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2018-07-09

10.1038/s41440-018-0070-0

Peer reviewed
Simultaneous GLP-1 receptor activation and angiotensin receptor blockade increase natriuresis independent of altered arterial pressure in obese OLETF rats

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Received: 20 June 2017 / Revised: 11 January 2018 / Accepted: 14 January 2018 © The Japanese Society of Hypertension 2018

Abstract
Obesity is associated with an inappropriately activated renin–angiotensin–aldosterone system, suppressed glucagon-like peptide-1 (GLP-1), increased renal Na⁺ reabsorption, and hypertension. To assess the link between GLP-1 and angiotensin receptor type 1 (AT₁) signaling on obesity-associated impairment of urinary Na⁺ excretion (UNaV) and elevated arterial pressure, we measured mean arterial pressure (MAP) and heart rate by radiotelemetry and metabolic parameters for 40 days. We tested the hypothesis that stimulation of GLP-1 signaling provides added benefit to blockade of AT₁ by increasing UNaV and further reducing arterial pressure in the following groups: (1) untreated Long–Evans Tokushima Otsuka (LETO) rats (n = 7); (2) untreated Otsuka Long–Evans Tokushima Fatty (OLETF) rats (n = 9); (3) OLETF + ARB (ARB; 10 mg olmesartan/kg/day; n = 9); (4) OLETF + GLP-1 receptor agonist (EXE; 10 µg exenatide/kg/day; n = 7); and (5) OLETF + ARB + EXE (Combo; n = 6). On day 2, UNaV was 60% and 62% reduced in the EXE and Combo groups, respectively, compared with that in the OLETF rats. On day 40, UNaV was increased 69% in the Combo group compared with that in the OLETF group. On day 40, urinary angiotensinogen was 4.5-fold greater in the OLETF than in the LETO group and was 56%, 62%, and 58% lower in the ARB, EXE, and Combo groups, respectively, than in the OLETF group. From day 2 to the end of the study, MAP was lower in the ARB and Combo groups than in the OLETF rats. These results suggest that GLP-1 receptor activation may reduce intrarenal angiotensin II activity, and that simultaneous blockade of AT₁ increases UNaV in obesity; however, these beneficial effects do not translate to a further reduction in MAP.

Introduction
Obesity is a major health concern, with 35% of males and 40% of females in the United States currently classified as obese [1]. Obesity is associated with many adverse health outcomes such as metabolic syndrome, insulin resistance, attenuated glucagon-like peptide-1 (GLP-1) secretion, and hypertension [2–5]. Although many factors are associated with the development of hypertension in obesity, increased renal Na⁺ reabsorption is among the primary factors [6, 7]. The mechanisms by which obesity increases renal Na⁺ reabsorption are multifaceted and involve the physical compression of the kidneys by fat, increased sympathetic nervous system activity, and inappropriate activation of the renin–angiotensin–aldosterone system (RAAS) [8, 9]. Because of the many factors associated with the development of hypertension in obesity, treatment-resistant hypertension is more prevalent in obese individuals [10, 11]. Some treatment options make matters worse, because they can lead to the development or worsening of hyperglycemia [12, 13]. Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are among the treatment options that do not lead to the development or

Electronic supplementary material
The online version of this article (https://doi.org/10.1038/s41440-018-0070-0) contains supplementary material, which is available to authorized users.

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Published online: 09 July 2018
worsening of hyperglycemia. In contrast, ACEi and ARB decreased new-onset type 2 diabetes mellitus (T2DM) in individuals with cardiovascular risk factors or cardiovascular disease [14, 15]. Furthermore, in individuals without cardiovascular disease but with impaired fasting glucose or impaired glucose tolerance, treatment with an ACEi (ramipril) increased the regression to normoglycemia [16], suggesting that disrupting RAAS may prove beneficial for managing hypertension in those with metabolic derangements.

