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Attenuated diuresis and natriuresis in response to glucagon-like peptide-1 in hypertensive rats are associated with lower expression of the glucagon-like peptide-1 receptor in the renal vasculature

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ABSTRACT

Accumulating evidence from clinical and experimental studies indicates that the incretin glucagon-like peptide-1 (GLP-1) elicits blood-pressure lowering effects via its diuretic, natriuretic and vasodilatory properties. The present study investigated whether acute infusion of GLP-1 induces diuresis and natriuresis in spontaneously hypertensive rats (SHRs). Additionally, we examined whether GLP-1 influences the vascular reactivity of the renal arteries of normotensive and hypertensive rats and elucidated the underlying mechanisms. We found that the increase in urinary output and urinary sodium excretion in response to systemic infusion of GLP-1 for 30 min in SHRs was much less pronounced than in normotensive rats. The diuretic and natriuretic actions of GLP-1 in normotensive rats were accompanied by increases in GFR and RBF and a reduction in RVR through activation of the cAMP signaling pathway. However, no changes in renal hemodynamics were observed in SHRs. Similarly, GLP-1 induced an endothelium-independent relaxation effect in the renal arteries of normotensive rats, whereas the renal vasculature of SHRs was unresponsive to this vasodilator. The absence of a GLP-1-induced renal artery vasodilator effect in SHRs was associated with lower expression of the GLP-1 receptor, blunted GLP-1-induced increases in cAMP production and higher activity and expression of the GLP-1 inactivating enzyme dipeptidyl peptidase IV relative to the renal arteries of normotensive rats. Collectively, these results demonstrate that the renal acute responses to GLP-1 are attenuated in SHRs. Thus, chronic treatment with incretin-based agents may rely upon the upregulation of GLP-1/GLP-1 receptor signaling in the kidneys of hypertensive patients and experimental models.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is an incretin hormone produced through post-translational cleavage of the proglucagon gene product in the intestinal L cells in response to food intake. GLP-1 exerts a variety of actions that contribute to the maintenance of blood glucose, including stimulation of insulin secretion, reduction of glucagon release, slowing of gastric emptying, improvement of insulin sensitivity and diminution of food intake (Degn et al., 2004; Holst, 2013; Hvidberg et al., 1994; Perley and Kipnis, 1967). As such, this peptide constitutes a potential therapeutic target for the treatment of type 2 diabetes mellitus. However, the clinical use of native GLP-1 is limited due to its rapid inactivation by the enzyme dipeptidyl peptidase IV (DPPIV), which cleaves GLP-1 from the penultimate position at the N-terminal region to yield the GLP-1 (9-36) truncate form (Deacon et al., 1995), which does not act at the GLP-1 receptor. Therefore, inhibitors of DPPIV activity, which enhance the bioavailability of endogenous GLP-1, and DPPIV-resistant GLP-1 receptor agonists, have been developed for clinical use.

Accumulating evidence from experimental studies and clinical trials suggests that incretin-based therapeutic agents reduce blood pressure in both diabetic and non-diabetic patients and experimental models, suggesting that these drugs exert anti-hypertensive actions independent of their glucose lowering properties (Giannocco et al., 2013; Hirata et al., 2009; Mistry et al., 2008; Okerson et al., 2010; Pacheco et al., 2011; Wang et al., 2013; Yu et al., 2003). Indeed, GLP-1 possesses diuretic, natriuretic and vasodilatory actions that may reduce blood pressure or attenuate blood pressure increases (Tanaka et al., 2011).

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The natriuretic actions of GLP-1 are mediated through inhibition of the Na^+/H^+ exchanger isoform 3 (NHE3) in the proximal tubule and increased renal blood flow (RBF) and glomerular filtration rate (GFR) (Crajoinas et al., 2011; Moreno et al., 2002; Schlatter et al., 2007; Thomson et al., 2012). Inhibition of NHE3 requires activation of the GLP-1 receptor, which leads to increased intracellular cAMP accumulation, PKA activation and phosphorylation of the C-terminal region of NHE3 at the PKA consensus sites, serines 552 and 605 (Crajoinas et al., 2011; Farah et al., 2016; Rieg et al., 2012). Nevertheless, it remains to be established whether a similar signaling cascade is triggered to reduce renal vascular resistance, leading to an increase in RBF and GFR. In fact, the vasodilatory actions of GLP-1 are known to occur in vascular smooth cells and/or endothelial cells by GLP-1 receptor-dependent (Nystrom et al., 2005) and -independent mechanisms (Ban et al., 2008). The latter are mediated by the GLP-1 metabolite GLP-1 (9-36) and require the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) signaling pathway.

The present study was undertaken to investigate whether GLP-1 is capable of acutely affecting the renal function of spontaneously hypertensive rats (SHR) and to test the hypothesis that the effects of GLP-1 on renal vascular resistance are dependent on the cAMP signal transduction pathway. In addition, we investigated whether GLP-1 exerts direct vascular actions on the renal arteries of normotensive and hypertensive rats and elucidated the underlying mechanisms.

2. Materials and methods

2.1. Materials

GLP-1 (7-36), exendin-9, and the DPPIV substrate Gly-Pro-p-nitroanilide tosylate were purchased from Bachem (Torrance, CA). The monoclonal antibody (mAb) against DPPIV, clone 5E8, was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Polyclonal antibodies against GLP-1 receptor were obtained from Santa Cruz Biotechnology and Abcam (Cambridge, MA). The mAb to actin (JLA20) was purchased from Merck Millipore (Billerica, MA). MAbs directed against the catalytic and regulatory subunits of protein kinase A (PKA) were purchased from BD Biosciences (San Jose, CA). Secondary antibodies were purchased from Life Technologies Corporation (Carlsbad, CA). All other chemicals were acquired from Sigma Chemical Company (St. Louis, MO) unless otherwise noted.

