The physiological role of glucagon-like peptide-1 in the regulation of renal function

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The glycemic effects of GLP-1 are primarily mediated via binding to its G protein-coupled receptor and triggering of intracellular cAMP generation (27). Activation of the GLP-1R also elicits extraglycemic effects. Continuous infusion of GLP-1 or administration of GLP-1R agonists induces diuresis and natriuresis in humans and experimental models (4, 11, 18, 21, 23). We previously showed that pharmacological doses of GLP-1 reduce Na+/H+ exchanger isoform 3 (NHE3)-mediated NaHCO3 reabsorption in the renal proximal tubule and increase intrarenal cAMP generation, PKA activation, and phosphorylation of NHE3 at the PKA consensus sites serines 552 and 605 (NHE3pS552, NHE3pS605) (4). In addition, the GLP-1/GLP-1R agonists are associated with reduced resistance of preglomerular vessels (14, 26), increased glomerular blood flow, and glomerular filtration rate (GFR) in rodents (4, 14, 18, 26).

While these studies demonstrate that pharmacological doses of GLP-1 and GLP-1R agonists induce diuresis and natriuresis, a physiological role for endogenous GLP-1 in regulating basal renal function remains an open question. Therefore, this study tested the hypothesis that physiological activation of GLP-1R under basal condition exerts a tonic effect on sodium and water handling via modulation of proximal tubule NHE3.

METHODS

Reagents and antibodies. All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise noted. GLP-1 and the GLP-1R antagonist exendin-9 were purchased from Bachem (Philadelphia, PA). The following antibodies were used: a monoclonal antibody (mAb) to NHE3 (Biemesderfer and Aronson, Yale University, New Haven, CT), mAb anti-NHE3pS552 (Santa Cruz Biotechnology), mAb anti-actin (JLA20, Merck Millipore), a rabbit polyclonal to horseradish peroxidase-conjugated secondary antibodies (Life Technologies).

Experimental animals. All experiments were performed in accordance with the ethical principles of the Brazilian College of Animal Experimentation and approved by the Institutional Animal Care and Use Committee. Male Wistar rats (8–10 wk old, State University of Campinas, São Paulo, Brazil) were housed at the Heart Institute (InCor) animal facility at a constant temperature and a 12:12-h dark-light cycle. The acute renal effects of GLP-1R blockade were evaluated after 8 h of fasting to avoid possible differences in post-prandial circulating levels of endogenous GLP-1. Rats were anesthetized with thiopental (60 mg/kg ip) and placed on a heated surgical table to maintain a body temperature of 37°C. Supplemental doses of the anesthetic were administered as required. After a tracheotomy, polyethylene catheters were inserted into the jugular vein (drug infusion), urinary bladder (urine collection), and right carotid (mean
blood pressure and blood sampling). After an equilibration period of 45 min, exendin-9 (100 μg·kg⁻¹·min⁻¹) or vehicle (4% BSA/saline) was intravenously infused at a rate of 40 μl/min for 30 min. Experiments designed to evaluate the effects of GLP-1R blockade on renal NHE3 phosphorylation levels were also performed in rats infused with 1 μg·kg⁻¹·min⁻¹ GLP-1 or 1 μg·kg⁻¹·min⁻¹ GLP-1 plus 100 μg·kg⁻¹·min⁻¹ exendin-9. Anesthetized rats were euthanized by decapitation, and the kidneys were immediately removed, and arterial blood collected.

**Blood and urine analysis.** Blood glucose, blood and urinary sodium concentrations, and pH were measured on a Radiometer ABL5 blood-gas analyzer (Radiometer). Blood arterial samples for measurements of active GLP-1 were also collected into Vacutainer K2 EDTA tubes (BD Biosciences) containing 10 μM DPP4 inhibitor sitagliptin and centrifuged at 3,000 rpm at 4°C for 15 min. Plasma was collected and stored at −80°C. Endogenous lithium concentrations were determined by flame photometry (Micronal B262, São Paulo, Brazil). Urinary creatinine concentration was determined with a kit (Labtest, Lagoa Santa, Brazil), and plasma creatinine was measured on a Beckman Coulter Synchrom CX7 Analyzer (Beckman Coulter). An ELISA was employed to determine plasma active GLP-1 (7–36), insulin (Merck Millipore), and urinary cAMP (Arbor Assays).

