The natriuretic effect by exendin-4, but not the DPP-4 inhibitor alogliptin, is mediated via the GLP-1 receptor and preserved in obese type 2 diabetic mice

Timo Rieg, Maria Gerasimova, Fiona Murray, Takahiro Masuda, Tong Tang, Michael Rose, Daniel J Drucker and Volker Vallon

Am J Physiol Renal Physiol published online 25 July 2012

The natriuretic effect by exendin-4, but not the DPP-4 inhibitor alogliptin, is mediated via the GLP-1 receptor and preserved in obese type 2 diabetic mice

Timo Rieg [1,2], Maria Gerasimova [2], Fiona Murray [3], Takahiro Masuda [1,2], Tong Tang [1,2], Michael Rose [2], Daniel J. Drucker [4], and Volker Vallon [1,2,3,*]

[1] Department of Medicine, University of California San Diego, La Jolla, CA 92093, USA

[2] VA San Diego Healthcare System, San Diego, CA 92161, USA

[3] Department of Pharmacology, University of California San Diego, La Jolla, CA 92093, USA

[4] Department of Medicine, Samuel Lunenfeld Research Institute, Mt Sinai Hospital, University of Toronto, Toronto, Ontario, Canada

* To whom correspondence should be addressed:

Volker Vallon, MD; Department of Medicine & Pharmacology, Division of Nephrology/Hypertension; University of California San Diego & VA San Diego Healthcare System; 3350 La Jolla Village Drive (9151); San Diego, CA 92161; Phone: 858-552-8585 ext. 5945; Fax: 858-642-438; E-mail: vvallon@ucsd.edu.

Running title: GLP-1 receptor, DPP-4, and renal function

Abstract

Activation of the glucagon-like peptide-1 receptor (GLP-1R) and inhibition of dipeptidyl peptidase-4 (DPP-4) are new anti-diabetic strategies. The GLP-1R and DPP-4 are also expressed in the renal proximal tubular brush border where they may regulate Na^+ reabsorption. Exendin-4 (EX4) is a naturally occurring antidiabetic polypeptide (from the venom of a lizard *Heloderma suspectum*) and GLP-1R agonist, however, part of its non-glucoregulatory effects are through GLP-1R-independent mechanisms. DPP-4 cleaves and inactivates GLP-1 and, thus, the natriuretic effect of DPP-4 inhibition may be mediated by the GLP-1R. We report that parenteral application of EX4 in wild-type mice induced a diuresis and natriuresis which were associated with increases in glomerular filtration rate, fractional urinary fluid and Na^+ excretion, and renal membrane expression of Na^+/H^+-exchanger NHE3 phosphorylated at serine residues 552 and 605, established consensus sites for cAMP-dependent protein kinase A. These effects were absent in mice lacking the GLP-1R and independent of adenylyl cyclase 6. In comparison, parenteral application of the DPP-4 inhibitor, alogliptin, reduced plasma DPP-4 activity by 95% and induced a diuresis and natriuresis independent of the presence of the GLP-1R or changes in phosphorylated NHE3. The inhibitory effect on renal fluid and Na^+ reabsorption of EX4, but not that of alogliptin, was preserved in diabetic db/db mice and associated with a modest reduction in blood pressure. These results reveal mechanistic differences in how exendin-4 vs. DPP-4 inhibition induce diuresis and natriuresis under normal states, with preservation of GLP-1R but not DPP-4 inhibitor-dependent natriuretic mechanisms in a mouse model of obese type 2 diabetes.

Key words: glucagon-like peptide-1, dipeptidyl peptidase-4, NHE3, cAMP, proximal tubule,

Introduction

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted from enteroendocrine L cells in the intestine that stimulates glucose-dependent insulin release and may promote preservation of beta-cell function in patients with type 2 diabetes (9; 10). As a consequence, GLP-1 has been a principal focus of clinical and basic diabetes research in recent years. After secretion, active GLP-1 is rapidly cleaved by the widely expressed enzyme dipeptidyl peptidase-4 (DPP-4, CD26), such that the half-life of bioactive GLP-1 is less than 3 minutes. Therefore, therapeutic manipulation of the GLP-1 system includes strategies that inhibit the degradation of GLP-1 by DPP-4 (i.e. DPP-4 inhibitors) or degradation-resistant GLP-1R agonists with a longer half-life, such as exendin-4 (EX4) or liraglutide.

In addition to its metabolic effects, GLP-1 affects kidney function. Analysis of GLP-1R expression in rats (6), porcine, and humans (34) localized the GLP-1R to the brush-border microvilli of proximal tubules. Intravenous infusion of GLP-1 increased GFR, inhibited proximal tubular reabsorption, and increased urine flow and Na^+ excretion in rats (6; 30). In healthy subjects, infusion of GLP-1 evoked a dose-dependent increase in urinary Na^+ excretion without changing GFR (20; 21). Studies in intact rat and porcine renal proximal tubules indicated that GLP-1 decreases Na^+-H^+ exchanger (NHE3)-mediated bicarbonate reabsorption (6; 34). Moreover, GLP-1 increased the expression of NHE3 phosphorylated at serine residues 552 and 605, two PKA consensus sites, without changing total NHE3 expression, in rat proximal tubule brush-border microvilli (6). Similar results on proximal tubular bicarbonate flux and NHE3 phosphorylation were obtained following EX4 administration in the rat (6). Thus, there is strong evidence that GLP-1 and related GLP-1R agonists not only mediate important effects on glucose homeostasis, but also stimulate renal excretion of fluid and Na^+ in rats and humans.

71 The current studies first tested whether EX4 also induces a natriuretic effect in the
72 mouse, and whether this includes effects on both GFR and fractional renal Na^+ excretion. It is
73 known that GLP-1R agonists may mediate non-glucoregulatory effects at least in part through
74 GLP-1R-independent mechanisms (1). Moreover, EX4 has only 54% amino acid identity to
75 GLP-1 (18). Therefore, we determined whether the renal effects of EX4 are dependent on the
76 presence of an intact GLP-1R by using a mouse model that lacks this receptor.

77 DPP-4 inactivates GLP-1, is present in plasma and the surface of capillary endothelial
78 cells, and is one of the major proteins expressed in the apical brush-border membrane of the
79 proximal tubule (25), where it assembles with NHE3 (15). The DPP-4 inhibitors Lys [$Z(NO_2)$]-
80 pyrrolidide and P32/98 inhibited NHE3-mediated Na^+ reabsorption in rat renal proximal tubule
81 *in vivo* (6; 16). Studies in an opossum kidney proximal tubule cell line indicated that the
82 enzymatic activity of DPP-4 in the proximal tubular brush border may locally affect the
83 generation or breakdown of endogenous factors that regulate Na^+ reabsorption via effects on
84 NHE3 activity (17). Here we tested in the mouse the renal effects of alogliptin (ALG), a novel,
85 high affinity, high-specificity DDP-4 inhibitor (5; 7; 13). We hypothesized that the natriuretic
86 effect of ALG involves inhibiting the breakdown of GLP-1 and thus depends on an intact GLP-
87 1R.

88 Studies in humans demonstrate that obese men responded to GLP-1 infusion with a
89 natriuresis, but, in contrast to healthy subjects, this was associated with a decrease in GFR (21)
90 indicating that factors related to body mass may affect the renal response to EX4 and/or DPP-4
91 inhibition. Therefore, we also compared the effect of EX4 and ALG on renal function in obese
92 type 2 diabetic db/db mice.
93

Materials and Methods

Animal experiments were conducted according to the protocols reviewed and approved by the Institutional Animal Care and Use Committee of the Veterans Affairs San Diego Healthcare System. Heterozygote breeding strategies were used to yield *Glp1r*-/- and littermate WT mice (35) as well as adenylyl cyclase 6 (AC6) -/- and littermate WT mice (37). Homozygous C57BLKS/J db/db mice were used as an obese type 2 diabetic model (The Jackson Laboratories, Bar Harbor, ME) and littermate heterozygote db/- mice served as controls. Age- and gender-matched adult mice were used.

Metabolic cage experiments in awake mice. Mice were randomized to application of EX4 (10 µg/kg i.p.; Sigma-Aldrich, St. Louis, MO), ALG (10 mg/kg i.p.; Takeda Pharmaceuticals, Oak Grove, IL) or vehicle (2µl/g bw of 0.85% NaCl i.p.). After emptying the bladder, the mice were NaCl loaded by oral gavage (0.85% NaCl; 30 µl/g bw; ~30% of daily NaCl intake) and placed in metabolic cages for quantitative urine collection over 3 hours without access to food or water (32; 33), followed by blood glucose measurements by tail snip.