2.2. Experimental animals

All experiments were carried out in accordance with the ethical principles in animal research of the Brazilian College of Animal Experimentation and were approved by the Institutional Animal Care and Use Committee. Male spontaneously hypertensive rats (SHRs) (14–16 weeks old) and age-matched Wistar rats were obtained from State University of Campinas, São Paulo, Brazil. Rats were housed at the University of São Paulo Medical School animal facility at a constant temperature with a 12 h dark/light cycle. Systolic blood pressure (SBP) was measured by noninvasive tail-cuff plethysmography (BP-2000 Blood Pressure Analysis System, Visitech Systems, Apex, NC). Rats were euthanized by decapitation and their renal arteries were immediately removed.

2.3. Renal function studies

The effects of GLP-1 on renal function were examined in a separate group of normotensive (n = 22) and hypertensive rats (n = 23). On the day of the study, rats were anesthetized with ketamine-xylazine-acepromazine (64.9, 3.20, and 0.78 mg/kg sc, respectively) and placed on a heated surgical table to maintain a body temperature of 37 °C. After tracheotomy, polyethylene catheters were inserted into the jugular vein and the urinary bladder for drug infusion and urine collection, respectively. To evaluate the mean blood pressure (MBP) and allow blood sampling, a PE-60 catheter was inserted into the right carotid. After an equilibration period of 30–45 min, GLP-1 (1.0 μg/kg/min) or vehicle (4% BSA/saline) was intravenously infused at a rate of 40 μl/min for 30 min. At the end of the experiment, the left renal artery was carefully dissected to allow placement of a nanoprobe that was connected to a perivascular transonic ultrasonic flow sensor to measure renal blood flow (RBF) as previously described (Luchi et al., 2015). Left renal vascular resistance (RVR) was calculated as the mean arterial pressure divided by the left RBF. Urine samples collected during the 30 min were used to measure urine output, urinary sodium and creatinine. Urine output was measured gravimetrically. Creatinine clearance was used to estimate GFR. Serum and urinary creatinine concentrations were measured by a kinetic method (Labtest, Minas Gerais, Brazil) using a ThermoPlate Analyzer Plus (ThermoPlate, São Paulo, Brazil). Sodium was measured on a Radiometer ABL800 Flex blood gas analyzer (Radiometer Medical, Brønshøj, Denmark). Urinary cAMP was measured using the Cyclic AMP Direct Enzyme Immunoassay Kit (Arbor Assays, Ann Arbor, MI), according to the manufacturers' specifications. Renal function was also assessed in Wistar rats infused with both GLP-1 and the adenylyl cyclase inhibitor SQ-22536 (5 μg/kg/min) (n = 10) or SQ-22536 alone (n = 8). In this last series of experiments, both GLP-1 + SQ-22536 and SQ-22536-treated rats were pre-infused for 30 min with the SQ22536 compound.

2.4. Vascular reactivity

For isometric tension recording, renal arteries removed from SHRs and Wistar rats were dissected free, cleaned of connective tissue and divided into cylindrical segments (3 mm in length). Subsequently, each renal arterial ring was mounted in an isolated tissue chamber containing Krebs-Henseleit solution (in mM: 118 NaCl, 4.7 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$-$2H_2O$, 1.2 KH_2PO_4, 1.2 $MgSO_4$-$7H_2O$, 11 glucose, and 0.01 EDTA) gassed with 95% O_2 and 5% CO_2. Two metal wires were passed through the lumen of the segments. One wire was attached to a fixed support, while the other was connected to a moveable holder connected to an isometric tension transducer (Letica TRI 210, Spain). The renal arterial rings were placed under a resting tension of 500 mg. After a 60-min equilibration period, the rings were exposed to 125 mM KCl to assess maximal tension. After a washout period with Krebs-Heinseleit and a subsequent 30-min stabilization period, the rings were contracted with a concentration of phenylephrine inducing 50–70% of the contraction induced by KCl (75 mM). After reaching a plateau, cumulative concentration-response curves to GLP-1 (10^{-10} to 10^{-6} M) were obtained in the presence or absence of 100 μM of the GLP-1 receptor antagonist exendin-9 (Serre et al., 1998) or 100 μM of the nonselective nitric oxide synthase (NOS) inhibitor N-nitro-L-arginine methyl ester (L-NAME). In some experiments, the endothelium was mechanically removed by gently rubbing the intimal surface with a stainless-steel rod. The effectiveness of endothelium removal was confirmed by the absence of relaxation induced by acetylcholine (10^{-6} M).

2.5. Extraction of proteins from renal arteries

Renal arterial rings were added to microcentrifuge tubes containing 1 mM EDTA, 1 mM EGTA, 2 mM $MgCl_2$, 5 mM KCl, 25 mM HEPES (pH 7.5), 2 mM DTT, 1 mM PMSF, 0.1% Triton X-100, 1:300 proteases inhibitors cocktail (Sigma), 1:300 phosphatases inhibitors cocktail I (Sigma) and 1:300 phosphatases inhibitors cocktail II (Sigma). The tubes were then agitated continuously at 4 °C for 12 h. Protein concentration was determined by the Lowry method (Lowry et al., 1951).