学位論文

Effects of an SGLT2 inhibitor on salt sensitivity of blood pressure and sympathetic nerve activity in a non-diabetic rat model of chronic kidney disease

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医学専攻

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Effects of an SGLT2 inhibitor on salt sensitivity of blood pressure and sympathetic nerve activity in a non-diabetic rat model of chronic kidney disease

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Abstract

The glucose-lowering effect of sodium-glucose cotransporter 2 (SGLT2) inhibitors is reduced in patients with diabetes who have chronic kidney disease (CKD). In the present study, we examined the effect of an SGLT2 inhibitor on the salt-sensitivity of blood pressure (BP), circadian rhythm of BP, and sympathetic nerve activity (SNA) in non-diabetic CKD rats. Uninephrectomized Wistar rats were treated with adenine (200 mg/kg/day) for 14 days. After stabilization with a normal salt diet (NSD, 0.3% NaCl), a high salt diet (HSD, 8% NaCl) was administered. Mean arterial pressure (MAP) was continuously monitored using a telemetry system. We also analyzed the low frequency (LF) of systolic arterial pressure (SAP), which reflects SNA. In adenine-induced CKD rats, HSD for 5 days significantly increased the mean MAP from 106 ± 2 to 148 ± 3 mmHg. However, MAP was decreased to 96 ± 3 mmHg within 24 hours after switching back to NSD (n=7). Treatment with an SGLT2 inhibitor, luseogliflozin (10 mg/kg/day, p.o., n=7), significantly attenuated the HSD-induced elevation of MAP, which was associated with a reduction in LF of SAP. These data suggest that treatment with an SGLT2 inhibitor attenuates the salt-sensitivity of BP, which is associated with SNA inhibition in non-diabetic CKD rats.

Keywords

Sodium-glucose cotransporter 2 inhibitor, chronic kidney disease, blood pressure, salt, sympathetic nerve activity, rat

Introduction

Diabetes mellitus is the leading cause of chronic kidney disease (CKD) and hypertension.[1] Sodium-glucose cotransporter 2 (SGLT2) inhibitors are antihyperglycemic drugs used for the treatment of diabetes,[2] and have been shown to improve diabetic complications in patients with type 2 diabetes,[3] including cardiovascular (CV) death.[4,5] Furthermore, several clinical studies have shown that the administration of an SGLT2 inhibitor remarkably decreases blood pressure (BP), and meta-analyses have indicated that both systolic BP (SBP) and diastolic BP (DBP) are significantly decreased by treatment with an SGLT2 inhibitor.[6,7] More recently, a randomized, double-blind, placebo-controlled clinical trial reported that treatment with an SGLT2 inhibitor significantly decreased 24-hour ambulatory BP in patients with uncontrolled nocturnal hypertension and type 2 diabetes who were treated with antihypertensive agents.[8]

Interestingly, several clinical studies have shown that the SGLT2 inhibitor-induced reduction in BP is not concomitant with a notable change or compensatory increase in heart rate (HR).[8-15] Sano et al.,[16] reported that treatment with the selective SGLT2 inhibitor luseogliflozin effectively decreased HR in patients with type 2 diabetes with high HR (≥ 70 beats/min before treatment). These data suggest that SGLT2 inhibitors can attenuate sympathetic nerve activity (SNA). Our previous animal studies using a telemetry

system showed that treatment with an SGLT2 inhibitor significantly decreased BP and SNA in high salt-treated obese rats,[9] and metabolic syndrome rats.[10] Yoshikawa et al.,[17] utilized similar methods to show that an SGLT2 inhibitor significantly lowered sympathetic vasoconstrictor activity during active periods in streptozotocin-induced diabetic rats. SGLT2 inhibitors have also been shown to significantly decrease norepinephrine production and turnover in brown adipose tissue in mice,[18] and high fat-induced elevation of norepinephrine levels in the kidney and heart in rats.[19] Jordan et al.,[20] administered an SGLT2 inhibitor for 4 days to patients with type 2 diabetes treated with metformin and showed that there was no change in muscle SNA, despite a significant reduction in BP. Although the precise mechanism responsible for SNA reduction is unclear, several studies have reported that SGLT2 inhibitors can modulate urinary sodium excretion.[9,21,22] Clinical studies have indicated that the blood glucose-lowering effect induced by SGLT2 inhibitors was significantly attenuated in patients with diabetes and CKD with a reduced glomerular filtration rate.[23] However, the effects of an SGLT2 inhibitor on BP and SNA in non-diabetic patients with CKD have not previously been investigated. Therefore, we examined the effects of an SGLT2 inhibitor on BP and SNA in non-diabetic adenine-induced CKD rats. To determine the salt-sensitivity of BP, we administered a high salt diet (HSD) to non-diabetic adenine-induced CKD rats.

Methods

Animals

All experimental procedures were carried out according to the guidelines for care and use of animals established by Kagawa University. Wistar rats were purchased from Japan SLC Inc. (Shizuoka, Japan) and were housed in specific pathogen-free animal facilities at a controlled temperature (24±2 °C) and humidity (55±5%) with a 12-hour light-dark cycle. Rats were given standard chow prior to telemetry implantation and had access to water *ad libitum*.