**Stationary microperfusion.** In the experiments designed to investigate the role of GLP-1R blockade on NHE3-mediated HCO₃⁻ reabsorption (JHCO₃⁻), proximal tubules were perfused with control solution (HCO₃⁻ saline-stained solution) in the presence of 20 nM GLP-1, 2 μM exendin-9, 20 nM GLP-1 plus 2 μM exendin-9 or vehicle, as previously described (4, 19).

**Renal cortical membrane protein isolation.** Kidneys were removed, cut in half, and the cortices were isolated and homogenized as previously described (20). Protein concentration was determined by the Lowry method (17).

**SDS-PAGE and immunoblotting.** Membrane proteins were solubilized in Laemmli sample buffer and resolved using 7.5% SDS-PAGE gels. Immunoblotting was performed as previously described (3). Relevant bands were digitized using an ImageScanner III (GE HealthCare) and quantified using Scion Image Software (Scion, Frederick, MD).

**Statistical analysis.** Results are reported as means ± SE, with n indicating the number of rats, unless otherwise stated. Comparisons between two groups were performed using unpaired t-tests. If more than two groups were compared, statistical significance was determined by using one-way ANOVA followed by the Tukey post hoc test. P < 0.05 was considered significant.

## RESULTS

**Acute systemic infusion of the GLP-1R antagonist exendin-9 reduces renal GLP-1R signaling and alters renal function.** To test the hypothesis that endogenous activation of the GLP-1R influences renal function at baseline, we infused the GLP-1R antagonist exendin-9 in overnight-fasted anesthetized Wistar rats for 30 min at a dose of 100 μg·kg⁻¹·min⁻¹. This dose was chosen based on binding studies performed in Chinese hamster ovary cells expressing rat GLP-1R which showed that exendin-9 at a concentration 100 times higher than that of GLP-1 completely blocks GLP-1R activation (7). The exendin-9 dose chosen corresponds to 100 times the pharmacological dose of GLP-1 shown to induce natriuresis and diuresis in rodents (4).

The levels of fasting blood glucose, insulin, and GLP-1 were similar between exendin-9- and vehicle-infused rats (Table 1). As seen in Fig. 1A, rats infused with exendin-9 excreted lower levels of urinary cAMP compared with vehicle-infused rats (8.8 ± 1.7 vs. 14.5 ± 1.4 pmol/min, P = 0.02). In addition, phosphorylation of PKA substrates in the renal cortex was lower in exendin-9-infused vs. vehicle-infused rats (73 ± 3 vs. 100 ± 2%, P < 0.001) (Fig. 1B).

The lower renal GLP-1R/cAMP/PKA activation induced by exendin-9 was associated with a 50% reduction in urine output and urinary sodium excretion (Table 1). The antidiuretic and antinatriuretic actions of GLP-1R blockade were accompanied by a reduction in GFR. In addition, fractional excretion of sodium, urinary pH, and lithium clearance (an index of volume flow out of the proximal tubule) were significantly reduced in exendin-9- vs. vehicle-treated rats. A trend toward increased blood pressure in 30-min exendin-9-infused rats was evident but not significant (P = 0.10) (Table 1). Taken together, these results suggest that acute blockade of the renal GLP-1R/cAMP/PKA pathway exerts strong antinatriuretic and antidiuretic effects that can be attributed, at least in part, to lower GFR and higher renal proximal tubule sodium reabsorption.

**Effects of acute GLP-1R blockade by exendin-9 on activity and phosphorylation of proximal tubule NHE3.** We previously reported that luminal perfusion of proximal tubules with GLP-1 inhibits NHE3 transport activity (4). Therefore, we hypothesized that GLP-1R antagonism would stimulate NHE3-mediated Na⁺/H⁺ exchange. As shown in Fig. 2A, NHE3-mediated JHCO₃⁻, in proximal tubules perfused with 2 μM exendin-9, measured by stationary in situ microperfusion, was very similar to those tubules perfused with control solution (2.18 ± 0.07 vs. 2.00 ± 0.08 nmol·cm⁻²·s⁻¹). At first glance, this finding appears to refute the hypothesis that endogenous GLP-1 is a tonic inhibitor of NHE3. However, during stationary in situ microperfusion, the tubular flow is interrupted and the proximal tubule cell is solely exposed to the perfusion solution. Since the proximal tubule does not synthesize or secrete GLP-1, the blockade of GLP-1R in the absence of its ligand is not expected to affect proximal tubular function. Indeed, proximal tubular perfusion with exendin-9 in the presence of its ligand completely blocked the inhibitory effect of GLP-1 on JHCO₃⁻ (1.19 ± 0.11 vs. 2.00 ± 0.08 nmol·cm⁻²·s⁻¹, P < 0.001). To evaluate whether GLP-1R blockade could alter NHE3 phosphorylation levels at the PKA consensus site serine 552 (PS552), rats were infused with exendin-9 (100 μg·kg⁻¹·min⁻¹), GLP-1 (1 μg·kg⁻¹·min⁻¹), or GLP-1 (1

