-1 7-36amide and the metabolite demonstrated that both moieties were extracted ($P < 0.001$ and $P = 0.012$, respectively), with the fraction for the metabolite being slightly higher than that of intact GLP-1 (60% vs. 43%; Fig. 2, B and C). During saline infusion, a statistically significant renal extraction of GLP-1 could not be demonstrated.

Effects of GLP-1 on arterial blood glucose and plasma insulin. During GLP-1 infusion, arterial plasma insulin levels increased transiently with a maximum at 20 min ($P = 0.027$; Fig. 3, A–C) and blood glucose levels were transiently reduced ($P = 0.004$; Fig. 3, D and E) with a nadir of 4.34 ± 0.14 mmol/l at 40 min and a range of 3.89–5.70 mmol/l within the first 60 min (Fig. 3D). None of the subjects developed symptoms of hypoglycemia.

Effects of GLP-1 on vasoactive hormones. Plasma epinephrine concentrations tended to increase transiently with a maximum at 60 min concomitantly with a reduction in plasma glucose concentrations during GLP-1 infusion but remained unchanged during saline (Fig. 4, A and B). Plasma norepineph-
raine concentrations were unchanged in both experiments (Fig. 4, C and D). During GLP-1 infusion, plasma renin concentrations decreased ($P = 0.037$) but remained unchanged during saline infusion (Fig. 4, E and F). Plasma proANP and ANP concentrations remained constant in both experiments (Fig. 4, G–J).

Effects of GLP-1 on central and renal hemodynamics. Effects of GLP-1 on blood pressure, heart rate, and cardiac output are shown in Fig. 5.

During GLP-1 infusion, systolic blood pressure and arterial pulse pressure increased within 60 min ($P = 0.011$ and $P < 0.001$, respectively; Fig. 5, B and H), and both pressures remained increased throughout GLP-1 infusion with a mean increase of $5 \pm 1 \text{ mmHg}$ ($P = 0.015$ and 0.002, respectively; Fig. 5, A–C and G–I). Diastolic blood pressure remained unchanged in both experiments (Fig. 5, D–F). Heart rate increased within 60 min ($P = 0.039$; Fig. 5K) and remained elevated throughout GLP-1 infusion with a mean increase of $5 \pm 1 \text{ beats/min}$ ($P = 0.005$; Fig. 5, J–L). Mean heart rate range (heart rate variability) was measured in the end of the infusion period and was similar during GLP-1 and saline infusion ($33 \pm 6$ and $33 \pm 9 \text{ beats/min}$, $P = 0.888$). Cardiac output increased within 60 min ($P = 0.038$; Fig. 5N) by 18% ($P = 0.016$; Fig. 5, M–O).

Effects of GLP-1 on renal hemodynamics are shown in Fig. 6. Renal plasma flow and glomerular filtration rate, as measured continuously by $^{51}$Cr-EDTA infusion and arterial and renal venous concentrations, were unaffected by GLP-1 infusion (Fig. 6, A–D).

Average renal electrolyte clearances and urinary secretion rates. In the present study, differences in arteriovenous plasma concentrations of Na$^+$, K$^+$, H$^+$, and Li$^+$ could not be demonstrated (data not shown). Furthermore, using samples from urine collected throughout each experiment ($446 \pm 18$ and $465 \pm 13$ min), renal electrolyte excretions as well as clearances were not statistically different (Table 2).

DISCUSSION

The major finding in the present study is a sustained increase in cardiac output during an intravenous GLP-1 infusion. This was due to a simultaneous increase in stroke volume and heart rate. Despite GLP-1 being extracted by the kidneys, it was not possible to demonstrate any significant effect on renal plasma flow and glomerular filtration rate. Furthermore, plasma renin activity decreased during GLP-1 infusion, whereas it was not possible to demonstrate an effect on ANP levels.

Plasma levels of GLP-1 in the present study were in the slightly supraphysiological range (5, 7).

To our knowledge, this is the first human study investigating the acute effects of GLP-1 on blood pressure invasively. Systolic blood pressure increased significantly during GLP-1 infusion, whereas diastolic blood pressure remained unchanged, indicating an increase in stroke volume, which, along with a significant increase in heart rate, led to an increase in cardiac output. The mechanisms behind the increase in arterial pulse pressure remain unclear. Since the GLP-1 receptor probably may be exclusively localized in the sinoatrial node (25), a direct GLP-1 receptor-mediated positive inotropic effect, generating a subsequent increase in stroke volume, seems unlikely.