GLP-1 is an incretin hormone that is released into circulation during food intake and binds the GLP-1 receptor (GLP-1r) in the pancreas, where it stimulates insulin secretion in a glucose-dependent manner [17]. In addition to the pancreas, the GLP-1r is expressed in the lung, hypothalamus, hippocampus, cerebral cortex, gastrointestinal tract, heart, vasculature, and kidneys [18, 19], suggesting that GLP-1 may exert a variety of biological actions. To this end, GLP-1r agonists and dipeptidyl peptidase-4 inhibitors have been shown to increase urinary sodium excretion (UNaV) and decrease blood pressure [20, 21], implicating GLP-1 signaling in the regulation of volume-dependent hypertension. The majority of studies that report a natriuretic and/or blood pressure-lowering effect examined the acute effects of these agents [22–27]. Furthermore, the few studies that assessed the chronic effects of GLP-1r agonists showed mixed results [28, 29]. Therefore, the objectives of this study were to evaluate (1) the potential added benefits of targeting two signaling pathways (GLP-1 and AT1) that contribute to renal Na+ handling and (2) if these benefits in renal Na+ handling translate into amelioration of obesity-associated hypertension. To accomplish these objectives, we treated Otsuka Long–Evans Tokushima Fatty (OLETF) rats, a model of diet-induced obesity [30], with a GLP-1r agonist (exenatide) and an ARB (olmesartan). Moreover, we tested the hypothesis that stimulation of GLP-1 signaling provides added benefit to blockade of AT1 by increasing renal Na+ excretion and further reducing arterial pressure in obese OLETF rats.

Methods

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California, Merced.

Animals

Eight-week-old male, lean Long–Evans Tokushima Otsuka (LETO) and obese OLETF rats were studied (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan). Rats were divided into the following groups: (1) untreated LETO (n = 7) + vehicle (0.5% methylcellulose by oral gavage once daily); (2) untreated OLETF (n = 9) + vehicle; (3) OLETF + ARB (ARB; 10 mg olmesartan/kg by oral gavage once daily; n = 9); (4) OLETF + GLP-1r agonist (EXE; 10 µg exenatide/kg by osmotic mini-pumps for 42 days; n = 7); and (5) OLETF + ARB + Exe (Combo; n = 6). OLETF rats were assigned to groups, so that mean body mass (BM) at the onset of the study was within 5% of each other. Once assigned, a subset of animals from each group (LETO n = 4, OLETF n = 6, ARB n = 4, EXE n = 5, Combo n = 5) were surgically implanted with radio-telemeters (PA-C40; DSI, St. Paul, MN), as previously described [31, 32]. Animals were allowed to recover from the surgery for 7 days. Following the recovery period, all animals were transferred to metabolic cages and allowed to acclimate to the cages for 7 days. Treatments were started following the acclimation period. In the OLETF groups, mean arterial pressure (MAP) and heart rate were measured continuously for 30 s at 5 min intervals and are presented as the means for the 12 h light and 12 h dark cycles. In the LETO group, MAP and heart rate were measured continuously for 2 min once a day during the light cycle. Urine was collected at baseline and 2, 7, 13, 21, 28, and 40 days following the start of the treatments. At collection, urine volumes were recorded, samples were centrifuged (3000 × g × 15 min at 4 °C), and an aliquot transferred to a cryovial and immediately stored at −20 °C. All animals were housed in a specific pathogen-free facility under controlled temperature (23 °C) and humidity (55%) conditions on a 12 h light–dark cycle. All animals were given free access to water and standard laboratory chow (Teklad Diets, Madison, WI).

BM, water, and food intake

BM was measured daily to calculate the appropriate ARB dose. Water and food intake were measured for 24 h once a week.

Tissue collection

After the 6-week study period, animals were fasted for 12 h and tissues were collected the following morning. After measuring BM, animals were decapitated and trunk blood was collected into chilled vials containing 50 mM EDTA and a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Immediately following, the left kidney was removed, snap-frozen in liquid nitrogen, and stored at −80 °C until analyzed. Blood samples were centrifuged (3000 × g × 15 min at 4 °C) and plasma was transferred to cryovials and stored at −80 °C for later analyses.
Western blotting