Two-period clearance experiments to assess GFR and absolute and fractional renal excretion. Mice were anesthetized with thiobutabarbital (100 mg/kg i.p., 2 µl/g bw; Sigma-Aldrich, St. Louis, MO) and ketamine (100 mg/kg i.m., 2 µl/g bw; Butler, Dublin, OH) and prepared for renal clearance experiments as described (31; 32). The jugular vein was cannulated for continuous infusion of 2.25% bovine serum albumin in 0.85% NaCl at a rate of 0.4 ml h^{-1} 30^{-1} g bw. For assessment of two-kidney GFR by inulin clearance, [^{3}H]inulin was added to the infusion to deliver 5 µCi h^{-1} 30^{-1} g bw. GFR and urinary excretion of fluid, Na^+ and K^+ were assessed by quantitative urine collection via a bladder catheter in two 30-min periods: after completion of a basal period, EX4 (10 µg/kg i.v.), ALG (10 mg/kg i.v.) or vehicle (1 µl/g bw of

117 0.85% NaCl i.p.) were given by i.v. bolus application and 5 min later the second period was
118 started. Blood samples (50 µl) were drawn midway in each period from an arterial catheter,
119 which was also used to monitor blood pressure and heart rate. Concentrations of [^{3}H]inulin in
120 plasma and urine were measured by liquid scintillation counting. Plasma and urine were
121 analyzed for Na^+ and K^+ concentrations by flame photometry (Cole-Parmer Instrument Co.,
122 Vernon Hills, IL, USA). cAMP concentrations were assessed by radioimmunoassay (33). In a
123 separate set of two-period clearance studies in WT mice, parathyroid hormone (10 µg/kg i.v.)
124 was given by i.v. bolus application and 5 min later the second period was started to measure
125 urinary cAMP excretion.

126 *Expression of total and phosphorylated NHE3 in renal membranes.* Kidneys were
127 harvested 1 hour after application of EX4 (10 µg/kg i.p.), ALG (10 mg/kg i.p.), or vehicle (2µl/g
128 bw of 0.85% NaCl). Renal membranes were prepared in the presence of protease and
129 phosphatase inhibitors as previously described (33; 39). Immunoblotting was performed at 4°C
130 overnight with the primary NHE3-Ab (Millipore Inc., Billerica, MA), pS552 NHE3-Ab (Novus
131 Biologicals, Littleton, CO), and pS605 NHE3-Ab (Santa Cruz Biotechnology Inc., Santa Cruza,
132 CA) diluted 1:1000,1:1000 and 1:200, respectively. The latter two antibodies only recognize
133 NHE when the serine 552 or serine 605 is phosphorylated (27). Chemiluminescent detection was
134 performed using a 1:5000 dilution of ECL donkey anti-rabbit IgG and anti-mouse IgG linked to
135 horseradish-peroxidase and ECL detection reagent (GE Healthcare, Buckinghamshire, UK). To
136 verify equal protein loading, the membrane was stripped (0.2 M NaOH for 5 minutes) and
137 reprobed with monoclonal anti–β-actin Ab (Sigma-Aldrich).

138 *DPP-4 activity in plasma*. Plasma DPP-4 activity was measured using a homogeneous
139 luminescent assay, DPPIV-Glo Protease Assay (Promega, San Luis Obispo, CA), according to

the manufacture's instruction. For each sample, the specific DPP-4 activity was determined by comparing measurements in the presence and absence of adding alogliptin (1 μM) to the incubation.

Statistical Analysis. The data are expressed as mean ± SEM. Unpaired and paired *t*-test were performed, as appropriate, to analyze for statistical differences between and within groups. *P* values <0.05 were considered statistically significant. The contribution of GLP-1R in the renal response to EX4 was determined by comparing changes in WT versus *Glp1r*-/- mice.

Results

Basal kidney function in Glp1r-/- mice. *Glp1r*-/- and littermate wild-type (WT) mice had similar body weight (27±1 vs 27±1 g), food and fluid intake (determined in regular cages; 0.14±0.01 vs. 0.14±0.01 g day^{-1} g bw^{-1}; 0.15±0.01 vs. 0.15±0.01 ml day^{-1} g bw^{-1}; n=22-26; NS), and plasma aldosterone concentration (879±115 vs 935±65 pg/ml; n=9; NS). Clearance studies under thiobutabarbital/ketamine anesthesia revealed similar blood pressure, heart rate, hematocrit, plasma Na^+ and K^+ concentrations, and absolute and fractional urinary excretion of H_2O, Na^+ and K^+, while GFR was modestly greater in *Glp1r*-/- compared with WT mice (Table 1).

Exendin-4-induced diuresis, natriuresis, and NHE3 phosphorylation in awake mice requires an intact GLP-1 receptor. Studies in metabolic cages revealed that urinary excretion of H_2O, Na^+, K^+, and Cl^- (Figure 1A) and blood glucose levels (154±9 vs. 147±8 mg/dl) were not different between WT and *Glp1r*-/- mice following an oral NaCl and water load with subsequent quantitative urine collection for 3 hours (vehicle groups). EX4 (10 µg/kg i.p.), the 39 amino acid naturally occurring GLP-1R agonist (12), that is highly resistant to the proteolytic action of DPP-4 in vivo (3; 8), lowered blood glucose levels in WT mice vs. vehicle treatment (104±8 vs. 154±9 mg/dl; $P<0.05$) and increased urinary excretion of H_2O and Na^+, whereas K^+ excretion was not significantly changed (Figure 1A). In comparison, EX4 did not affect blood glucose levels (146±6 vs. 147±8 mg/dl, NS) or urinary H_2O or Na^+ excretion in *Glp1r*-/- mice (Figure 1A).

Total renal membrane expression of NHE3 was not different in WT vs. *Glp1r*-/- mice and was not altered by EX4 (Figure 2A). EX4 increased the amount of NHE3 phosphorylated at serines 552 and 605 in renal membranes of WT but not *Glp1r*-/- mice (Figure 2B & C).

171 *Alogliptin-induced diuresis and natriuresis are independent of an intact GLP-1 receptor*
172 *and changes in NHE3 phosphorylation in awake mice.* ALG (10 mg/kg i.p.) administered in
173 metabolic cage studies increased urinary excretion of fluid and Na^+ in both WT and *Glp1r*-/-
174 mice, whereas K^+ excretion was not significantly changed (Figure 1B). In contrast to EX4, ALG
175 did not affect the amount of NHE3 phosphorylated at serines 552 and 605 in renal membranes
176 (Figure 2B & C) although the drug strongly inhibited plasma DPP-4 activity in both WT and
177 *Glp1r*-/- mice (Figure 2D).
178 *Exendin-4-induced increases in GFR and fractional urinary Na^+ excretion in*
179 *anesthetized mice depend on an intact GLP-1 receptor.* Two-period clearance experiments under
180 anesthesia were performed in *Glp1r*-/- and WT mice with a basal period followed by EX4
181 administration 5 min prior to the second period. Figure 3A shows the changes in the second
182 period versus the basal period. Changes in blood pressure and heart rate in the second period
183 were not different between WT and *Glp1r*-/- mice (9±2 vs. 9±4% and 5±2 vs. 5±2%,
184 respectively; NS for both). EX4 increased GFR, urinary flow rate, and absolute and fractional
185 urinary Na^+ excretion in WT relative to *Glp1r*-/- mice (Figure 3A). Thus, consistent with the
186 metabolic cage studies in awake mice, the EX4-induced natriuresis depends on an intact GLP-1R
187 and was the consequence of an increase in GFR and a modest decrease in fractional Na^+
188 reabsorption.
189 *Exendin-4-induced natriuresis and NHE3 phosphorylation occurs without changes in*
190 *urinary cAMP and is not affected by the absence of adenylyl cyclase 6*. Basal urinary excretion
191 of cAMP in clearance studies under anesthesia was increased in *Glp1r*-/- compared with WT
192 mice (Table 1). EX4 did not significantly change urinary excretion of cAMP in clearance studies
193 (Figure 3A) or in metabolic cage studies (Figure 4A). In contrast, parathyroid hormone increased

194 urinary cAMP excretion in WT mice in the experimental setting of the clearance studies (Figure
195 3B). EX4-induced diuresis and natriuresis and phosphorylation of NHE3 were not affected by
196 the absence of AC6 (Figure 4).

197 *The inhibitory effect of exendin-4 on renal Na^+ reabsorption but not the stimulatory effect*
198 *on GFR is preserved in db/db mice*. Two-period clearance experiments under anesthesia were
199 performed in hyperglycemic db/db mice and heterozygous non-diabetic db/- mice (control): a
200 basal period was followed by administration of EX4 (10 µg/kg i.v.) or vehicle 5 min prior to the
201 second period. Table 2 compares data obtained in basal periods in db/db and control mice. Db/db
202 mice, as expected, were heavier compared with control mice and hyperglycemic. Hematocrit,
203 blood pressure, heart rate, and plasma Na^+ concentration were similar between groups. Absolute
204 GFR and renal excretion of H_2O and Na^+ were not different between groups, but lower in db/db
205 versus controls when related to body weight. Lower plasma K^+ concentrations in db/db mice
206 were associated with greater renal fractional K^+ excretion and urinary K^+ to Na^+ ratios.

207 Figure 5 shows the effects of EX4 vs. vehicle in control and db/db mice. Changes in
208 blood pressure were not different between treatments in control mice. Similar to the responses
209 observed in C57BL/6 mice (WT, see above), EX4 lowered blood glucose levels and increased
210 GFR, urinary flow rate, and absolute and fractional urinary Na^+ excretion without altering renal
211 K^+ excretion in controls when compared with vehicle. EX4 lowered blood glucose levels in
212 control and db/db mice (Figure 5). EX4 modestly lowered blood pressure in db/db mice but did
213 not alter GFR compared with vehicle; yet and as observed in controls, EX4 increased fractional
214 renal excretion of H_2O and Na^+ in db/db mice without altering renal K^+ excretion.