| Body wt, g | 254 ± 5 (18) | 253 ± 9 (16) |
| Glucose, mg/dl | 106 ± 8 (14) | 113 ± 12 (12) |
| Insulin, ng/ml | 0.41 ± 0.04 (14) | 0.44 ± 0.07 (12) |
| GLP-1, pg/ml | 11.5 ± 0.6 (14) | 11.3 ± 0.9 (12) |
| MAP, mmHg | 105 ± 5 (14) | 116 ± 4 (12) |
| Urinary flow, μl·min⁻¹·kg⁻¹ | 130 ± 11 (14) | 47 ± 3** (12) |
| Urinary Na⁺, μeq·min⁻¹·kg⁻¹ | 2.62 ± 0.34 (14) | 1.22 ± 0.26* (12) |
| GFR, ml·min⁻¹·kg⁻¹ | 8.7 ± 0.5 (14) | 7.0 ± 0.6** (12) |
| FE Na⁺, % | 1.06 ± 0.20 (14) | 0.55 ± 0.09** (12) |
| Urinary pH | 6.36 ± 0.15 (14) | 5.90 ± 0.15* (12) |
| Li⁺ clearance, ml·min⁻¹·kg⁻¹ | 2.08 ± 0.12 (14) | 1.56 ± 0.10* (12) |
| FE Li⁺, % | 29 ± 2 (14) | 21 ± 1** (12) |

Table 1. Effects of acute GLP-1R blockade by exendin-9 on glycemia and renal function of overnight-fasted rats
μg·kg\(^{-1}\)·min\(^{-1}\) plus exendin-9 (100 μg·kg\(^{-1}\)·min\(^{-1}\)) for 30 min, and renal cortical membrane proteins were isolated from these rats and subjected to SDS-PAGE and immunoblotting. As illustrated in Fig. 2B, acute exendin-9 infusion reduced the levels of NHE3pS552 in the renal cortex compared with vehicle-infused rats (71 ± 5 vs. 100 ± 4%, P < 0.01). Consistent with our previous findings, GLP-1 increased renal cortical NHE3pS552 (147 ± 4%). In line with our data from the stationary in situ microperfusion, exendin-9 was capable of preventing the effects of GLP-1 on NHE3 (94 ± 2 vs. 147 ± 4%, P < 0.001). Collectively, these results suggest that acute systemic administration of exendin-9 reduces NHE3pS552, which is consistent with higher NHE3 activity and the lower estimated with endogenous lithium clearance.

DISCUSSION

The diuretic and natriuretic effects of GLP-1R activation have been consistently reported by numerous studies (4, 11, 18, 21). However, these studies did not address whether endogenous GLP-1 plays a physiological role in the basal regulation of sodium balance. Herein, we provide novel evidence that baseline levels of GLP-1R signaling tonically influence renal sodium reabsorption. Thus GLP-1R activation is now added to the list of factors that, working in concert, match sodium and volume output to intake.

The physiological role of endogenous GLP-1 in the regulation of renal function was established by acutely blocking the GLP-1R with its peptide antagonist exendin-9, which lowered...
RENAL EFFECTS OF GLP-1R BLOCKADE

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AUTHOR CONTRIBUTIONS

Author contributions: L.X.F., V.V., T.D.P., and G.M. performed experiments; L.X.F., V.V., T.D.P., G.M., A.A.M., and A.C.C.G. interpreted results of experiments; L.X.F., V.V., T.D.P., G.M., A.A.M., and A.C.C.G. approved final version of manuscript; A.A.M. and A.C.C.G. edited and revised manuscript; A.C.C.G. provided conception and design of research; A.C.C.G. prepared figures; A.C.C.G. drafted manuscript.

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