A mid-transverse section of the kidney was used to extract proteins using RIPA buffer containing NP-40, EDTA, and protease and phosphatase inhibitor cocktail (Thermo, Waltham, MA). Tissue homogenate was centrifuged (20,000 × g × 15 min at 4 °C), and the aqueous layer was transferred to a separate tube and stored at 80 °C for later analyses. Total protein content was measured by Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein were separated on 6–15% Bis-Tris gels. Proteins were transferred to the Bio-Rad Trans-Blot SD semi-dry cell onto 0.45 µm polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA). Membranes were blocked with Odyssey blocking solution (LI-COR Biosciences, Lincoln, NE). After blocking, membranes were incubated overnight with the following primary antibodies against phosphorylated (p)-IGF-1 receptor-β (Tyr1135/1136)/(p)-insulin receptor (IR)-β (Tyr1150/1151), p-Akt (ser473), Akt (Cell Signaling, Danvers, MA), β-actin, IR-β (Santa Cruz Biotechnology, Santa Cruz, CA), γ-epithelial Na⁺ channel (γENaC) [33], renal outer medullary K⁺ channel (ROMK) (342HNFGKTVEVETPHCAMCYLEKDATRKMKG-YDNPENFLSE VDETDDTQMA391), and aquaporin 2 (AQP2) (254RQSVELHSPQSLPGRSKA271; Alomone Laboratories, Jerusalem, Israel) in Odyssey blocking solution + 0.2% Tween 20 at 4 °C. Membranes were washed, incubated for 1 h with specific secondary antibodies (IRDye: LI-COR Biosciences) in TBS-T + 5% non-fat milk + 0.01% SDS, re-washed, and scanned in an Odyssey infrared imager (LI-COR Biosciences). In addition to consistently loading the same amount of total protein per well, densitometry values were further normalized by correcting for the densitometry values of β-actin.

Biochemical analyses and homeostasis model assessment of insulin resistance

Fasting plasma glucose (FPG) and triglycerides (TGs) were measured using an Analox GM7 analyzer (Analox Instruments, London, UK). Fasting plasma non-esterified fatty acids (NEFA) (WAKO, Osaka, Japan), creatinine (Sigma-Aldrich), insulin (FPI), and leptin (EMD Millipore) were measured using commercially available kits. All samples were analyzed in duplicate and run in a single assay with intra-assay coefficients of variability of <10%. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR = (FPG × FPI)/2.430, with FPG in milligrams per deciliter and FPI in microunits per milliliter, as previously described [34].

Urine analyses

Plasma and urinary Na⁺ and K⁺ were measured using an EasyLyte analyzer (Medica, Bedford, MA). Urinary creatinine was measured using a commercially available colorimetric kit (Cayman Chemical, Ann Arbor, MI). Urinary aldosterone and corticosterone were extracted as previously described [35, 36] and measured using commercially available RIA kits (Siemens Healthcare Diagnostics, Los Angeles, CA, and MP Biomedicals, Solon, OH, respectively). Urinary angiotensinogen (Takara Bio USA, Mountain View, CA) was measured using a commercially available enzyme-linked immunosorbent assay kit. Urinary excretion values were calculated as the product of concentration and urine volume, and normalized by correcting for urinary creatinine excretion. Glomerular filtration rate (GFR) was estimated from samples taken on the last day of the study and calculated as follows: GFR = (Ucr × UV) ÷ Pcr, where Ucr is urinary creatinine, UV is urine volume, and Pcr is plasma creatinine. Fractional excretions (FEs) for Na⁺ and K⁺ were calculated as follows: FE = [(U × Pcr) ÷ (P × Ucr)] × 100, where x is the electrolyte.

Statistical analyses

Means (±SE) were calculated for all samples unless otherwise noted. One-way analysis of variance (ANOVA) followed by Tukey’s honest significant difference or Games–Howell post hoc test were used to analyze differences between groups for protein expression and for end-of-study plasma and urinary analyses. For data measured repeatedly, we used a two-factor repeated-measures ANOVA with time as a within-subjects factor and group as a between-subjects factor. When significant interactions were observed, pair-wise comparisons were carried out using a Bonferroni correction. Relationships between dependent and independent variables were evaluated by linear regression analysis, and correlations were evaluated using Pearson’s correlation coefficients. Means, regressions, and correlations were considered significant at p < 0.05 and analyses were performed with SPSS version 24 (IBM, Armonk, NY).

Results

Effects of GLP-1r activation alone and in combination with AT₁ blockade on MAP and heart rate

MAP and heart rate were measured daily by telemetry to accurately assess the effects of chronic GLP-1r agonism and AT₁ blockade on cardiovascular function.
**Light cycle**

Baseline MAP was greater in OLETF rats than in LETO rats, remaining so for the remainder of the study (Fig. 1a). From day 2 to the end of the study, MAP was decreased in the ARB and Combo groups compared with that in the OLETF group (Fig. 1a). Exenatide treatment transiently decreased MAP, reaching its nadir on day 11, after which time, MAP returned to and hovered around its baseline levels (Fig. 1a). Baseline heart rate was greater in LETO rats, remaining so for the duration of the study (Fig. 1b). From day 4 to day 7, heart rate increased in the Combo group compared with that in the OLETF group; after that, heart rate hovered around baseline levels (Fig. 1b). On day 25, heart rate decreased below baseline levels in the EXE group and remained lower for the remainder of the study (Fig. 1b).