Protocols

Five-week old male Wistar rats were subjected to right nephrectomy. After 1 week of acclimatization, rats were treated with vehicle (0.5% carboxymethylcellulose, p.o., n=6) or adenine (200 mg/kg per day, p.o., n=14) for 2 weeks. Next, a radiotelemetry device was implanted in all animals. Thereafter, rats received a normal salt diet (NSD, 0.3% NaCl, OYC, Tokyo, Japan) for 9 days. Subsequently, the chow was switched to a HSD (8% NaCl, OYC, Tokyo, Japan), and adenine-treated rats were treated with vehicle (n=7) or luseogliflozin (10 mg/kg per day, p.o., n=7). After 5 days, the chow was again switched to NSD in all animals (Supplemental Figure 1).

Sample collection

At the end of the observation period, overnight-fasted animals were sacrificed by overdose of sodium pentobarbital (250 mg/kg, i.p.). Blood was collected for the measurement of blood urea nitrogen (BUN) level. Left-kidney tissue was harvested, fixed in 10% formalin (pH 7.4) for 48 hours, and embedded in paraffin for periodic acid-Schiff (PAS) staining.

Telemetry system

A radio-telemetry system (Data Science International, Saint Paul, Minnesota, USA) was used for the measurement of BP in conscious animals at 8 weeks of age. A telemetry catheter was inserted into the femoral artery and animals were maintained under stress-free conditions for 7 days for recovery (Supplemental Figure 1). All animals underwent a 24-hour acclimatization period on a telemetry receiver panel. Twenty four-hour BP measurement was undertaken during the experimental period. Moreover, we calculated the 12-hour dark-period (18:00–06:00) and 12-hour light-period (06:00–18:00) mean arterial pressure (MAP) to evaluate the BP circadian rhythm. We also investigated SNA with low frequency (LF) of SBP, locomotor activity (LA), and their circadian rhythm using the telemetry system as previously described. [9,17]

Statistical analysis

Data are presented as the mean ± SEM. Statistical comparisons of the differences between values were performed using a t-test, one-way analysis of variance followed by Tukey's multiple comparison test, or two-way ANOVA analysis of variance. P-values <0.05 were considered statistically significant.

Results

Effects of luseogliflozin on renal injury and salt-induced BP elevation in adenine-induced CKD rats

Compared with the vehicle-treated rats, adenine-induced CKD rats exhibited reduced body weight. Treatment with luseogliflozin did not affect body weight in adenine-induced CKD rats (data not shown). In adenine-induced CKD rats, severe renal histological injury was observed (Figure 1a) and plasma BUN levels were significantly increased, while luseogliflozin did not affect histological changes and plasma BUN levels (Figure 1b).

[Figure 1 here]

In vehicle-treated rats, HSD significantly increased the average 24-hour MAP from 111±1 to 126±1 mmHg. In adenine-induced CKD rats, the HSD-induced increase in MAP was significantly enhanced (from 106±2 to 148±3 mmHg). In both animal groups, MAP decreased immediately after switching to NSD. Treatment with luseogliflozin significantly attenuated the salt-induced increase in MAP (134±4 mmHg) in adenine-induced CKD rats (Figure 2).

[Figure 2 here]

Figures 3a–3c show the average 12-hour MAP during the light (inactive) and dark (active) period, respectively. In vehicle-treated rats, a significant difference in MAP was observed

between the inactive and active periods before initiating HSD treatment (114±1 vs. 109±0.4 mmHg respectively, Figure 3a). In contrast, MAP did not differ significantly between the inactive and active periods in adenine-induced CKD rats, indicating a non-dipper pattern of BP. After 5 days of HSD, a difference in MAP was observed between the inactive and active periods in vehicle- and adenine-treated rats. In luseogliflozin-treated adenine-induced CKD rats, a difference in MAP was also observed between inactive and active periods, although these changes were not statistically significant (Figure 3b). After switching from HSD to NSD, the BP circadian rhythm patterns immediately returned to the levels at baseline (Figure 3c). The average values for the difference of MAP between the inactive and active periods are summarized in Figure 3d. As shown in Supplemental Figures 2–5, the changes in SBP and DBP were similar to those of MAP.

[Figure 3 here]

Effects of luseogliflozin on SNA in adenine-induced CKD rats

We assessed the effects of luseogliflozin on LF (0.25–0.75 Hz) of SBP, which reflects the level of sympathetic vasoconstrictor activity.[9,24] Figure 4a shows the average 24-hour LF of SBP. During feeding with NSD, adenine-induced CKD rats showed significantly

reduced LF of SBP compared with that of vehicle-treated rats (3.1±0.3 vs. 4.8±0.2 $mmHg^2$, respectively), which was in response to an increase in BP (Figure 2). Feeding with HSD did not change SNA in all animals. In adenine-induced CKD rats, luseogliflozin-treated CKD rats exhibited a lower SNA compared with that of vehicle-treated CKD rats on the fifth day of HSD (2.1±0.1 $mmHg^2$ vs. 2.6±0.3 $mmHg^2$, respectively). In all groups, SNA returned to the basal values within 24 hours after switching to NSD. However, the luseogliflozin-induced reduction in SNA persisted until the end of the experimental period in CKD rats (2.8±0.3 $mmHg^2$ vs. 4.2±0.5 $mmHg^2$, adenine + luseogliflozin vs. adenine), indicating the presence of a continuing efficacy in luseogliflozin.

