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Renal effects of glucagon-like peptide in rats

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Abstract

The present study examined the effects of recombinant glucagon-like peptide-1-(7-36)amide (rGLP-1) on renal hemodynamics and excretory function in innervated and denervated kidneys of anesthetized rats. Intravenous infusion of rGLP-1 at a dose of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ increased urine flow and Na^+ excretion 13-fold in the innervated kidney. The natriuretic and diuretic response to rGLP-1 was attenuated in the denervated kidney in which urine flow and Na^+ excretion only increased 3-fold. Fractional excretion of Li^+, an index of proximal tubular reabsorption, increased 219% in the innervated kidney but only 54% in the denervated kidney during infusion of rGLP-1. The diuretic and natriuretic response to rGLP-1 was associated with an increase in glomerular filtration rate (39%) in the innervated kidney, but it had no effect on glomerular filtration rate in the denervated kidney. These results indicate that the natriuretic and diuretic effects of rGLP-1 are due to inhibition of Na^+ reabsorption in the proximal tubule. It also increases glomerular filtration rate in kidneys with an intact renal innervation.

Keywords: rGLP-1 (glucagon-like peptide-1-(7-36)amide), recombinant; Kidney; Hemodynamics, renal; Proximal tubule

1. Introduction

Glucagon-like peptide 1 (GLP-1) is a hormone produced by L-type cells in the intestine. It possess multiple actions, including stimulation of glucose-dependent insulin secretion, inhibition of glucagon release and the regulation of nutrient assimilation by inhibiting gastric emptying and food intake (Drucker, 2001; Holst, 2000). GLP-1 has been suggested to have potential for the treatment of diabetes, since it improves glucose utilization in patients with type 2 diabetes by increasing the secretion of insulin and inhibiting the secretion of glucagon (Vella et al., 2000).

GLP-1 has a short half-life due to rapid renal clearance and inactivation by dipeptidyl-peptidase IV (Ruiz-Grande et al., 1990; Kieffer et al., 1995). The major form of circulating GLP-1 is glucagon-like peptide-1-(7-36)amide (rGLP-1). Receptors for GLP-1 have been identified in pancreatic islets cells, gastrointestinal tract, lung, heart, central nervous system and in the kidney (Bullock et al., 1996). Beside its effects on insulin and glucagon secretion, GLP-1 alters the secretion of vasopressin (Larsen et al., 1997) and the production of pulmonary surfactant by type II cells in the lung (Benito et al., 1998). GLP-1 also affects the cardiovascular system, as it has been reported to increase arterial pressure in man and rats (Barragan et al., 1999; Edwards et al., 1998). This effect is thought to be mediated centrally since administration of the GLP-1 receptor antagonist, exendin-(9-39), into cerebral ventricles attenuates the cardiovascular effects of GLP-1 (Barragan et al., 1999).

Glucagon is a renal vasodilator that increases the excretion of Na^+, water and K^+ (Ahloulay et al., 1995). However, the mechanism of action of glucagon on renal tubular and vascular function remains uncertain. Receptors for GLP-1 are highly expressed in the kidney, but the effects of GLP-1 on renal function have not been examined. Therefore, the present study evaluated the effect of rGLP-1 on the renal function of rats with both innervated and denervated kidneys.

2. Materials and methods

Experiments were performed on 13 male Sprague–Dawley rats (230–300 g) purchased from Harlan Laboratories (Indianapolis, IN). The rats were housed in Animal Care Facility at the Medical College of Wisconsin that is approved by the American Association for the Accreditation of Laboratory Animal Care. All protocols comply with the

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European Community guidelines for the use of experimental animals and were reviewed and approved by the Animal Care Committee of the Medical College of Wisconsin.

Rats were anesthetized with ketamine (30 mg·kg^{-1}, i.m.) and thiobutabarbital (50 mg·kg^{-1}, i.p.) and placed on a heated table to maintain body temperature at 36 °C. Cannulas were placed in right femoral artery and vein for measurement of mean arterial pressure and intravenous infusions. The ureters were also cannulated for urine collection. The rats received an intravenous infusion of 0.9% NaCl solution at a rate of 3 ml·h^{-1} throughout the experiment. [^{3}H]inulin (1 μCi·ml^{-1}) and LiCl (20 mM) were included in the infusion solution to allow for measurement of glomerular filtration rate and the fractional excretion of Li^+, an index of proximal tubular reabsorption (Thomsen et al., 1981). The left kidney was denervated, a 2-mm electromagnetic flow probe (Carolina Instruments, NC) was placed around the left renal artery for measurement of renal blood flow, and a fiber optic probe was inserted in the renal medulla for measurement of renal medullary blood flow by laser Doppler flowmetry (Perimed KB, Sweden), as previously described (Mattson et al., 1993). In addition, a polyethylene matrix capsule was implanted in the renal cortical interstitium for measurement of renal interstitial hydrostatic pressure, as previously described (Fenoy et al., 1992).