**Dark cycle**

From day 1 to the end of the study, MAP was decreased in the ARB and Combo groups compared with that in the OLETF group (Fig. 1c). Exenatide treatment initially increased MAP, peaking on day 1, then gradually decreasing to OLETF levels, where it remained for the rest of the study (Fig. 1c). From day 2, heart rate was increased in the EXE and Combo groups, peaking on day 3 in the EXE group and day 5 in the Combo group, then gradually decreasing to the levels observed in the OLETF rats by day 18 in the EXE group and day 30 in the Combo group (Fig. 1d). Collectively, these results suggest that the beneficial effects of GLP-1r activation on MAP may be more prominent during the light cycle and that the addition of an AT1 inhibitor does not lead to added benefits.

**GLP-1r activation in combination with AT1 blockade decreases BM but does not improve the biochemical parameters of metabolic syndrome**

BM, food intake, FPG, FPI, leptin, TG, and NEFA were measured, and HOMA-IR was calculated to assess whether GLP-1r activation alone or in combination with AT1 blockade would protect against the development of metabolic syndrome and insulin resistance. From baseline, BM was greater in OLETF rats than in LETO rats (Fig. 2a). On day 2, BM was lower in the EXE and Combo groups than in the OLETF group, remaining lower in the Combo group for the remainder of the study (Fig. 2a). From baseline, food
Simultaneous GLP-1 receptor activation and angiotensin receptor blockade increase natriuresis.

Exenatide in conjunction with olmesartan treatment decreased food intake (Fig. 2). On day 2, food intake was 46% and 71% lower in the EXE and Combo, respectively, compared with that in the OLETF group (Fig. 2b). From day 7, food intake was 41% and 70% lower in the EXE and Combo groups, respectively, compared with that in the OLETF group (Fig. 2b). On day 21, relative food intake was 14% greater in the Combo group than in the OLETF group; notwithstanding, there was no difference in relative food intake between the OLETF group and the treatment groups for the remainder of the study (Fig. 2c). Mean FPG and FPI were 32% and 128% higher, respectively, in the OLETF group compared with those in the LETO group, and FPG was 9% lower in the ARB group compared with that in the OLETF group. Neither exenatide nor combination treatment had a significant effect on these parameters (Table 1). Mean fasting plasma TG and leptin were increased 133% and 3.6-fold, respectively, in the OLETF group compared with those in the LETO group, but none of the treatments had a significant effect on TG (Table 1). Mean fasting plasma NEFA did not differ in any of the groups (Table 1). HOMA-IR was 2.4-fold higher in the OLETF group than in the LETO group, but none of the treatments had a significant effect on HOMA-IR (Table 1). These data suggest that the decrease in BM in the Combo group did not translate into profound or sustained improvements in the biochemical parameters of metabolic syndrome or insulin resistance. In addition, these data suggest that the arterial pressure-reducing effects of the treatments are not secondary to BM reduction, as the improvements in MAP did not match the changes in BM.

**Obesity linked to elevated RAAS components but not corticosterone**

Urinary aldosterone excretion ($U_{\text{aldo}}$), urinary corticosterone excretion ($U_B$), and urinary angiotensinogen excretion ($U_{\text{Agt}}$) were measured to assess the contribution of RAAS and corticosterone to anti-natriuresis in obesity, and to determine whether GLP-1r activation alone or in combination with AT$_1$ blockade would ameliorate this effect. From baseline, $U_{\text{aldo}}$ was greater in OLETF rats than in LETO rats (Fig. 3a). On day 2, $U_{\text{aldo}}$ was 124% higher in the EXE group than in OLETF rats and was 37% lower in the Combo group than in the OLETF group, remaining lower for the rest of the study (Fig. 3a). On day 7, $U_{\text{aldo}}$ was 61% less in the EXE group compared with that in the OLETF (Fig. 3a). From the onset to the end of the study, there was no difference in $U_B$ between LETO and OLETF rats or between OLETF rats and the treatment groups (Fig. 3b). BM was positively correlated with $U_{\text{aldo}}$ [$U_{\text{aldo}}$ = 0.131 BM − 19.047 ($R$ = 0.621; $P$ = 0.0001)], but not with $U_B$ [$U_B$ = −0.141 BM + 409.91 ($R$ = 0.078; $P$ = 0.375)]. On day 40, $U_{\text{Agt}}$ was 4.5-fold greater in OLETF rats than in LETO rats and was 56%, 62%, and 58% lower in the ARB, EXE, and Combo groups, respectively, compared with that in the OLETF group (Fig. 4). Collectively, these data suggest that obesity is associated with an inappropriate elevation in RAAS components but not corticosterone levels in OLETF rats, and that combination treatment reversed these effects.
Effects of GLP-1r activation alone and in combination with AT₁ blockade on renal electrolyte handling