[Figure 4 here]

Figure 4b–4d shows the average 12-hour LF of SBP during inactive and active periods. During feeding with NSD, the circadian rhythm of LF of SBP (Figure 4b) was similar to that of MAP (Figure 3a). Specifically, a significant difference between inactive and active periods of LF of SBP (6.4±0.3 $mmHg^2$ vs. 3.0±0.2 $mmHg^2$, respectively) was observed in vehicle-treated rats, while adenine-induced CKD rats exhibited a non-dipper pattern of LF of SBP and treatment (Figure 4b). After 5 days of HSD feeding, both vehicle- and adenine-treated CKD rats showed reduced LF of SBP in the active period but not in the inactive period. Interestingly, the LF value of SBP in luseogliflozin-treated CKD rats in the

active period was significantly lower than that of vehicle-treated CKD rats (1.9±0.1 $mmHg^2$ vs. 2.6±0.4 $mmHg^2$, respectively, Figure 4c). After switching from HSD to NSD, the LF of SBP during both the inactive and active periods did not change in vehicle-treated rats. However, a difference in LF of SBP between the inactive and active periods was apparent in adenine-induced CKD rats. Furthermore, the increase in LF of SBP observed in adenine-induced CKD rats during the active period was inhibited by luseogliflozin treatment (Figure 4d).

Effects of luseogliflozin on LA in HS-treated adenine-induced CKD rats

Figure 5a shows the average 24-hour LA. During feeding with NSD, the average 24-hour LA was lower in adenine-induced CKD rats than that in vehicle-treated rats. After 5 days of HSD feeding, LA was markedly increased from 2.7±0.1 to 4.4±0.2 counts/minute in vehicle-treated rats. However, the HSD-induced increase in LA was significantly lower in vehicle- or luseogliflozin-treated adenine-induced CKD rats (2.6±0.3 and 2.2±0.2 counts/minute, respectively). After switching from HSD to NSD, LA immediately returned to the respective baseline level in all groups.

[Figure 5 here]

Figure 5b–5d depicts the average 12-hour LA during the inactive and active periods. During feeding with NSD, LA was significantly greater in the active period than in the inactive period (Figure 5b). Moreover, both vehicle- and luseogliflozin-treated adenine-induced CKD rats exhibited decreased LA during the active period. After 5 days of HSD treatment, LA was significantly increased during the inactive and active periods in vehicle-treated rats but not in adenine-induced CKD rats. In these animals, luseogliflozin did not significantly affect LA (Figure 5c). After switching from NSD to HSD, LA levels returned to the respective basal levels in all animals (Figure 5d).

Discussion

The blood glucose-lowering effect induced by SGLT2 inhibitors is directly proportional to the glomerular filtration rate.[23] The present study examined the effect of an SGLT2 inhibitor on the salt-sensitivity of BP and SNA in non-diabetic CKD rats. Experiments were performed in non-diabetic adenine-induced CKD rats to avoid the influence of changes in blood sugar level. Our results showed that administration of the SGLT2 inhibitor luseogliflozin significantly attenuated the HSD-induced elevation of BP in adenine-induced CKD rats, and was associated with a reduction in SNA. These data suggest that treatment with an SGLT2 inhibitor decreases the salt-sensitivity of BP by attenuating SNA in non-diabetic CKD rats, independent of blood glucose changes.

Impaired renal function predominantly contributes to the pathogenesis of salt sensitivity of BP.[25] In patients with CKD whose glomerular ultrafiltration capability is reduced, BP becomes salt sensitive.[25,26] In the present study, we confirmed that HSD-induced increases in BP were significantly augmented in adenine-induced CKD rats. Interestingly, treatment with luseogliflozin significantly attenuated the development of HSD-induced hypertension in non-diabetic adenine-induced CKD rats. Since blood sugar levels were not influenced by luseogliflozin, BP reduction appears to be induced via a blood glucose-independent mechanism. These data are consistent with those from previous studies

showing that treatment with empagliflozin prevented salt-induced increases in BP without changing blood sugar levels in obese rats with normal kidney function.[27] As indicated in previous studies,[27-29] SGLT2 inhibitors induce natriuresis, which may mediate the SGLT2 inhibitor-induced reduction in BP in hypertensive subjects. However, a deficiency of the present study is the failure to identify the luseogliflozin-induced natriuresis, given the difficulties in monitoring the accurate urinary excretion rate of sodium in adenine-treated uninephrectomized rats fed with an 8% NaCl diet because of severe polyuria.

It has also been reported that SNA plays an important role in the development of salt-dependent hypertension.[30,31] In the present study, treatment with luseogliflozin significantly attenuated the HSD-induced BP elevation in CKD rats, which was associated with a reduction in SNA. These data are consistent with previous reports showing that SGLT2 inhibitors decrease SNA in salt-treated obese rats,[9] and metabolic syndrome rats.[10] The precise mechanism responsible for the SGLT2 inhibitor-induced reduction in SNA is not clear. However, our data indicate that this reduction is not accompanied by reductions in blood sugar levels and LA (Figure 5). We therefore speculate that the SGLT2 inhibitor-induced reduction in SNA is associated with the effect of the inhibitor on natriuresis, as reported by other investigators.[32,33] However, further studies are required to clarify the precise molecular mechanism for this effect.