215 *The inhibitory effect of alogliptin on renal H_2O and Na^+ reabsorption is blunted in db/db*
216 *mice.* Two-period clearance experiments with application of ALG (10 mg/kg i.v.) prior to the

second period revealed that changes in blood pressure were not different between ALG and vehicle treatment in db/db and control mice. In controls, ALG tended to increase GFR compared with vehicle (P=0.058); this was associated with an increase in absolute and fractional urinary H_2O and Na^+ excretion without altering renal K^+ excretion (Figure 5). As observed in controls, intravenous application of ALG did not significantly lower blood glucose levels in db/db mice compared with vehicle (Figure 5). In contrast to EX4, ALG did not significantly affect renal H_2O or Na^+ excretion in db/db mice (Figure 5). Additional metabolic cage experiments in awake mice confirmed that ALG (10 mg/kg i.p.) increased urinary Na^+ excretion in controls but not in db/db mice (Figure 6). Moreover, ALG at a dose of 30 mg/kg i.p. did not lower blood glucose levels in control (109±4 mg/dl; n=10; NS) or db/db mice (408±40 mg/dl; n=10; NS) compared with vehicle at 3 hours after application and significantly increased Na^+ excretion in controls (22.6±1.0 vs. 19.1±1.1 nmol/min/g; P<0.05 vs vehicle) but not in db/db mice (23.6±1.6 21.9±1.4 nmol/min/g; NS).

Discussion

The present study shows that EX4 induces a diuresis and natriuresis due to increasing GFR and reducing fractional renal fluid and Na^+ reabsorption. These findings in mice are in accordance with previous studies in rats (6). Using gene knockout and WT mice, the studies further show that this response requires a functional GLP-1R.

In rats, the increase in GFR in response to GLP-1 and EX4 has been associated with an increase in renal blood flow (6) indicating a primary vascular effect. Both GLP-1 and GLP-1(9-36) induced vasodilation and increased coronary flow in constant pressure–perfused isolated hearts (1). These effects were at least in part NO/cGMP-dependent and maintained in *Glp1r*-/- mice, suggesting a GLP-1R-independent mechanism. Notably, EX4 did not produce any vasodilatation or cGMP release in that preparation (1). In comparison, GLP-1 induced vasorelaxation in the rat aorta through classic GLP-1R-dependent adenylyl cyclase-coupled mechanisms (19). Here we show that the effect of EX4 on GFR, and thus potentially on the renal vasculature, is dependent on an intact GLP-1R.

Natriuretic effects of GLP-1 and EX4 in the rat have been linked to the phosphorylation and inhibition of NHE3 in the proximal tubule (6). We show that the natriuretic effect of EX4 in the mouse is also associated with phosphorylation of NHE3 at serines 552 and 605 and that the changes in NHE3 phosphorylation are dependent on an intact GLP-1R. Serines 552 and 605 are two consensus sites for PKA on NHE3 that are physiologically regulated both in vitro (27) and in vivo (26; 27). However, phosphorylation of these sites does not directly alter NHE activity (26). Thus, GLP-1R activation induces phosphorylation of NHE3 at serines 552 and 605, however, further studies are needed to understand the relevance and role of NHE3 phosphorylation for the natriuretic effects of GLP-1 and EX4.

The GLP-1R is a G protein-coupled receptor whose activation stimulates the formation of cAMP (14). Systemic application of GLP-1 and EX4 at 1-5 μg/kg increased urinary cAMP excretion in rats by 20-fold and the PKA inhibitor H89 prevented the inhibitory effect of GLP-1 on bicarbonate reabsorption in renal proximal tubule (6). We found that the EX4-induced and GLP-1R-mediated natriuresis and NHE3 phosphorylation in the mouse is not associated with an increase in urinary cAMP excretion and persisted in mice lacking AC6. The latter is the most abundant AC isoform in rat whole kidney (36) and mouse medulla (33) based on mRNA expression, and the dominant AC isoform with regard to forskolin-induced cAMP formation in mouse kidney (4) and inner medulla (33). It could be that the impact of proximal tubule release on total urinary cAMP excretion is lower in mice versus rats, although application of parathyroid hormone, which is thought to activate proximal tubular AC, increased urinary cAMP/creatinine ratios 3-fold in mice (43), a finding confirmed in the present studies. One may speculate that activation of proximal tubular AC by different receptors causes cAMP to enter the tubular lumen at different rates. Furthermore, an AC isoform other than AC6 may be activated by GLP-1Rs in the proximal tubule, which in the rat expresses AC 2, 3, 6, 7, and 9 (2).

Alogliptin at a dose of 10 mg/kg i.p. inhibited plasma DPP-4 activity by about 95% and induced a diuresis and natriuresis in non-diabetic mice that was associated with an increase in fractional renal excretion of fluid and Na^+. Alogliptin tended to increase GFR, but, similar to a previous report for the DPP-4 inhibitor, P32/98, in the rat (6), the effect did not quite reach statistical significance (P=0.058). Overall, it appears that DPP-4 inhibition can induce a small increase in GFR (and renal plasma flow (6)) in non-diabetic rats and mice but this effect appears to be smaller compared with direct GLP-1 receptor activation (this study and (6)).

276 Studies in an opossum kidney proximal tubule cell line showed that inhibitors of DPP-4
277 (diprotin A and P32/98) significantly reduced NHE3 activity (17). Treating rats with the DPP-4
278 inhibitor Lys [Z(NO_2)]-pyrrolidide for 7 days decreased NHE activity in isolated proximal tubule
279 microvillar membrane vesicles, redistributed NHE3 from the apical enriched microvillar
280 membranes to the intermicrovillar microdomain of the brush border, and increased fractional Na^+
281 excretion and lithium clearance (16). Moreover, intratubular application of GLP-1 or EX4 but
282 not the DPP-4 inhibitor P32/98 reduced the rate of bicarbonate flux during stationary in vivo
283 microperfusion in proximal convoluted tubules, i.e. during the absence of flow and glomerular
284 filtrate reaching the site of study (6). All these findings indicated that the enzymatic activity of
285 DPP-4 in the proximal tubular brush border may locally affect the generation or breakdown of
286 endogenous factors that regulate Na^+ reabsorption via effects on NHE3 and that this factor may
287 derive from the systemic circulation via glomerular filtration. The present study shows that, in
288 contrast to EX4, the alogliptin-induced diuresis and natriuresis was preserved in mice lacking the
289 GLP-1R, indicating a GLP-1R independent mode of action. These data provide the first evidence
290 that DPP-4 and its inhibition may regulate an endogenous substrate other than GLP-1 to affect
291 renal reabsorption of fluid and Na^+. Alternatively, GLP-1 accumulating in response to DPP-4
292 inhibition exerts natriuretic activity through a receptor other than the known GLP-1R.

293 High doses of the DPP-4 inhibitor, P32/98 (50 mg/kg), increased the expression of NHE3
294 phosphorylated at S552 and S605 in proximal tubular microvilli of the rat, although this response
295 was smaller than the response to EX4 (6). In the present study, alogliptin lowered plasma DPP-4
296 activity by 95% and induced a diuresis and natriuresis but did not affect NHE3 phosphorylation.
297 The relative extent of the diuresis and natriuresis (and GFR effects) observed for alogliptin vs.
298 EX4 in the present study was similar to the differences observed between P32/98 and EX4 in the

299 previous rat study (6). Moreover, increasing the dose of alogliptin from 10 to 30 mg/kg in awake
300 mice did not increase the natriuresis. Whereas higher doses of a DPP-4 inhibitor may affect
301 additional peptides and pathways and/or induces stronger effects, the present findings indicate
302 that the diuretic and natriuretic effect of the selective DPP-4 inhibitor alogliptin can occur
303 independent of changes in NHE3 phosphorylation at serines 552 and 605 in mice.

304 Proximal tubular hyperreabsorption via the physiology of tubuloglomerular feedback has
305 been proposed to enhance glomerular filtration rate in the early diabetic kidney, a risk factor for
306 the progression to diabetic nephropathy (38). Therefore, antidiabetic drugs that lower proximal
307 tubular hyperreabsorption may have additional beneficial effects on the kidney beyond blood
308 glucose control. We observed that the acute inhibitory effect of EX4 on renal Na^+ reabsorption
309 was preserved in db/db mice. Moreover, the EX4-induced increase in GFR observed in non-
310 diabetic mice was blunted in db/db mice. This may in part be due to a modest reduction in blood
311 pressure in db/db mice by EX4. Anti-hypertensive effects of EX-4/GLP-1 have been previously
312 reported in db/db mice (22), a rat model of the metabolic syndrome (28), and in Dahl salt-
313 sensitive rats (42). In addition, EX4 may lower GFR by inhibiting proximal reabsorption via the
314 physiology of tubuloglomerular feedback. Along these lines, mice lacking the GLP-1R have an
315 increased basal GFR. We speculate that knockout of the GLP-1R removes its tonic inhibitiory
316 influence on proximal tubular reabsorption, which lowers the NaCl concentration at the macula
317 densa and increases GFR via tubuloglomerular feedback. Further studies are needed to follow up
318 on the hypothesis that changes in proximal tubular reabsorption explain the enhanced basal GFR
319 in the *Glp1r*-/- mice and the blunted GFR effect of EX4 under diabetic conditions.