During the transient decrease in plasma glucose, a transient and statistically nonsignificant increase in plasma epinephrine concentrations was seen. Thus, the sustained positive chronotropic effect was probably not due to the hypoglycemic activation of the sympathetic nervous system. Furthermore, the transient increase in plasma insulin concentrations subsided within 1 h. An insulin-mediated activation of the sympathetic nervous system is therefore unlikely. Therefore, the increase in
Fig. 4. Effects of GLP-1 or saline infusion on arterial plasma concentrations of epinephrine (A and B), norepinephrine (C and D), renin (E and F), proatrial natriuretic peptide (proANP; G and H), and ANP (I and J). A, C, E, G, and I: time courses of concentrations during the infusion from 0 to 180 min. B, D, F, H, and J: integrated effects during the infusion from 0 to 180 min. Data are presented as means ± SE.
Fig. 5. Effects of GLP-1 or saline infusion on intra-arterial blood pressure (A–I), heart rate [in beats/min (bpm); J–L], and cardiac output (M–O). A, D, G, J, and M: time courses of measurements during the infusion from 0 to 180 min. B, C, E, F, H, I, K, L, N, and O: integrated effect during the infusion from 0 to 60 min (B, E, H, K, and N) and 0 to 180 min (C, F, I, L, and O). Data are presented as means ± SE.
A novel finding in the present study is that GLP-1 is extracted in the human kidneys, comparable to the renal extraction of GLP-1 in pigs (27). Clearly, the extraction exceeds what can be explained by glomerular filtration. In this context, it is of interest that the GLP-1 receptor has also been localized to the renal afferent arterioles, and binding to these could be involved in the additional clearance (25). However, the metabolite, which is a weak antagonist of the receptor but may importantly influence blood vessels (1), was extracted at an even higher rate, suggesting that additional mechanisms might be involved. Despite this extraction, we were not able to measure any change in Na\(^+\), K\(^+\), H\(^+\), or Li\(^+\) clearance or excretion rate during GLP-1 infusion, in agreement with a constant renal plasma flow and glomerular filtration rate. Nevertheless, plasma renin activity decreased during GLP-1 infusion. It can be argued that there may be a slight underestimation of GFR by \(\sim 10\%\) using \(^{51}\text{Cr-EDTA}\) in the present study. This is due to a small amount (12.15 \(\pm\) 0.59\%) of \(^{51}\text{Cr-EDTA}\) plasma protein bound, and, consequently, this may affect their ultrafiltration (8, 13). However, in the present experimental setup, arterial \(^{51}\text{Cr-EDTA}\) levels were constant during the experiments, implying that also the protein-bound fraction of \(^{51}\text{Cr-EDTA}\) must have been constant.