In agreement with previous studies in patients with CKD,[34] adenine-induced CKD rats in the present study showed a non-dipper pattern in BP circadian rhythm. However, after short-term feeding with HSD, BP elevation was greater in the active period than in the inactive period, resulting in a significant difference in BP between the active and inactive periods. These data are consistent with those from previous studies in which BP elevation induced by short-term treatment with HSD was significantly greater in the active period than in the inactive period in Dahl salt-sensitive hypertensive rats.[35] It remains unclear why short-term HSD elicits greater BP elevation in the active period, although the effect may be attributable to differences in drinking water volume and urinary volume between the active and inactive periods. It is also possible that HSD alters the eating habits and rhythm in rodents, which can have a marked influence on the BP circadian rhythms.[36]

In conclusion, our data indicate that treatment with an SGLT2 inhibitor decreases the salt-sensitivity of BP, which is associated with the attenuation of SNA in CKD rats. BP control is the one of the most important aspects of cardiovascular disease management in patients with CKD.[37,38] In this regard, Wanner et al.,[39] showed that although treatment with an SGLT2 inhibitor elicits less glucose-lowering effect in patients with type 2 diabetes and CKD compared with the type 2 diabetic patients without CKD, its cardioprotective effect is not influenced by reduced renal function. The effect of an SGLT2 inhibitor in non-

diabetic CKD patients is currently being investigated in a large clinical study (https://www.astrazeneca.com/media-centre/press-releases/2016/astrazeneca-announces-two-new-phase-IIIb-trials-for-Forxiga-in-chronic-kidney-disease-and-chronic-heart-failure-120920161.html#). Although the primary endpoint of this study is the renal outcome, the effects of the SGLT inhibitor on BP are expected to be clarified in this patient population.

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Declaration of Conflicting Interests

A.N. has received honoraria for educational meetings conducted on behalf of Taisho Co., Ltd. The authors declare that there is no other conflict of interest.

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AUTHOR CONTRIBUTIONS

This study was performed at Kagawa University. All authors were involved in the acquisition, analysis, or interpretation of data. A.N. was involved in the conception and design of the study. N.W. wrote the manuscript and A.R. and A.N. revised it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All people designated as authors qualify for authorship.

Fig. 1

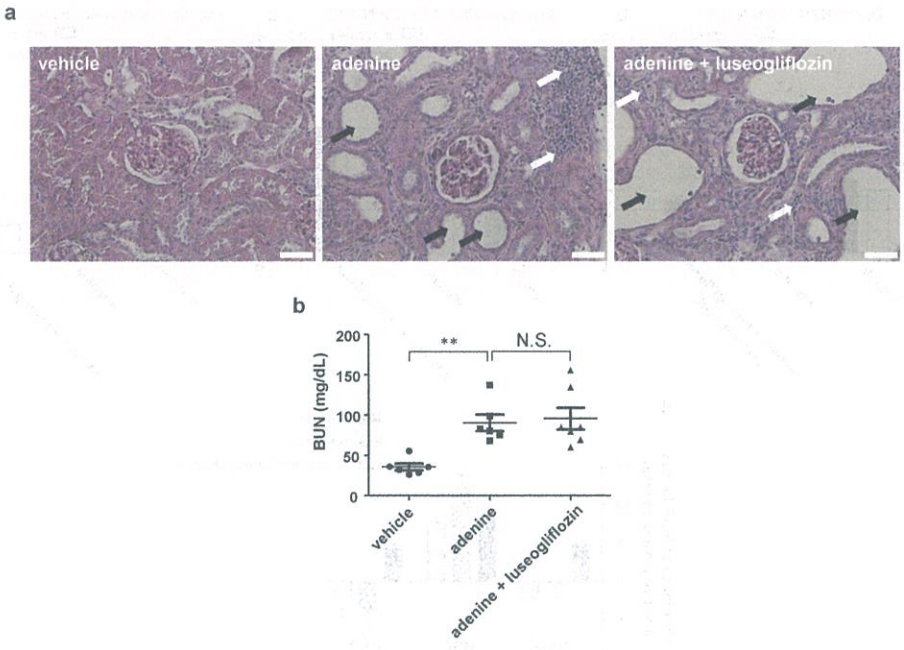

a
vehicle
adenine
adenine + luseogliflozin
b
BUN (mg/dL)
200
150
100
50
0
**
N.S.
vehicle
adenine
adenine + luseogliflozin

Fig. 2

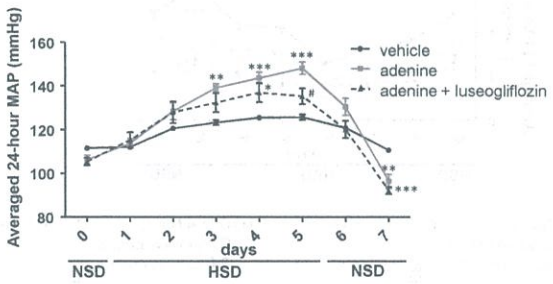

Averaged 24-hour MAP (mmHg)
160
140
120
100
80
0
1
2
3
4
5
6
7
days
NSD
HSD
NSD
vehicle
adenine
adenine + luseogliflozin
**
***
***
*
#
**
***