320 In contrast to EX4, the inhibitory effect of alogliptin on renal fluid and Na^+ reabsorption
321 was abolished in db/db mice. This was observed in awake mice and in clearance studies under

anesthesia. The results indicate that the inhibition of renal Na^+ reabsorption induced by acute GLP-1R activation is preserved in diabetic db/db mice whereas the endogenous natriuretic pathway induced by DPP-4 inhibition is rendered insensitive and/or ineffective. The reasons for the latter remain unclear. Plasma DPP-4 activity is increased in patients with type 1 and type 2 diabetes (29). Urinary DPP-4 excretion is enhanced in patients with noninsulin-dependent diabetes mellitus (24). Renal DPP-4 expression and activity (41) as well as urinary excretion of DPP-4 (23) are increased in hyperglycemic rats. Thus, changes in DPP-4 expression and activity may not explain the present findings, pointing to potential changes in the availability of the DPP-4 substrate(s) or in the natriuretic signaling cascade induced by DPP-4 inhibition in db/db mice.

Whereas EX4 lowered blood glucose levels in nondiabetic controls and db/db mice (but not in *Glp1r*-/- mice), i.p. or i.v. application of alogliptin did not significantly alter blood glucose levels within the experimental time frame of 1-3 hours in any of these groups compared with vehicle application. We documented that i.p. application of alogliptin reduced plasma DPP-4 activity by about 95% when determined 1 hour later. It has been proposed that DPP-4 inhibition modulates glucose homeostasis through pathways distinct from those used by GLP-1R agonists in mice (11). Studies in mice proposed that the predominant mechanism through which DPP-4 inhibitors regulate blood glucose levels involves local inhibition of intestinal DPP-4 activity (40), a pathway that may not be sufficiently inhibited by the acute i.v. or i.p. application of alogliptin performed in the present studies. In addition, it may be that the degree of enteral glucose loading and secretion of gut peptides necessary to unmask acute effects of i.v. DPP-4 inhibitors on blood glucose control were not established in the unchallenged control and db/db mice in the present experiments.

Summary and perspectives. The current studies indicate that the acute natriuretic effect of intravenous and/or intraperitoneal application of EX4 but not that of alogliptin is mediated via the GLP-1R, and that only the acute natriuretic effect of EX4 is preserved in a mouse model of type 2 diabetes. Thus DPP-4 and its inhibition may regulate one or more substrates beyond GLP-1 to affect renal reabsorption of fluid and Na^+. Further studies are needed to better understand the natriuretic effect of GLP-1R agonists and DPP-4 inhibitors including the involved molecular mechanisms and their long-term effects on renal fluid and salt transport in the diabetic kidney. The physiological and pathophysiological relevance of an intestinal-renal GLP-1 system remains unclear. The present studies show that the absence of the GLP-1R increases GFR. Its absence appears not to affect the natriuresis and diuresis induced by salt loading via oral gavage, indicating a negligible contribution of the endogenous GLP-1/GLP-1R system in response to this maneuver.

Acknowledgement

This work was supported by National Institutes of Health (DK56248, HL094728, P30DK079337 to V.V.), American Heart Association (Grant in Aid 10GRNT3440038 to V.V.; Grant in Aid 11GRNT7610059 to T.T.; Scientist Development Grant 10SDG2610034 to T.R.), a Carl W. Gottschalk Research Grant of the American Society of Nephrology (to T.R.), the Heart and Stroke Foundation of Ontario, the Canada Research Chairs Program, and the Banting and Best Diabetes Centre-Novo Nordisk Chair in Incretin Biology (to DJD); the Department of Veterans Affairs, and Takeda Pharmaceuticals U.S.A., Inc. (to V.V.).

Disclosure

Dr. Vallon has received within the past 12 months research grant support for basic science studies from Takeda Pharmaceuticals U.S.A., Inc, Bristol-Myers Squibb, and Astra-Zeneca. Dr. Drucker has served as an advisor or consultant within the past 12 months to Amylin Pharmaceuticals, Arisaph Pharmaceuticals Inc., Diartis Pharmaceuticals, Eli Lilly Inc, Glaxo Smith Kline, Merck research Laboratories, Novo Nordisk Inc., NPS Pharmaceuticals Inc., Pfized, Takeda, and Transition Pharmaceuticals Inc.. Mt. Sinai hospital receives operating grant support for basic science studies in Dr Drucker's lab from GSK, Merck, and Novo Nordisk Inc..

379
380 Reference List

381

382 1. **Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ and Husain M**.
383 Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated
384 through both glucagon-like peptide 1 receptor-dependent and -independent pathways.
385 *Circulation* 117: 2340-2350, 2008.

386 2. **Bek MJ, Zheng S, Xu J, Yamaguchi I, Asico LD, Sun XG and Jose PA**. Differential
387 expression of adenylyl cyclases in the rat nephron. *Kidney Int* 60: 890-899, 2001.

388 3. **Bunck MC, Corner A, Eliasson B, Heine RJ, Shaginian RM, Wu Y, Yan P, Smith U,**
389 **Yki-Jarvinen H, Diamant M and Taskinen MR**. One-year treatment with exenatide vs.
390 insulin glargine: effects on postprandial glycemia, lipid profiles, and oxidative stress.
391 *Atherosclerosis* 212: 223-229, 2010.

392 4. **Chien CL, Wu YS, Lai HL, Chen YH, Jiang ST, Shih CM, Lin SS, Chang C and**
393 **Chern Y**. Impaired water reabsorption in mice deficient in the type VI adenylyl cyclase
394 (AC6). *FEBS Lett* 584: 2883-2890, 2010.

395 5. **Covington P, Christopher R, Davenport M, Fleck P, Mekki QA, Wann ER and Karim**
396 **A**. Pharmacokinetic, pharmacodynamic, and tolerability profiles of the dipeptidyl
397 peptidase-4 inhibitor alogliptin: a randomized, double-blind, placebo-controlled, multiple-
398 dose study in adult patients with type 2 diabetes. *Clin Ther* 30: 499-512, 2008.

6. **Crajoinas RO, Oricchio FT, Pessoa TD, Pacheco BP, Lessa LM, Malnic G and Girardi AC**. Mechanisms mediating the diuretic and natriuretic actions of the incretin hormone glucagon-like peptide-1. *Am J Physiol Renal Physiol* 301: F355-F363, 2011.

7. **DeFronzo RA, Fleck PR, Wilson CA and Mekki Q**. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor alogliptin in patients with type 2 diabetes and inadequate glycemic control: a randomized, double-blind, placebo-controlled study. *Diabetes Care* 31: 2315-2317, 2008.

8. **DeFronzo RA, Okerson T, Viswanathan P, Guan X, Holcombe JH and MacConell L**. Effects of exenatide versus sitagliptin on postprandial glucose, insulin and glucagon secretion, gastric emptying, and caloric intake: a randomized, cross-over study. *Curr Med Res Opin* 24: 2943-2952, 2008.

9. **Drucker DJ**. The biology of incretin hormones. *Cell Metab* 3: 153-165, 2006.

10. **Drucker DJ and Nauck MA**. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 368: 1696-1705, 2006.

11. **Duez H, Smith AC, Xiao C, Giacca A, Szeto L, Drucker DJ and Lewis GF**. Acute dipeptidyl peptidase-4 inhibition rapidly enhances insulin-mediated suppression of endogenous glucose production in mice. *Endocrinology* 150: 56-62, 2009.

12. **Eng J, Kleinman WA, Singh L, Singh G and Raufman JP**. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem* 267: 7402-7405, 1992.

13. **Feng J, Zhang Z, Wallace MB, Stafford JA, Kaldor SW, Kassel DB, Navre M, Shi L, Skene RJ, Asakawa T, Takeuchi K, Xu R, Webb DR and Gwaltney SL**. Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV. *J Med Chem* 50: 2297-2300, 2007.

14. **Gallwitz B, Witt M, Folsch UR, Creutzfeldt W and Schmidt WE**. Binding specificity and signal transduction of receptors for glucagon-like peptide-1(7-36)amide and gastric inhibitory polypeptide on RINm5F insulinoma cells. *J Mol Endocrinol* 10: 259-268, 1993.

15. **Girardi AC, Degray BC, Nagy T, Biemesderfer D and Aronson PS**. Association of Na(+)-H(+) exchanger isoform NHE3 and dipeptidyl peptidase IV in the renal proximal tubule. *J Biol Chem* 276: 46671-46677, 2001.

16. **Girardi AC, Fukuda LE, Rossoni LV, Malnic G and Reboucas NA**. Dipeptidyl peptidase IV inhibition downregulates Na+ - H+ exchanger NHE3 in rat renal proximal tubule. *Am J Physiol Renal Physiol* 294: F414-F422, 2008.

17. **Girardi AC, Knauf F, Demuth HU and Aronson PS**. Role of dipeptidyl peptidase IV in regulating activity of Na+/H+ exchanger isoform NHE3 in proximal tubule cells. *Am J Physiol Cell Physiol* 287: C1238-C1245, 2004.

18. **Goke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J and Goke B**. Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. *J Biol Chem* 268: 19650-19655, 1993.

19. **Green BD, Hand KV, Dougan JE, McDonnell BM, Cassidy RS and Grieve DJ**. GLP-1 and related peptides cause concentration-dependent relaxation of rat aorta through a pathway involving KATP and cAMP. *Arch Biochem Biophys* 478: 136-142, 2008.

20. **Gutzwiller JP, Hruz P, Huber AR, Hamel C, Zehnder C, Drewe J, Gutmann H, Stanga Z, Vogel D and Beglinger C**. Glucagon-like peptide-1 is involved in sodium and water homeostasis in humans. *Digestion* 73: 142-150, 2006.