After surgery and a 60-min equilibration period, glomerular filtration rate, urine flow, Na^+ excretion, K^+ excretion and fractional excretion of Li^+ were measured in both kidneys during a 20-min control period. Renal blood flow, renal medullary blood flow and renal interstitial hydrostatic pressure were also measured in left (denervated) kidney.

After the control period, the rats received an intravenous infusion of rGLP-1 (BioNebraska, NE) at a dose of 0.2 μg·kg^{-1}·min^{-1}. After a 10-min equilibration period, urine and plasma samples were collected during a 20-min experimental period. The dose of rGLP-1 was then increased to 1.0 μg·kg^{-1}·min^{-1} and urine and plasma samples were again collected during a second 20-min clearance period. Control rats received intravenous infusion of vehicle (10 mM acetic acid and 5.07% D-mannitol, pH 4.5) during the experimental periods. A sample of blood (2 ml) was collected via the arterial catheter at the end of the experiment and the plasma frozen at −80 °C for measurement of plasma levels of total GLP-1, and the kidneys were removed and weighed.

2.1. Analytical techniques

The concentration of [^{3}H]inulin in urine and plasma samples was determined using a liquid scintillation counter. Urine flow rate was determined gravimetrically. Glomerular

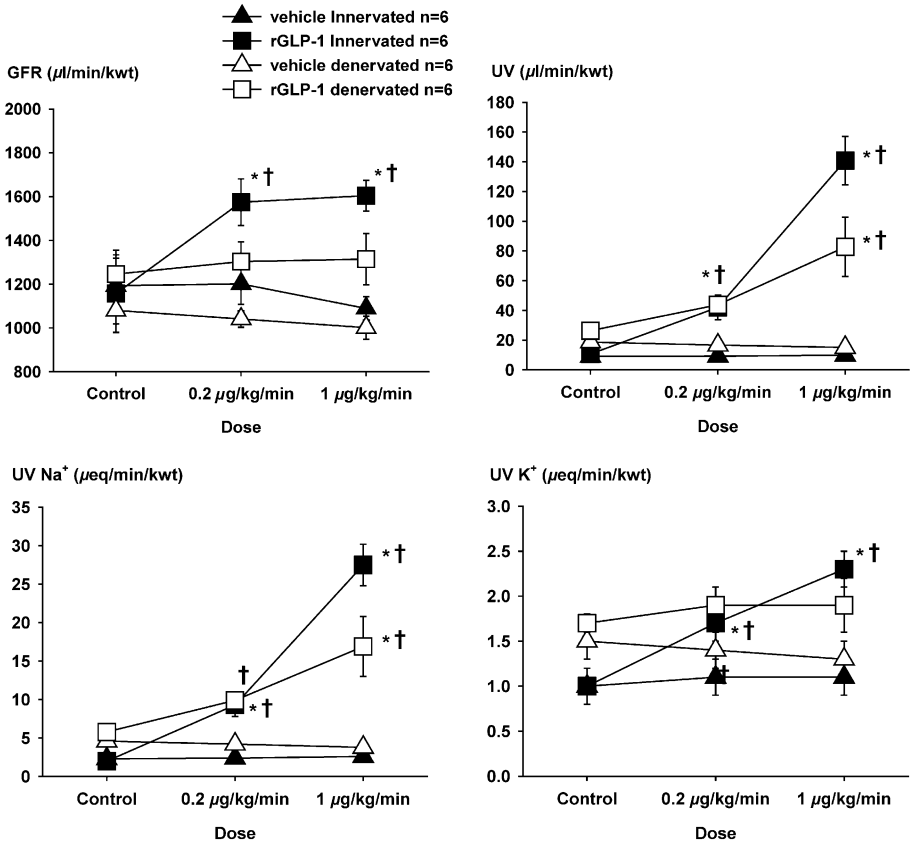

Fig. 1. Effects of saline (△, $N=6$) or glucagon-like peptide-1-(7-36)amide (rGLP-1) (□, $N=7$) at 0.2 and 1 μg·kg^{-1}·min^{-1} on glomerular filtration rate (GFR), urine flow (UV), Na^+ excretion (UV Na^+) and K^+ excretion (UV K^+). Close symbols reflect values for innervated kidneys. * Significant difference from the control period within the same group. † Significant difference from the corresponding value in the time control group.