Fig. 3

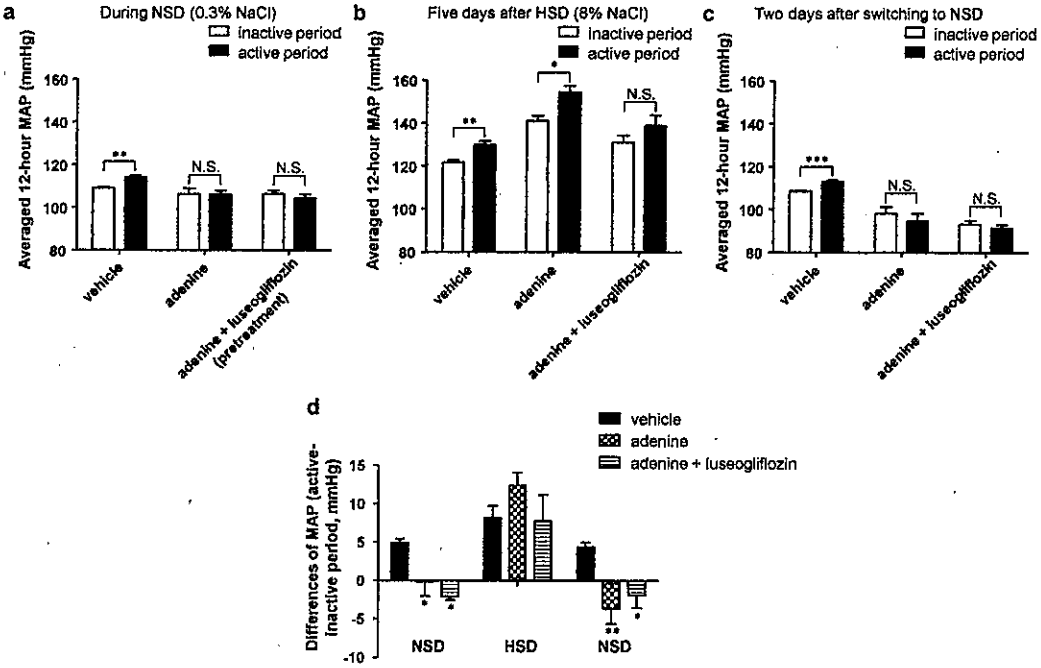

a
During NSD (0.3% NaCl)
inactive period
active period
Averaged 12-hour MAP (mmHg)
160
140
120
100
80
**
N.S.
N.S.
vehicle
adenine
adenine + luseogliflozin (pretreatment)
b
Five days after HSD (8% NaCl)
inactive period
active period
Averaged 12-hour MAP (mmHg)
**
*
N.S.
vehicle
adenine
adenine + luseogliflozin
c
Two days after switching to NSD
inactive period
active period
Averaged 12-hour MAP (mmHg)
***
N.S.
N.S.
vehicle
adenine
adenine + luseogliflozin
d
vehicle
adenine
adenine + luseogliflozin
Differences of MAP (active-inactive period, mmHg)
15
10
5
0
-5
-10
NSD
HSD
NSD