Urinary Na⁺ excretion (U_{NaV}), urinary K⁺ excretion (U_{KV}), U_{NaV}/Na⁺ intake, U_{KV}/K⁺ intake, Na⁺ balance, K⁺ balance, and urinary Na⁺/K⁺ ratio were calculated to assess the effects of GLP-1r activation and AT₁ blockade on renal function and electrolyte balance. From baseline to the end of the study, there were no differences in U_{NaV}, U_{KV}, U_{NaV}/Na⁺ intake, U_{KV}/K⁺ intake, Na⁺ balance, K⁺ balance, or urinary Na⁺/K⁺ ratio between LETO and OLETF rats (Fig. 5a-c and Figure S1 and 2a, b). On day 2, U_{NaV}, U_{NaV}/Na⁺ intake, and the urinary Na⁺/K⁺ ratio were 60%, 40%, and 61% lower, respectively, in the EXE group compared with those in the OLETF group, and U_{NaV} and the urinary Na⁺/K⁺ ratio were 62% and 53% lower, respectively, in the Combo group compared with that in the OLETF group (Fig. 5a, c and Figure S1 and 2a, b). On day 2, U_{KV}/K⁺ intake was 132% greater in the EXE group and 167% greater in the Combo group compared with that in the OLETF group (Fig. 5c and Figure S1A). In contrast, on day 40, U_{KV}/K⁺ intake was 132% greater in the EXE group and 167% greater in the Combo group compared with that in the OLETF group (Fig. 5c). However, these effects disappeared when U_{NaV} and U_{KV} were corrected for Na⁺ and K⁺ intake (Figure S1A & B). Nevertheless, on day 40, Na⁺ balance was 122% lower in the Combo group than in the OLETF (Figure S2A). The

Table 1  Plasma parameters associated with the metabolic syndrome

<table>
<thead>
<tr>
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<th>LETO (n = 7)</th>
<th>OLETF (n = 9)</th>
<th>ARB (n = 9)</th>
<th>EXE (n = 7)</th>
<th>Combo (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.6 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>6.7 ± 0.1</td>
<td>7.1 ± 0.2</td>
<td>7.0 ± 0.1</td>
</tr>
<tr>
<td>Insulin (mmol/L)</td>
<td>130 ± 18</td>
<td>296 ± 31</td>
<td>229 ± 28</td>
<td>325 ± 45</td>
<td>300 ± 27</td>
</tr>
<tr>
<td>HOMA-IR (relative units)</td>
<td>0.8 ± 0.1</td>
<td>2.7 ± 0.4</td>
<td>1.6 ± 0.2</td>
<td>2.5 ± 0.4</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.6 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>0.54 ± 0.06</td>
<td>0.55 ± 0.04</td>
<td>0.62 ± 0.04</td>
<td>0.53 ± 0.05</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td>Leptin (µg/L)</td>
<td>2.8 ± 0.3</td>
<td>13.0 ± 2.5</td>
<td>6.6 ± 0.6</td>
<td>10.2 ± 1.5</td>
<td>7.2 ± 1.2</td>
</tr>
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*p < 0.05, vs. LETO

*p < 0.05 vs. OLETF. NEFA, non-esterified fatty acids
treatments had no detectable effect on plasma Na\(^+\) or K\(^+\) (Table S1). Furthermore, the effects on electrolyte excretion were independent of changes in absolute or relative water intake, urine volume, urinary creatinine excretion (Figure S3), estimated GFR, FE\(_{\text{Na}^+}\), and FE\(_{\text{K}^+}\) (Table S1). Collectively, these data demonstrate that prolonged GLP-1r activation in conjunction with AT\(_1\) blockade increase UNaV and UKV in response to increased Na\(^+\) and K\(^+\) intake; however, the increase in urinary Na\(^+\)/K\(^+\) ratio suggests that tubular K\(^+\) secretion may be blunted in this group.