21. **Gutzwiller JP, Tschopp S, Bock A, Zehnder CE, Huber AR, Kreyenbuehl M, Gutmann H, Drewe J, Henzen C, Goeke B and Beglinger C**. Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. *J Clin Endocrinol Metab* 89: 3055-3061, 2004.

22. **Hirata K, Kume S, Araki SI, Sakaguchi M, Chin-Kanasaki M, Isshiki K, Sugimoto T, Nishiyama A, Koya D, Haneda M, Kashiwagi A and Uzu T**. Exendin-4 has an anti-

453 hypertensive effect in salt-sensitive mice model. *Biochem Biophys Res Commun* 27: 44-49,
454 2009.

455 23. **Ishii N, Ikenaga H, Ogawa Z, Aoki Y, Saruta T and Suga T**. Effects of renal sorbitol
456 accumulation on urinary excretion of enzymes in hyperglycaemic rats. *Ann Clin Biochem*
457 38: 391-398, 2001.

458 24. **Ishii N, Ogawa Z, Itoh H, Ikenaga H and Saruta T**. Diagnostic significance of urinary
459 enzymes for diabetes mellitus and hypertension. *Enzyme Protein* 48: 174-182, 1994.

460 25. **Kenny AJ, Booth AG, George SG, Ingram J, Kershaw D, Wood EJ and Young AR**.
461 Dipeptidyl peptidase IV, a kidney brush-border serine peptidase. *Biochem J* 157: 169-182,
462 1976.

463 26. **Kocinsky HS, Dynia DW, Wang T and Aronson PS**. NHE3 phosphorylation at serines
464 552 and 605 does not directly affect NHE3 activity. *Am J Physiol Renal Physiol* 293: F212-
465 F218, 2007.

466 27. **Kocinsky HS, Girardi AC, Biemesderfer D, Nguyen T, Mentone S, Orlowski J and**
467 **Aronson PS**. Use of phospho-specific antibodies to determine the phosphorylation of
468 endogenous Na+/H+ exchanger NHE3 at PKA consensus sites. *Am J Physiol Renal Physiol*
469 289: F249-F258, 2005.

28. **Laugero KD, Stonehouse AH, Guss S, Landry J, Vu C and Parkes DG**. Exenatide improves hypertension in a rat model of the metabolic syndrome. *Metab Syndr Relat Disord* 7: 327-334, 2009.

29. **Mannucci E, Pala L, Ciani S, Bardini G, Pezzatini A, Sposato I, Cremasco F, Ognibene A and Rotella CM**. Hyperglycaemia increases dipeptidyl peptidase IV activity in diabetes mellitus. *Diabetologia* 48: 1168-1172, 2005.

30. **Moreno C, Mistry M and Roman RJ**. Renal effects of glucagon-like peptide in rats. *Eur J Pharmacol* 434: 163-167, 2002.

31. **Rieg T, Gerasimova M, Boyer JL, Insel PA and Vallon V**. $P2Y_2$ receptor activation decreases blood pressure and increases renal Na excretion. *Am J Physiol Regul Integr Comp Physiol* 301: R510-R518, 2011.

32. **Rieg T, Steigele H, Schnermann J, Richter K, Osswald H and Vallon V**. Requirement of intact adenosine A1 receptors for the diuretic and natriuretic action of the methylxanthines theophylline and caffeine. *J Pharmacol Exp Ther* 313: 403-409, 2005.

33. **Rieg T, Tang T, Murray F, Schroth J, Insel PA, Fenton RA, Hammond HK and Vallon V**. Adenylate cyclase 6 determines cAMP formation and aquaporin-2 phosphorylation and trafficking in inner medulla. *J Am Soc Nephrol* 21: 2059-2068, 2010.

487 34. **Schlatter P, Beglinger C, Drewe J and Gutmann H**. Glucagon-like peptide 1 receptor
488 expression in primary porcine proximal tubular cells. *Regul Pept* 141: 120-128, 2007.

489 35. **Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL and**
490 **Drucker DJ**. Glucose intolerance but normal satiety in mice with a null mutation in the
491 glucagon-like peptide 1 receptor gene. *Nat Med* 2: 1254-1258, 1996.

492 36. **Shen T, Suzuki Y, Poyard M, Miyamoto N, Defer N and Hanoune J**. Expression of
493 adenylyl cyclase mRNAs in the adult, in developing, and in the Brattleboro rat kidney. *Am*
494 *J Physiol* 273: C323-C330, 1997.

495 37. **Tang T, Gao MH, Lai NC, Firth AL, Takahashi T, Guo T, Yuan JX, Roth DM and**
496 **Hammond HK**. Adenylyl cyclase type 6 deletion decreases left ventricular function via
497 impaired calcium handling. *Circulation* 117: 61-69, 2008.

498 38. **Vallon V**. The proximal tubule in the pathophysiology of the diabetic kidney. *Am J Physiol*
499 *Regul Integr Comp Physiol* 300: R1009-R1022, 2011.

500 39. **Vallon V, Schroth J, Lang F, Kuhl D and Uchida S**. Expression and phosphorylation of
501 the Na+-Cl- cotransporter NCC in vivo is regulated by dietary salt, potassium, and SGK1.
502 *Am J Physiol Renal Physiol* 297: F704-F712, 2009.

503 40. **Waget A, Cabou C, Masseboeuf M, Cattan P, Armanet M, Karaca M, Castel J,**
504 **Garret C, Payros G, Maida A, Sulpice T, Holst JJ, Drucker DJ, Magnan C and**

Burcelin R. Physiological and pharmacological mechanisms through which the DPP-4 inhibitor sitagliptin regulates glycemia in mice. *Endocrinology* 152: 3018-3029, 2011.

41. **Yang J, Campitelli J, Hu G, Lin Y, Luo J and Xue C**. Increase in DPP-IV in the intestine, liver and kidney of the rat treated with high fat diet and streptozotocin. *Life Sci* 81: 272-279, 2007.

42. **Yu M, Moreno C, Hoagland KM, Dahly A, Ditter K, Mistry M and Roman RJ**. Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 21: 1125-1135, 2003.

43. **Zhao N and Tenenhouse HS**. Npt2 gene disruption confers resistance to the inhibitory action of parathyroid hormone on renal sodium-phosphate cotransport. *Endocrinology* 141: 2159-2165, 2000.

518 Table 1. Basal parameters in WT and *Glp1r*-/- mice during clearance experiments.

	WT (n=12)	*Glp1r*-/- (n=11)
Body weight (g)	29±1	30±1
Mean arterial blood pressure (mm Hg)	70±3	76±2
Heart rate (min^{-1})	445±6	449±13
Hematocrit (%)	43±1	43±1
Plasma Na^+ (mM)	148±3	150±3
Plasma K^+ (mM)	3.8±0.2	3.8±0.2
GFR (µl/min)	373±29	459±23*
Urinary H_2O excretion (nl min^{-1} g^{-1} bw)	45±5	47±5
Urinary Na^+ excretion (nmol min^{-1} g^{-1} bw)	7±1	10±2
Urinary K^+ excretion (nmol min^{-1} g^{-1} bw)	15±2	18±1
Fractional H_2O excretion (%)	0.38±0.06	0.30±0.03
Fractional Na^+ excretion (%)	0.39±0.04	0.41±0.05
Fractional K^+ excretion (%)	33±3	31±2
Urinary cAMP excretion (pmol min^{-1} g^{-1} bw)	0.67±0.16	1.18±0.15*

519

520 Values are mean ± SEM. * P<0.05 vs. WT.

521

522

Table 2. Basal parameters in db/db and control mice during clearance experiments.

	control (n=22)	db/db (n=25)
Body weight (g)	26±1	46±1*
Blood glucose (mg/dl)	156±9	441±20*
Hematocrit (%)	44±1	45±1
Mean arterial blood pressure (mm Hg)	89±2	88±3
Heart rate (min^{-1})	427±12	441±16
Plasma Na^+ (mM)	158±1	156±2
Plasma K^+ (mM)	4.2±0.1	3.7±0.1*
GFR (µl/min)	428±22	450±20
GFR (µl min^{-1} g^{-1} bw)	16.5±0.8	9.7±0.4*
Urinary H_2O excretion (nl min^{-1})	1.8±0.2	2.6±0.3
Urinary H_2O excretion (nl min^{-1} g^{-1} bw)	68±6	57±5
Urinary Na^+ excretion (nmol min^{-1})	558±56	488±77
Urinary Na^+ excretion (nmol min^{-1} g^{-1} bw)	21±2	11±2*
Urinary K^+ excretion (nmol min^{-1})	523±33	668±43
Urinary K^+ excretion (nmol min^{-1} g^{-1} bw)	20±1	14±1*
Fractional H_2O excretion (%)	0.43±0.04	0.58±0.05
Fractional Na^+ excretion (%)	0.83±0.08	0.69±0.10
Fractional K^+ excretion (%)	31±2	43±3*
Urinary K^+/Na^+	1.1±0.1	1.9±0.2*

Values are mean ± SEM. * $P<0.05$ vs. control.