Fig. 4

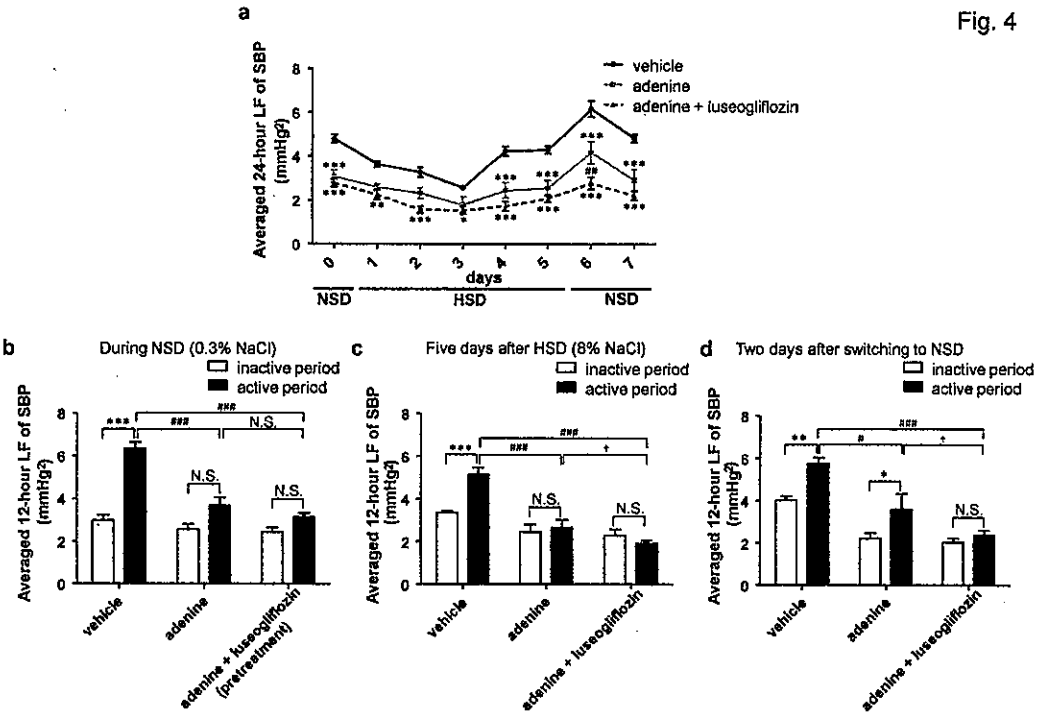
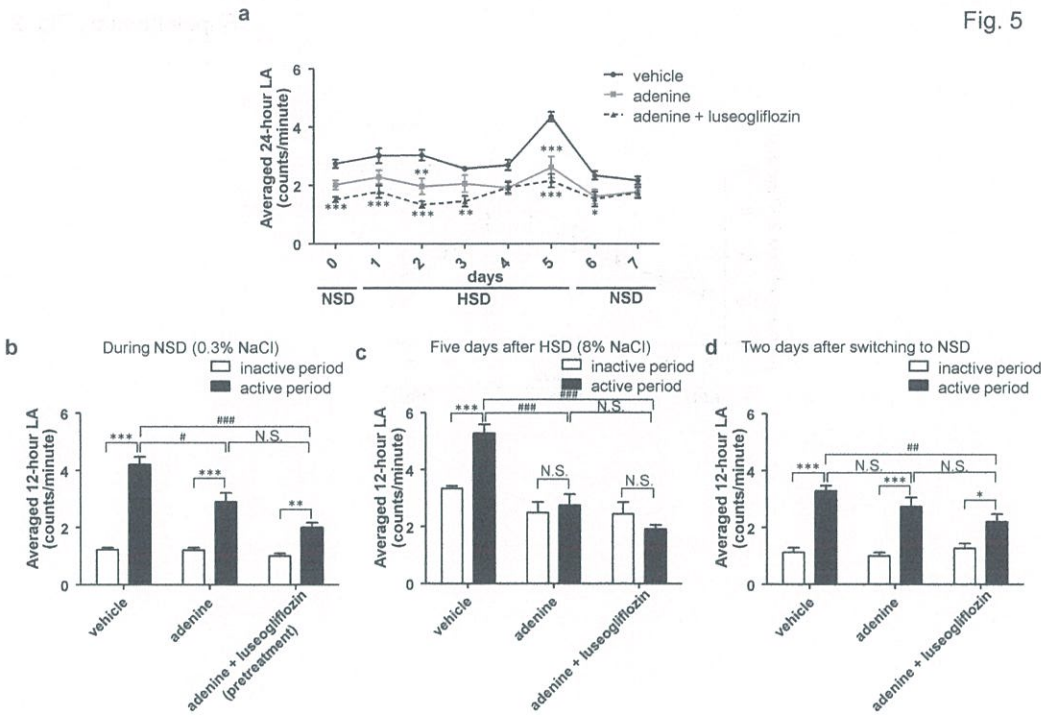
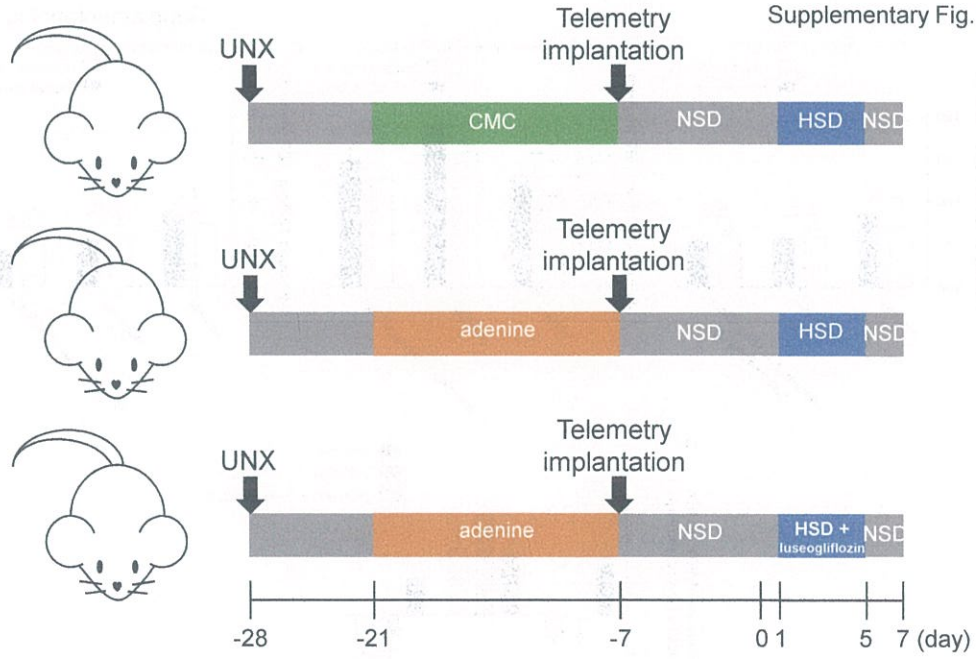

a
vehicle
adenine
adenine + luseogliflozin
Averaged 24-hour LF of SBP (mmHg²)
8
6
4
2
0
0
1
2
3
4
5
6
7
days
NSD
HSD
NSD
b
During NSD (0.3% NaCl)
inactive period
active period
Averaged 12-hour LF of SBP (mmHg²)
***
###
###
N.S.
N.S.
N.S.
vehicle
adenine
adenine + luseogliflozin (pretreatment)
c
Five days after HSD (8% NaCl)
inactive period
active period
Averaged 12-hour LF of SBP (mmHg²)
***
###
###
†
N.S.
N.S.
vehicle
adenine
adenine + luseogliflozin
d
Two days after switching to NSD
inactive period
active period
Averaged 12-hour LF of SBP (mmHg²)
**
###
#
†
*
N.S.
vehicle
adenine
adenine + luseogliflozin