filtration rate was calculated from the urine to plasma inulin concentration ratio times urine volume and was expressed per gram kidney weight (k wt). Na^+, K^+ and Li^+ concentrations in urine and plasma samples were measured by flame photometry (Instrumentation Laboratory, MA). Plasma concentrations of total rGLP-1 were determined using an Enzyme-Linked Immunosorbent Assay method (BioNebraska). The lower limit of detection (defined as the lowest concentration of rGLP-1 that could be fully recovered in rat plasma pools) was 125 $pg \cdot ml^{-1}$. The coefficient of variation for repeat measurements on rat plasma pool samples was < 6.2%.

2.2. Statistical methods

Data are expressed as means ± S.E.M. Statistical differences within and between groups were analyzed using analysis of variance for repeated measures followed by Student–Neuman–Keuls post hoc test. A $P < 0.05$ was considered statistically significant.

3. Results

Infusion of rGLP-1 (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) increased circulating levels of this peptide from < 125 $pg \cdot ml^{-1}$ in rats infused with vehicle ($N = 6$) to 18.8 ± 1.4 $ng \cdot ml^{-1}$ in rats receiving rGLP-1 ($N = 7$). Basal mean arterial pressure was similar in both control and experimental group (134 ± 3 and 130 ± 4 mm Hg, respectively). Infusion of rGLP-1 at either the 0.2 or the 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ dose had no significant effect on mean arterial pressure. Similarly, infusion of rGLP-1 at the low dose did not alter heart rate, but heart rate did

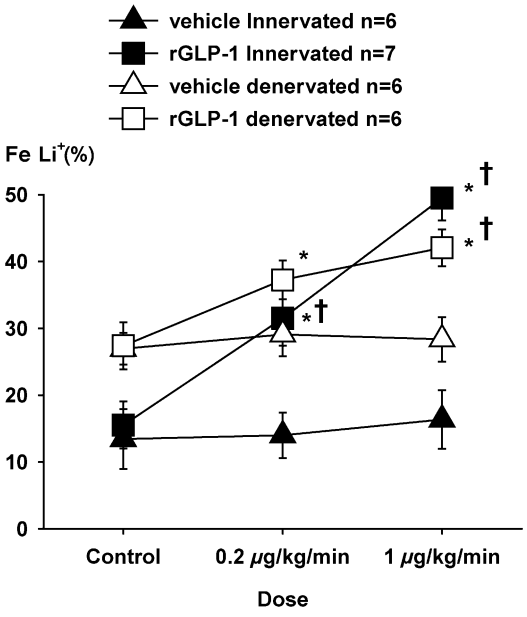

Fig. 2. Effects of saline (△, $N = 6$) or glucagon-like peptide-1-(7-36)amide (rGLP-1) (□, $N = 7$) at 0.2 and 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ on fractional Li^+ excretion (Fe Li^+). Close symbols reflect values for innervated kidneys. * Significant difference from the control period within the same group. † Significant difference from the corresponding value in the time control group.