Figure legends

Figure 1: *The natriuretic response to exendin-4, but not to alogliptin, is blunted in awake mice lacking the GLP-1 receptor. Glp1r*-/- and WT mice were randomized to application of exendin-4 (EX4; 10 µg/kg bw i.p.), alogliptin (ALG; 10 mg/kg bw i.p.) or vehicle (2 µl/g bw of 0.85% NaCl), followed by oral gavage with isotonic saline (30 µl/g; ~30% of daily NaCl intake) and determination of urinary excretion in metabolic cages over 3 hours. **A)** EX4 increased urinary flow rate and Na^+ excretion in WT but not in *Glp1r*-/- mice. **B)** The natriuretic effect of ALG was similar in both genotypes. **P*<0.05 vs. vehicle. n=5-6 per group.

Figure 2: *Effects of exendin-4 and alogliptin on phosphorylated NHE3 in renal membranes and plasma DPP-4 activity.* Kidneys and plasma were harvested 1 hour after application of exendin-4 (EX4; 10 µg/kg bw i.p.), alogliptin (ALG, 10 mg/kg i.p.), or vehicle (2 µl/g bw of 0.85% NaCl). **A-C)** The natriuretic response to EX4, but not to ALG, was associated with increased NHE3 phosphorylated at S552 and S605 in renal membranes. This effect of EX4 was absent in *Glp1r*-/- mice. **P*<0.05 vs. vehicle. n=5 per group. **D)** ALG inhibited DPP-4 activity in both WT and *Glp1r*-/- mice. Data are expressed relative to the mean of the vehicle-treated WT group, which was set as 100%. EX4 was without effect. **P*<0.05 vs. vehicle. n=5 per group.

Figure 3: *Exendin-4-induced increases in GFR and fractional urinary Na^+ excretion in anesthetized mice depend on an intact GLP-1 receptor.* Two-period clearance experiments under thiobutabarbital/ketamine anesthesia were performed with a basal period followed by application of exendin-4 (EX4; 10 µg/kg bw i.v.)(A) or parathyroid hormone (PTH; 10 µg/kg bw i.v.)(B) 5

570 min prior to the second period. Table 1 summarized the results from the basal periods of the EX4
571 series. Depicted here are the changes in the second period versus the basal period. EX4 increased
572 GFR, urinary flow rate, and absolute and fractional urinary Na^+ excretion in WT versus *Glp1r*-/-
573 mice. EX4 did not change urinary cAMP excretion (A). In comparison, PTH increased urinary
574 cAMP excretion in this experimental setting (B). **P*<0.05 vs. *Glp1r*-/-. n=8-12 per group.
575
576 **Figure 4:** *The natriuresis and increase in NHE3 phosphorylation in response to exendin-4 is*
577 *preserved in awake mice lacking adenylyl cyclase 6 (AC6).* A) AC6-/- and WT mice were
578 randomized to application of exendin-4 (EX4; 10 µg/kg bw i.p.) or vehicle (2 µl/g bw of 0.85%
579 NaCl), followed by oral gavage with isotonic saline (30 µl/g; ~30% of daily NaCl intake) and
580 determination of urinary excretion in metabolic cages over 3 hours. The natriuretic effect of EX4
581 was similar in both genotypes. EX4 did not change urinary cAMP excretion. **P*<0.05 vs.
582 vehicle. n=5-6 per group. B) Kidneys were harvested in AC6-/- mice 1 hour after application of
583 exendin-4 (EX4; 10 µg/kg bw i.p.) or vehicle (2 µl/g bw of 0.85% NaCl). Phosphorylation of
584 renal NHE3 at S552 and S605 was preserved in AC6-/- mice. **P*<0.05 vs. vehicle. n=6 per
585 group.
586
587 **Figure 5:** *Effects of exendin-4 and alogliptin on blood glucose, mean blood pressure and renal*
588 *function in diabetic db/db and non-diabetic db/- control mice*. Two-period clearance experiments
589 under thiobutabarbital/ketamine anesthesia were performed with a basal period followed by
590 application of exendin-4 (EX4; 10 µg/kg bw i.v.), alogliptin (ALG; 10 mg/kg bw i.v.), or vehicle
591 (2 µl/g bw of 0.85% NaCl) 5 min prior to the second period. Table 2 summarized the results
592 from the basal periods. This figure depicts the changes in the second period, i.e. in response to

593 EX4, ALG and vehicle. In non-diabetic control mice, both EX4 and ALG increased fractional
594 H_2O and Na^+ excretion versus vehicle without altering renal K^+ excretion; in addition, EX4
595 lowered blood glucose and increased GFR. In diabetic db/db mice, only EX4 lowered blood
596 glucose and increased fractional H_2O and Na^+ excretion; EX4 modestly lowered mean blood
597 pressure and did not increase GFR. * *P<0.05* vs. vehicle. n=6-10 per group.
598
599 **Figure 6:** *Alogliptin differentially affects urinary Na^+ excretion in awake control mice (A) vs.*
600 *db/db mice (B).* Mice were randomized to application of alogliptin (ALG; 10 mg/kg bw i.p.) or
601 vehicle (2 µl/g bw of 0.85% NaCl), followed by oral gavage with isotonic saline (30 µl/g bw)
602 and determination of urinary excretion in metabolic cages over 3 hours, followed by blood
603 glucose measurements. *P<0.05 vs. vehicle. n=10-15 per group.