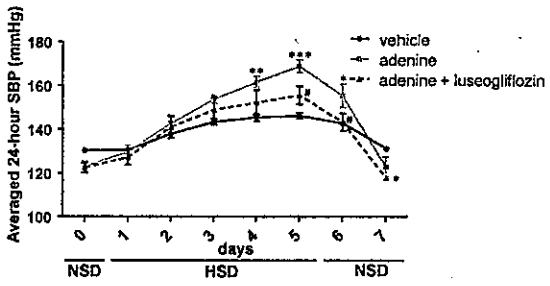
Fig. 5



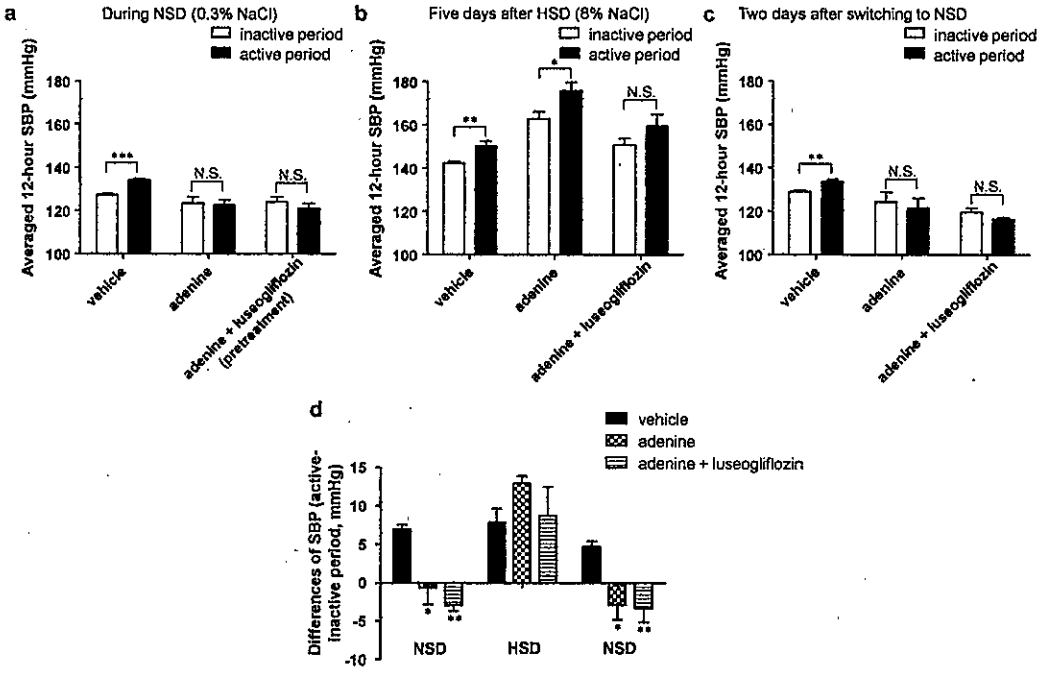
Supplementary Fig. 1



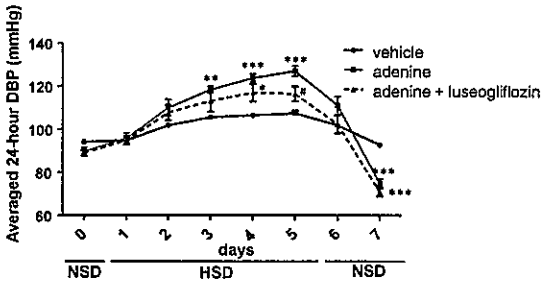
Supplementary Fig. 2



Supplementary Fig. 3



Supplementary Fig. 4



Supplementary Fig. 5

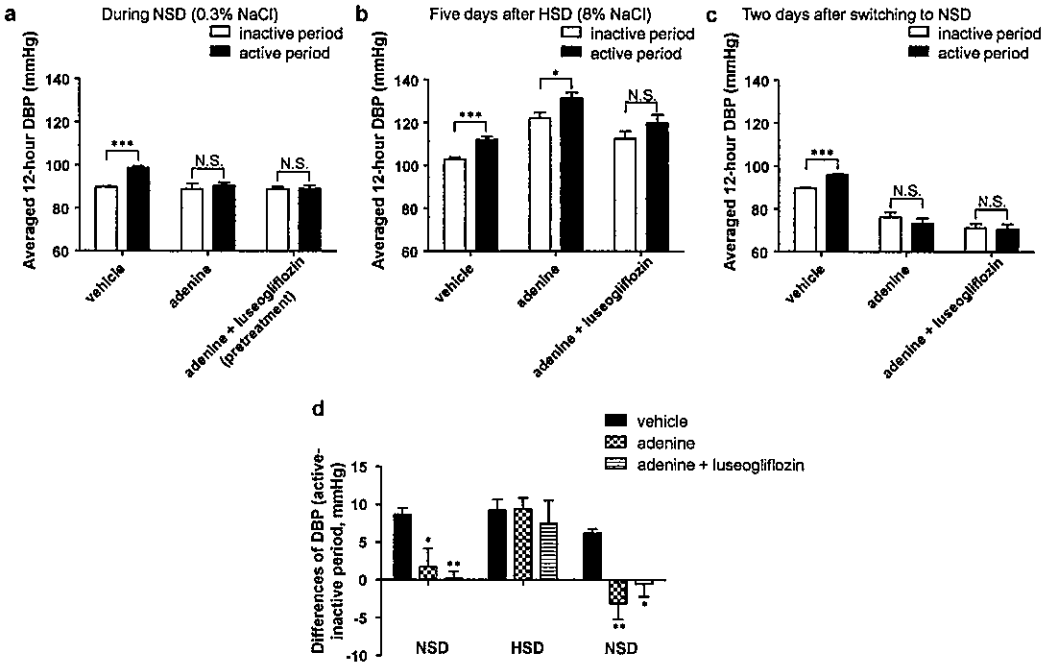


Figure legends

Fig. 1 Impact of luseogliflozin treatment on renal injury. Adenine-induced CKD rats fed a high-salt diet (HSD) exhibited (a) massive interstitial cellularity (white arrows), widespread tubular dilation (black arrows) in the kidneys and (b) increased level of blood urea nitrogen (BUN). Luseogliflozin treatment did not attenuate these changes. Bar = 50 μm. $^{**}P < 0.01$ vs. vehicle group.

Fig. 2 Impact of luseogliflozin treatment on mean arterial pressure (MAP). Average 24-hour MAP on normal salt diet (NSD, 0.3% NaCl), HSD (8% NaCl), and after switching back to NSD in adenine-induced CKD rats with or without luseogliflozin treatment. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle group; $^{\#}P < 0.05$ vs. adenine group.

Fig. 3 Impact of luseogliflozin treatment on MAP circadian rhythm. Average 12-hour MAP in the inactive and active period (a) during NSD feeding, (b) after 5 days on HSD, and (c) 2 days after switching to NSD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the inactive period of the respective group. (d) Differences in 12-hour MAP between the inactive and active period in adenine-induced CKD rats with or without luseogliflozin treatment. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle group.

Fig. 4 Impact of luseogliflozin treatment on sympathetic nerve activity (SNA) and circadian rhythm. Low frequency (LF) of systolic blood pressure (SBP) reflects the level of SNA. (a) Averaged 24-hour LF of SBP during feeding with NSD (0.3% NaCl), HSD (8% NaCl), and after switching back to NSD in adenine-induced CKD rats with or without luseogliflozin treatment. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle group; $^{\#\#}P < 0.01$ vs. adenine group. Average 12-hour LF of SBP in the inactive and active period (b) during feeding with NSD, (c) after 5 days of HSD, and (d) 2 days after switching to NSD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the inactive period of the respective group; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. the active period of the vehicle group; $^{\dagger}P < 0.05$ vs. the active period of the adenine group.