**Effects of GLP-1r activation alone and in combination with AT\(_1\) blockade on Na\(^+\) and K\(^+\) channels in the kidney**

Protein expression of γENaC, ROMK, and AQP2 was measured to assess whether the hyperinsulinemia and increased U\(_{\text{aldoV}}\) in OLETF rats altered the protein expression of Na\(^+\) and K\(^+\) channels in the kidney. In addition, the phosphorylation of IR and AKT were measured to assess the link between changes in renal electrolyte channels and insulin signaling. Cleaved and active γENaC migrated as a doublet band at ~60 kDa with an additional cleavage product migrating to ~50 kDa, as previously shown [33]. Mean protein expression of cleaved and active γENaC was not different among the groups. However, the mean protein expression of the 50–60 kDa multiband was decreased 28% in the ARB group compared with that in the OLETF group, and combination treatment prevented this effect (Fig. 6a). Mean protein expression of glycosylated ROMK was increased 98% in the OLETF group compared to that in the LETO group (\(P = 0.052\)) and was decreased 78% in the Combo compared with that in the OLETF group.
evaluate the potential added benefits of GLP-1r agonists decrease blood pressure by decreasing renal Na\(^+\) reabsorption by decreasing GLP-1 secretion [5], increases plasma insulin levels [44], and intrarenal Ang II [45], all of which are factors that increase NHE3 activity, leading to increased Na\(^+\) reabsorption [46-48]. In the present study, renal IR phosphorylation (activation) was not profoundly different among the groups, suggesting that insulin signaling likely did not contribute to the observed effects. Furthermore, blockade of AT1 and activation of GLP-1r (via exenatide) independently did not have profound effects on UNaV, despite having decreased U\(_{\text{AgT}}\)V (an index of intrarenal Ang II activity) [49], suggesting that intrarenal Ang II and/or AT1 activation likely did not alter NHE3 activity. However, UNaV was increased in the Combo group, suggesting that AT1 blockade and GLP-1r activation synergistically altered NHE3 activity to contribute to the natriuresis.

Along with increased UNaV, UrV was increased in the Combo group compared to that in the OLETF group. Nevertheless, the urinary Na\(^+\):K\(^+\) ratio was also increased in this group, suggesting that tubular K\(^+\) secretion may have been blunted. The decrease in protein expression of glycosylated ROMK in this group may contribute to the blunted secretion of tubular K\(^+\). Collectively, these results suggest that AT1 blockade in conjunction with GLP-1r activation increases UNaV and decreases UrV and that the natriuretic effect may be aldosterone independent. Nevertheless, the decrease in UrV may be aldosterone dependent through downregulation of the protein expression of glycosylated ROMK.

Obesity inappropriately activates RAAS, increasing circulating levels of angiotensinogen, renin, angiotensin -converting enzyme, and aldosterone [50]. In the present study, we evaluated U\(_{\text{AgT}}\)V as an index of intrarenal Ang II.
Simultaneous GLP-1 receptor activation and angiotensin receptor blockade increase natriuresis ...

In summary, the present study demonstrates that enhancing GLP-1 and blocking AT₁ signaling produce synergistic effects on renal Na⁺ and K⁺ regulation, resulting in increased UNaV and decreased UKV. Furthermore, GLP-1r activation alone reduced UaV, and this effect was not enhanced further by AT₁ blockade. Nonetheless, co-treatment decreased UaV for the duration of the study. Despite these beneficial effects, simultaneously targeting GLP-1r activation and AT₁ blockade did not translate to additive reductions in MAP. Interestingly, these effects were independent of improvements in hyperglycemia and hyperlipidemia in OLETF rats, suggesting that the obesity-associated impairments in renal electrolyte regulation are reconcilable during the early events contributing to the development of T2DM and not profoundly impacted by elevated glucose and TGs at this stage. Nonetheless, further studies are warranted in humans to confirm these findings.

Acknowledgements We thank S Duval Ruilova, M Thorwald, and G Vazquez for their assistance with the study. Olmesartan was kindly donated by Daiichi-Sankyo (Tokyo, Japan) to Dr. A Nishiyama.

Compliance with ethical standards

Conflict of interest The study was funded by AstraZeneca. At the time this work was performed, DGP was an employee and stockholder at Amylin Pharmaceuticals, Inc.

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