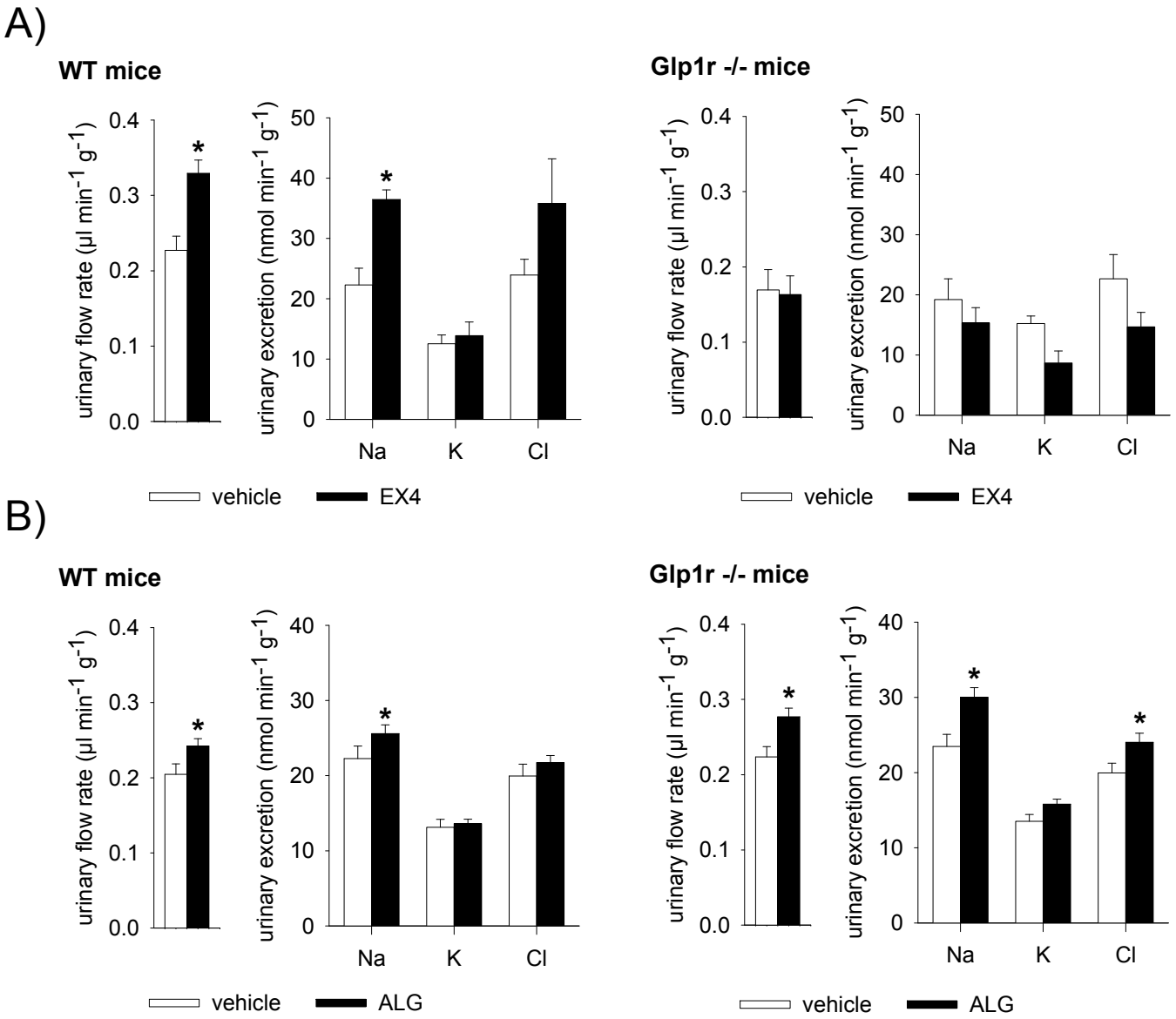

Figure 1

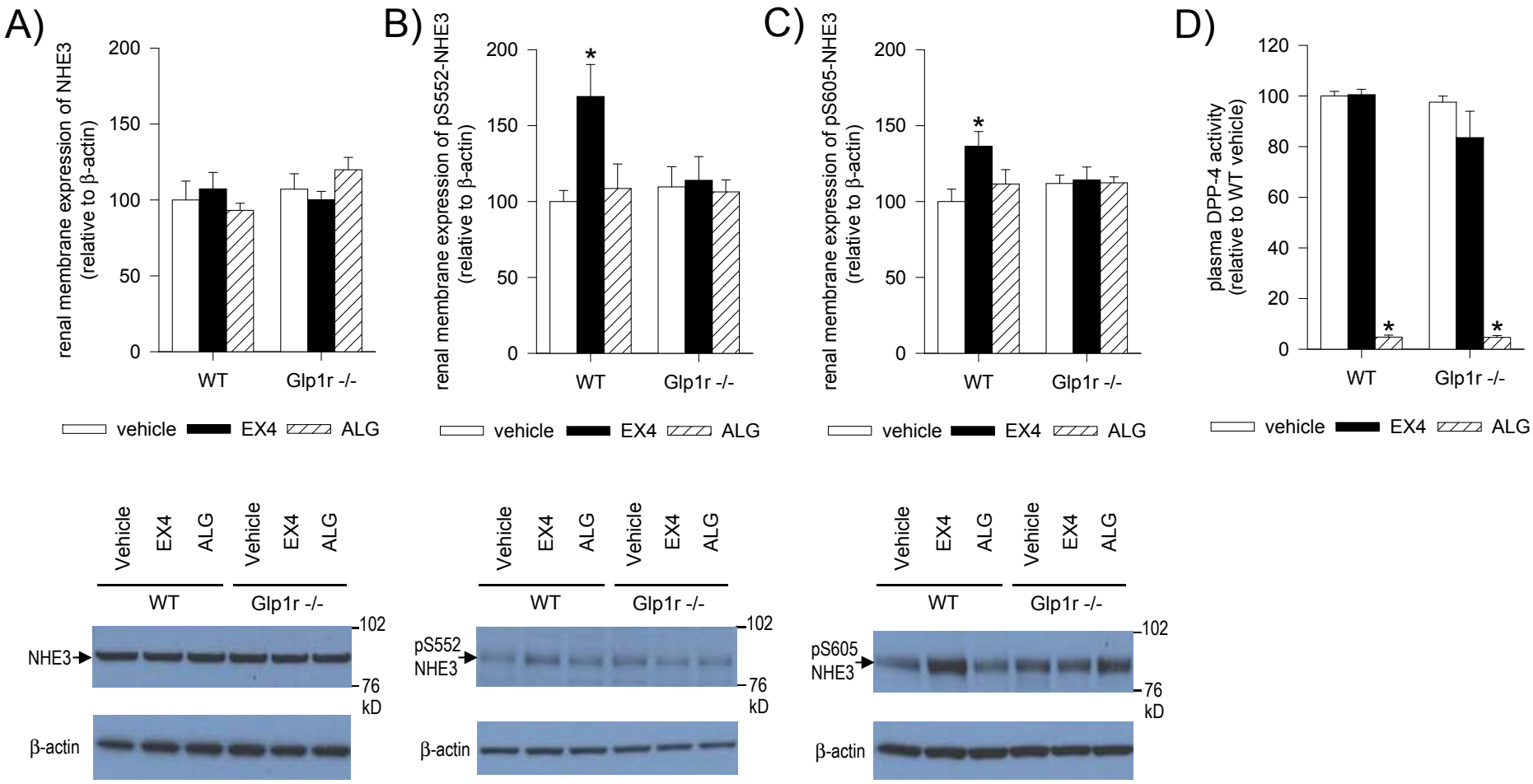

Figure 2

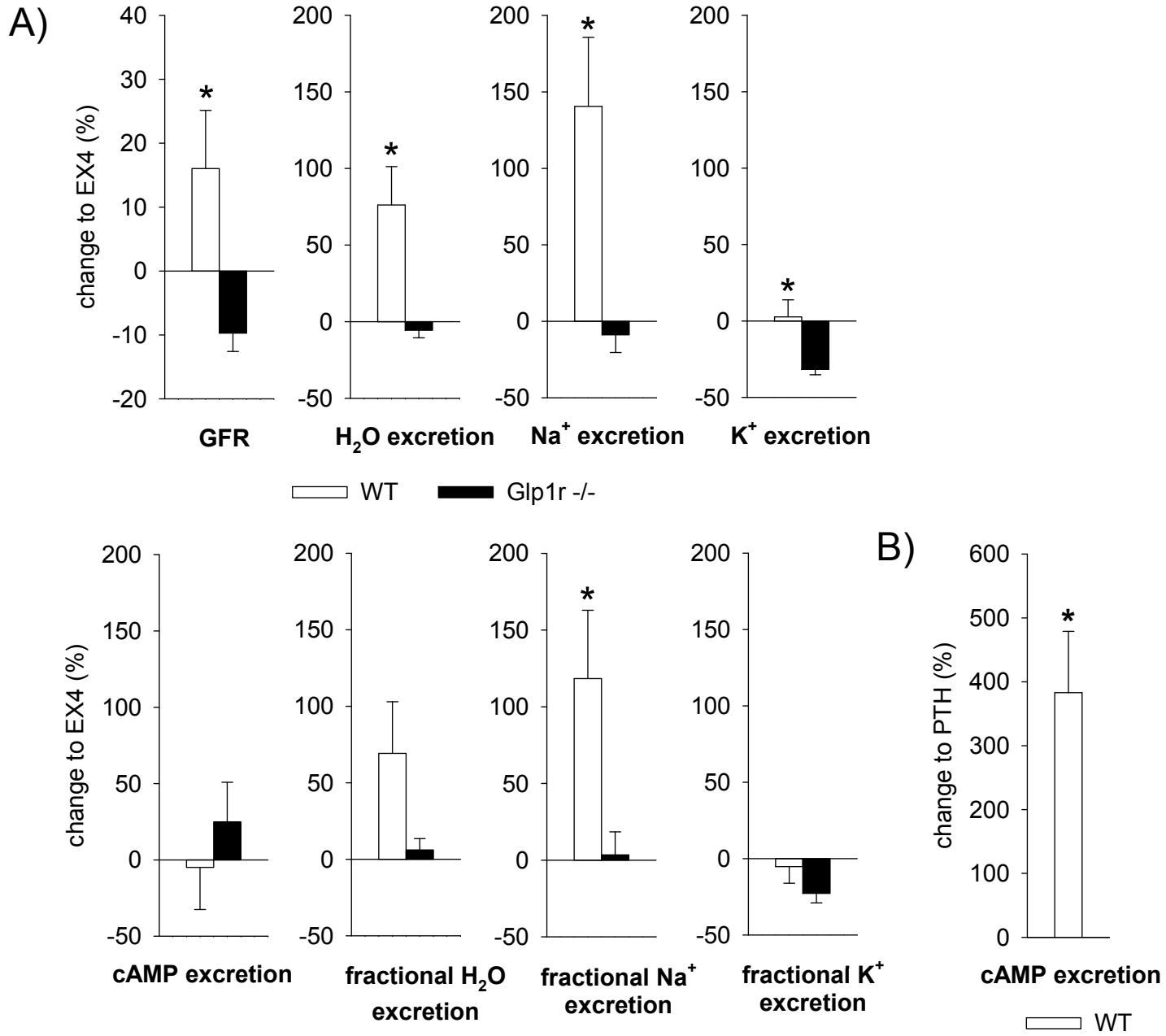

Figure 3