Fig. 5 Impact of luseogliflozin treatment on locomotor activity (LA) and circadian rhythm. (a) Average 24-hour LA during feeding with NSD (0.3% NaCl), HSD (8% NaCl), and after switching back to NSD in adenine-induced CKD rats with or without luseogliflozin treatment. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle group. Average 12-hour LA in the inactive and active period (b) during feeding with NSD, (c) after 5 days of HSD, and (d) 2 days after switching to NSD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the

inactive period of the respective group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the active period of the vehicle group.

Supplemental Fig. 1 Protocol for animal experiments. Following uninephrectomy (UNX), animals were randomized to one of three groups and treated with vehicle (CMC), adenine, or adenine+luseogliflozin. Animals were maintained on a normal salt diet (NSD, 0.3% NaCl) and then received a high salt diet (HSD, 8% NaCl) for 5 days before switching back to NSD.

Supplemental Fig. 2 Effect of luseogliflozin on systolic blood pressure (SBP). Average 24-hour SBP during feeding with NSD (0.3% NaCl), HSD (8% NaCl), and after switching back to NSD in adenine-induced CKD rats with or without luseogliflozin treatment. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle group; $^{\#}P < 0.05$ vs. adenine group.

Supplemental Fig. 3 Effect of luseogliflozin treatment on SBP circadian rhythm. Average 12-hour SBP in the inactive and active period (a) during feeding with NSD, (b) after 5 days of HSD, and (c) 2 days after switching to NSD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the inactive period of the respective group. (d) Differences in 12-hour MAP between the inactive and active period in adenine-induced CKD rats with or without luseogliflozin treatment. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle group.

Supplemental Fig. 4 Effect of luseogliflozin treatment on diastolic blood pressure (DBP). Average 24-hour DBP during feeding with NSD (0.3% NaCl), HSD (8% NaCl), and after switching back to NSD in adenine-induced CKD rats with or without luseogliflozin treatment. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle group; $^{\#}P < 0.05$ vs. adenine group.

Supplemental Fig. 5 Effect of luseogliflozin treatment on DBP circadian rhythm. Average 12-hour DBP in the inactive and active period (a) during feeding with NSD, (b) after 5 days of HSD, and (c) 2 days after switching to NSD. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. the inactive period of the respective group. (d) Differences in 12-hour DBP between the inactive and active period in adenine-induced CKD rats with or without luseogliflozin treatment. $^{*}P < 0.05$, $^{**}P < 0.001$ vs. vehicle group.

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