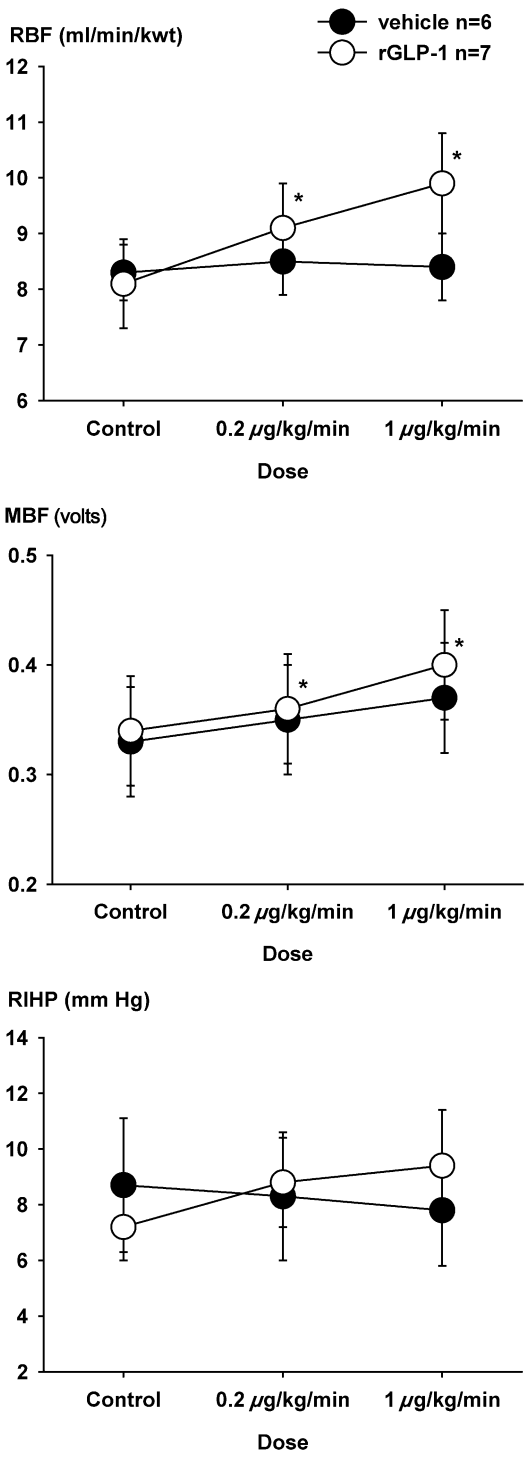

Fig. 3. Effects of saline (●, $N = 6$) or glucagon-like peptide-1-(7-36)amide (rGLP-1)(○, $N = 7$) at 0.2 and 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ on renal blood flow (RBF), renal medullary blood flow (MBF) and renal interstitial hydrostatic pressure (RIHP) in denervated kidneys. * Significant difference from the control period within the same group.

increase significantly when the rats were given the higher dose of rGLP-1 (from 362 ± 5.6 to 388 ± 7.9 bpm).

A comparison of the effects of infusion of rGLP-1 on water and electrolyte excretion in innervated and denervated kidneys are presented in Fig. 1. Urine flow, Na^+ and K^+ excretion and glomerular filtration rate were not significantly altered in either the innervated or denervated kidney in control rats infused with vehicle. Infusion of rGLP-1 produced significant and dose-dependent increases in urine flow (13-fold), Na^+ (13.5-fold) and K^+ excretion (2-fold) in the innervated kidney of rats. The diuretic and natriuretic response to infusion of rGLP-1 in kidneys with an intact innervation was associated with a 39% increase in glomerular filtration rate (Fig. 1) and a 3-fold increase in the fractional excretion of Li^+ (Fig. 2), a marker of proximal tubular reabsorption.

Renal denervation increased baseline urine flow, Na^+ and K^+ excretion (Fig. 1) and the fractional excretion of Li^+ (Fig. 2) but glomerular filtration rate was unaltered (Fig. 1). The diuretic and natriuretic response to infusion of rGLP-1 was attenuated in denervated kidneys relative to the response seen in kidneys with an intact innervation. Urine flow, Na^+ and K^+ excretion (Fig. 1) only rose by 3.1-, 2.9- and 1.1-fold, respectively, during infusion of the high dose of rGLP-1. Similarly, fractional excretion of Li^+ (Fig. 2) only increased by 54% in the denervated kidney during infusion of rGLP-1. In contrast to the results seen in kidneys with an intact renal innervation, rGLP-1 did not increase glomerular filtration rate in the denervated kidney.

The effects of rGLP-1 on renal hemodynamics in the denervated kidney are presented in Fig. 3. It was only possible to do these studies in the denervated kidney because placing of the electromagnetic flow probe requires stripping of the renal nerves from the renal artery and always results in some degree of renal denervation. Infusion of rGLP-1 increased renal blood flow by 22% but it had no significant effect on renal medullary blood flow or renal interstitial hydrostatic pressure when compared to the control rats infused with vehicle alone.