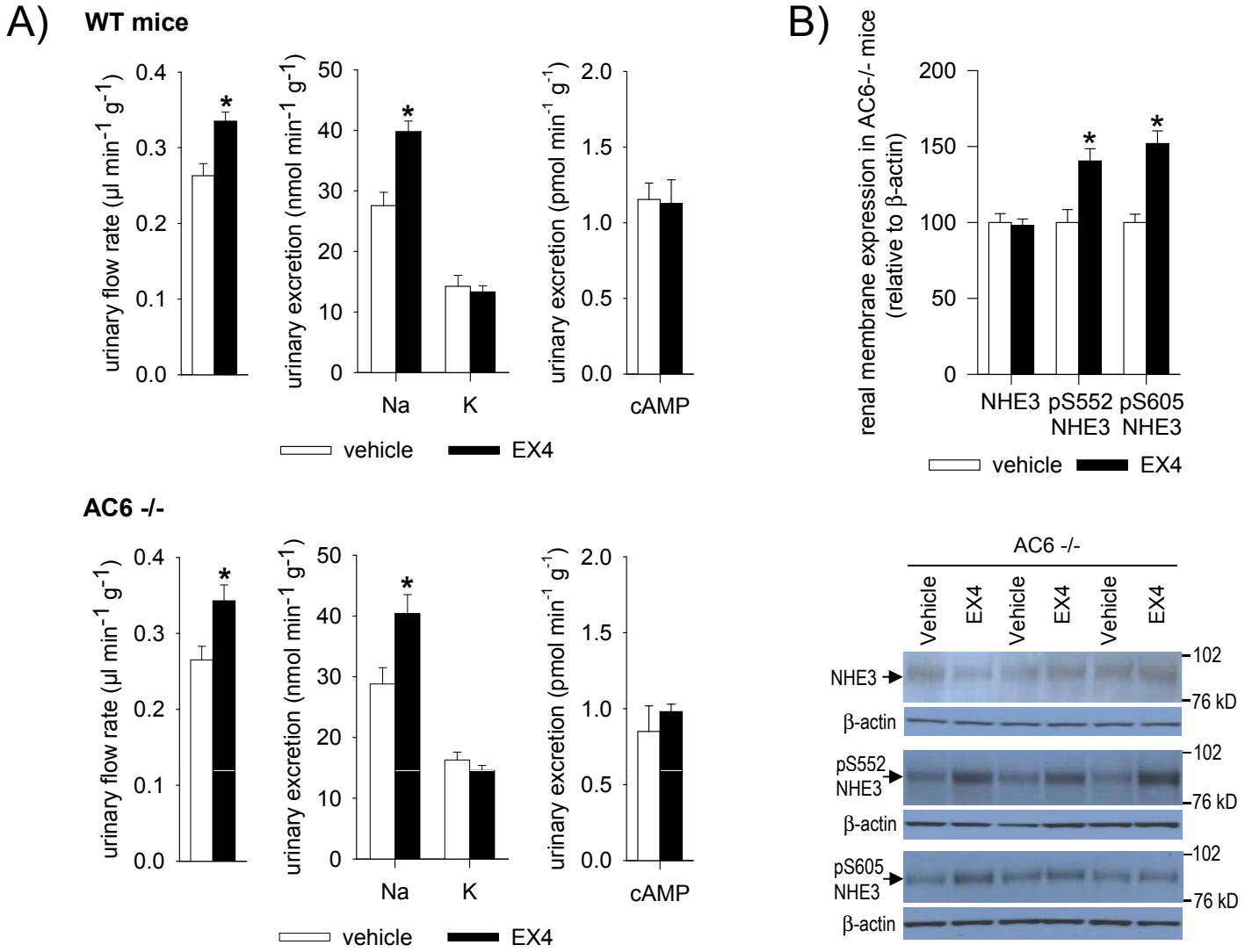

Figure 4

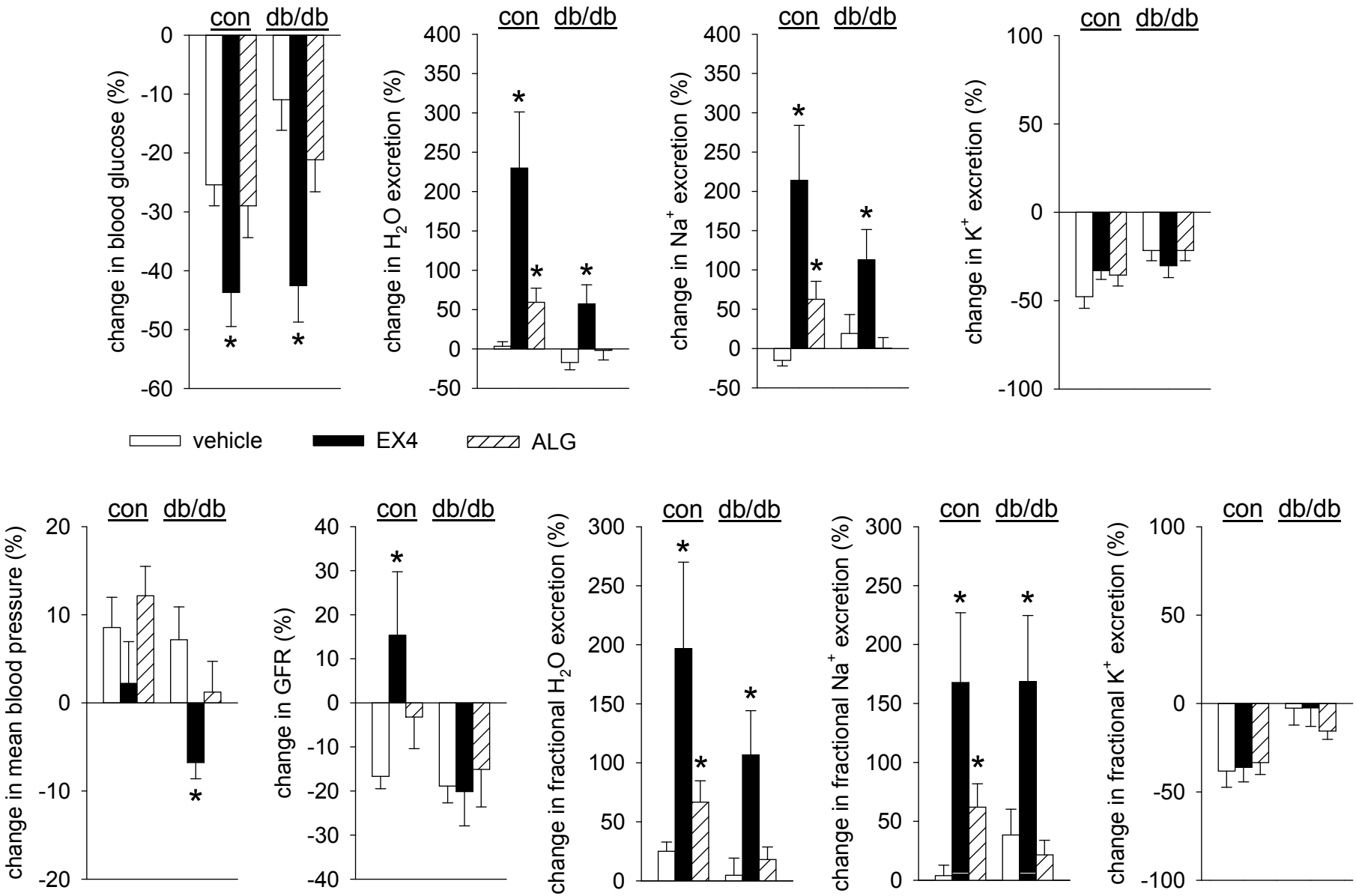

Figure 5

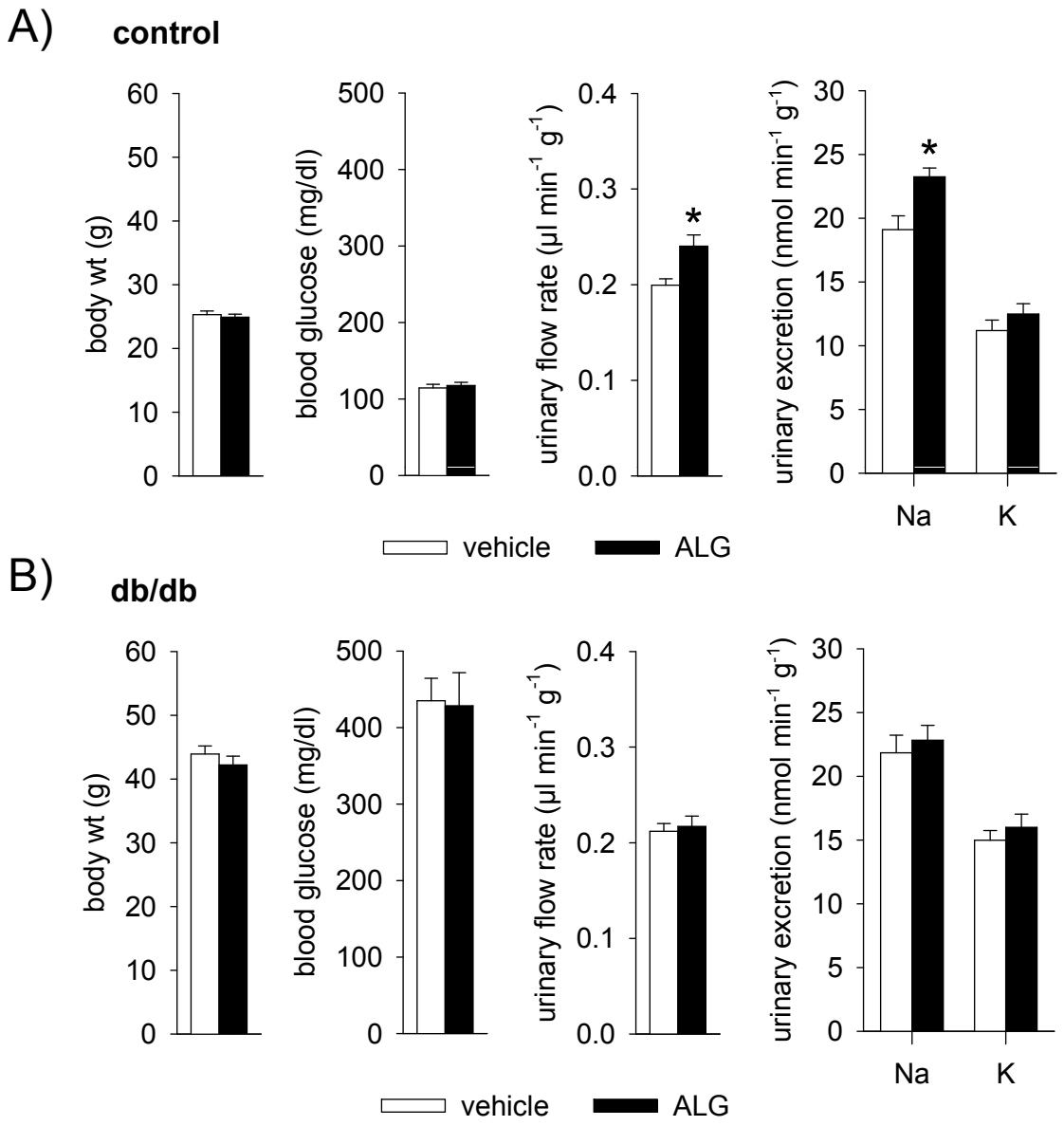

Figure 6