In contrast to the present study, Gutzwiller et al. (12) found that a 3-h GLP-1 infusion dose dependently increased renal Na\(^+\) excretion and decreased glomerular filtration rate, as measured as the renal clearance of creatinine. Due to a simultaneous decrease in renal H\(^+\) excretion, the authors suggested that GLP-1 has a direct effect on Na\(^+\)/H\(^+\) exchange at proximal tubular cells. Decreased Na\(^+\) reabsorption at a segment proximal to the macula densa would increase NaCl delivery to the macula densa and initiate a decrease in the glomerular filtration rate through tubuloglomerular feedback (24). GLP-1
receptors have been detected in porcine proximal tubular cells (26). However, it has not been possible to demonstrate the presence of GLP-1 receptors in proximal tubular cells in humans (17, 25). On the other hand, GLP-1 receptor expression in renin-secreting cells of the juxtaglomerular apparatus and in the afferent arteriole has been detected (25). In the present study, urine was collected ~2 h before and after the 3-h infusion, and subjects remained supine throughout the study. The mode of urine collection in the present study, beginning before the GLP-1 or saline infusion, did not allow any time resolution with respect to a possible natriuretic effect of GLP-1. Therefore, an initial natriuretic effect of GLP-1 cannot be excluded. However, with the sensitivity of the analytic method applied, it would have been possible to demonstrate an average natriuretic effect in humans of ~60%, as demonstrated by Gutzwiller et al. (12). Furthermore, based on this GLP-1-induced natriuresis (12), we would hypothetically expect a difference in the arteriovenous plasma concentration of Na⁺ by ~1.3 mmol/l. In the present study, the coefficient of variation of Na⁺ measurements in arterial and venous plasma was 0.004%, and the SE of the arteriovenous differences was calculated to be 0.21 mmol/L. Thus, despite a sufficient sensitivity of the analytic method applied, it was not possible to measure any exchange of plasma Na⁺ during GLP-1 or saline infusion. A study by Skov et al. (29), using an isotopic infusion technique coupled with urinary sampling, also showed an increase in renal Na⁺ clearance, compatible with the results of Gutzwiller et al. (12). Since the renal clearance of Li⁺ increased simultaneously, the authors suggested that the mechanism behind the possible GLP-1-mediated natriuresis is located to the proximal tubuli. On the other hand, glomerular filtration rate as well as renal plasma flow remained unchanged during GLP-1 infusion, similar to the present study. Nevertheless, the GLP-1-mediated natriuresis was not related to systemic hemodynamic effects, since blood pressure remained unchanged and heart rate only increased transiently, most likely related to a transient decrease in blood glucose levels. In the study of Skov et al., subjects changed to upright position every 20 min to urinate. Thus, high- and low-pressure baroreceptors were unloaded and thereby the renin-angiotensin-aldoosterone system was activated. Despite the fact that blood samples and blood pressure measurements were separated by 10 min from voiding, it is likely that an interindividual variation in the activation of the renin-angiotensin-aldoosterone system influenced the results of that study (10). This is supported by the finding that plasma renin concentrations remained unchanged during GLP-1 infusion, whereas only plasma ANG II concentrations surprisingly decreased. In the present study, we aimed at keeping the baroreceptors stimulated constantly by maintaining subjects in the supine position throughout the study. Furthermore, in studies by both Gutzwiller et al. (12) and Skov et al. (29), conditions (due to overhydration) were less physiological, and studies were not conducted under NaCl-standardized conditions before the experiments. The latter is particularly important in studies of acute renal effects potentially mediated by hormones (3). Natriuresis can be induced by pressure mechanisms and by volume mechanisms (4). In the study by Gutzwiller et al., subjects were given a 2.5% NaCl infusion for 120 min (0.03 ml·min⁻¹·kg⁻¹), corresponding to a fluid volume of 5,625 ml and a Na⁺ load of ~50 g. In the present study, a total volume load of 240 ml (0.9% NaCl) was given during each experiment, corresponding to a Na⁺ load of ~2 g. Thus, a remarkable difference between the present experiments and the studies by Gutzwiller et al. and Skov et al. is that subjects in the latter studies were hydrated to a larger extent. This suggests that GLP-1 may contribute to natriuresis elicited via volume-regulating mechanisms, whereas it does not play a role in natriuresis elicited by pressure-regulating mechanisms (4). Thus, these natriuretic effects may be related to the supraphysiological volume and Na⁺ load in contrast to the present study. Nevertheless, from the present study, it cannot be excluded that GLP-1 may have a small natriuretic effect under normal physiological conditions; however, if so, it seems to be of minor biological importance.

It has been suggested that GLP-1 receptor stimulation in the atria induces ANP secretion in mice (16). To our knowledge, so far only one other human study (30) has investigated a possible GLP1-ANP axis. In that study, MR-proANP and not ANP itself was analyzed, because the analysis was carried out post hoc. ANP is stored in the form proANP(1–126) and cleaved from the propeptide upon secretion. Thus, proANP(1–98) and active peptide [ANP = proANP(99–126)] are secreted in equimolar amounts. Nevertheless, due to a longer half-life and better preservation in plasma, proANP is today used clinically as a surrogate for cardiac ANP secretion. Similar to the study by Skov et al. (29), GLP-1-infusion in our study did not affect proANP levels in plasma, and also plasma ANP levels remained constant, which seem to exclude a GLP1-ANP axis in humans.

In conclusion, the present study demonstrates significant acute hemodynamic effects of GLP-1 in healthy men under highly standardized conditions. These effects are not due to changes in renal perfusion, which was unaffected by GLP-1. Changes in renal function in healthy men could not be demonstrated, although a renal extraction of total GLP-1 by ~55% was found.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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E649


REFERENCES


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