4. Discussion

The present study evaluated the effect of an intravenous infusion of rGLP-1 on renal function in rats. Intravenous infusion of rGLP-1 produced dose-dependent increases in Na^+ and water excretion in the absence of changes in mean arterial pressure. The diuretic and natriuretic response to rGLP-1 was associated with inhibition of proximal tubular reabsorption, as reflected by the marked elevation seen in the fractional excretion of Li^+. rGLP-1 also increased glomerular filtration rate and the filtered load of Na^+ in kidneys with an intact innervation, but it had no effect on glomerular filtration rate in denervated kidneys. The mechanism by which rGLP-1 increases glomerular filtration rate in innervated but not denervated kidneys could be related to an increase in renin release, since GLP-1 is known to activate sympathetic outflow (Barragan et al., 1999). The activation of the renin–angiotensin system would constrict the efferent arteriole and increase glomerular filtration rate. In denervated kidneys, rGLP-1 induced increase in sympathetic tone could not increase renin secretion. Therefore, the direct dilatory influence of rGLP-1 on the efferent arteriole may prevail, maintaining glomerular filtration rate despite an increase in renal blood flow. Two factors appear to explain the greater natriuretic and diuretic response to rGLP-1 in innervated versus denervated kidneys. First, rGLP-1 increased glomerular filtration rate only in innervated kidneys thus, the increase in the filtered load of Na^+ largely accounts for the greater diuretic and natriuretic effect of rGLP-1 seen in innervated versus denervated kidneys. Renal denervation also inhibited baseline proximal tubular reabsorption and increased fractional excretion of Li^+ and Na^+ and water. The increase in baseline Na^+ excretion also contributed to the smaller increment in Na^+ excretion seen during infusion of rGLP-1 in the denervated versus innervated kidney.

The mechanism by which infusion of rGLP-1 inhibits proximal tubular reabsorption remains to be determined. In the present study, the natriuretic response and increase in the fractional excretion of Li^+ excretion to rGLP-1 infusion was greater in innervated versus denervated kidneys. However, fractional excretion of Li^+ still rose to the same level during infusion of rGLP-1 in innervated and denervated kidneys. These observations suggest that the inhibitory effects rGLP-1 on proximal tubular reabsorption is not mediated by inhibition of renal sympathetic nerve activity but that the prevailing level of sympathetic tone likely modulates its response by altering basal levels of proximal tubular reabsorption.

Proximal tubular reabsorption is also influenced by changes in renal hemodynamics that elevate renal interstitial hydrostatic pressure and the backflux of Na^+ in the proximal tubule (Knox and Granger, 1992). In the present study, infusion of rGLP-1 increased renal blood flow, medullary blood flow and reduced filtration fraction in denervated kidneys. However, it had no significant effect on renal interstitial pressure which is thought to be the major mechanism by which changes in filtration fraction and renal medullary blood flow inhibits proximal tubular reabsorption. This observation suggests that the inhibitory effect of rGLP-1 on Na^+ transport in the proximal tubule is likely due to a direct effect rather than secondary to changes in renal hemodynamics that influence passive reabsorption of Na^+ in this nephron segment. The observation that receptors for GLP-1 are highly expressed in kidney (Bullock et al., 1996; Wei and Mojsov, 1995) is consistent with this view. However, at present time no information is currently available regarding the intrarenal distribution of GLP-1 receptors or whether they are localized to the proximal tubule.

Glucagon, like rGLP-1, also increases the excretion of Na^+, K^+ and water (Ahloulay et al., 1995). However, it remains to be determined whether the natriuretic response to

glucagon is due to a direct effect on epithelial transport or secondary to changes in renal hemodynamics and increases in renal blood flow and glomerular filtration rate. There is also a lack of agreement as to whether glucagon directly alters renal vascular tone or its effects are all secondary to stimulation of the release of cAMP from the liver. In this regard, Premen (1985) reported that a rise in circulating levels of cAMP mediates the vasodilatory effects of glucagon, because it had greater effect when infused in the portal circulation that it did when it was infused intrarenally. cAMP is also the second messenger for GLP-1 (Goke et al., 1989).

In summary, the results of the present study indicate that rGLP-1 is a potent diuretic and natriuretic agent. rGLP-1 acts by inhibiting reabsorption of Na^+ in the proximal tubule and to a lesser extent by increasing renal blood flow and/or glomerular filtration rate. Given the ability of GLP-1 to improve insulin resistance (Drucker, 2001) and the renal vasodilator and natriuretic effects of rGLP-1, it may have potential utility as a therapeutic agent in the treatment of heart failure and hypertension.

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