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Exendin-4 has an anti-hypertensive effect in salt-sensitive mice model

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ABSTRACT

The improvement of salt-sensitive hypertension is a therapeutic target for various vascular diseases. Glucagon-like peptide 1 (GLP-1), an incretin peptide, has been reported to have natriuretic effect as well as blood glucose lowering effect, although its exact mechanism and clinical usefulness remain unclear. Here, we examined anti-hypertensive effect of exendin-4, a GLP-1 analog, in salt-sensitive obese *db/db* mice and angiotensin II (angII)-infused C57BLK6/J mice. The treatment of exendin-4 for 12 weeks inhibited the development of hypertension in *db/db* mice. In *db/db* mice, the urinary sodium excretion was delayed and blood pressure was elevated in response to a high-salt load, whereas these were attenuated by exendin-4. In *db/db* mice, intra-renal angII concentration was increased. Furthermore, exendin-4 prevented angII-induced hypertension in non-diabetic mice and inhibited angII-induced phosphorylation of ERK1/2 in cultured renal cells. Considered together, our results indicate that exendin-4 has anti-hypertensive effects through the attenuation of angII-induced high-salt sensitivity.

It has been postulated that patients diagnosed as having obese type 2 diabetes mellitus or metabolic syndrome are at great risk for developing cardiovascular diseases [1,2]. It has been established that the blood pressure becomes salt-sensitive in patients with obesity, and salt-sensitivity has been reported to become greater as renal function declines [3]. Also, cardiovascular events occurred more frequently in patients with salt-sensitive hypertension [4,5]. Thus, salt-sensitivity seen in obese or diabetic patients is an independent risk factor for various vascular diseases, and its improvement is thought to be as a therapeutic target in these patients.

Glucagon-like peptide-1 (GLP-1) is an incretin peptide secreted from enteroendocrine L cells in the intestine, and is known to stimulate insulin secretion from pancreatic β-cells [6,7]. Thus, the stimulating GLP-1 signaling is considered as new therapeutic target in type 2 diabetes. However, GLP-1 is rapidly degraded by dipeptidyl peptidase-IV (DPP-IV) in the bloodstream. Therefore, GLP-1 itself is clinically difficult to apply. In contrast, exendin-4, a GLP-1 analog, is highly resistant to degradation by DPP-IV, is recently approved as an anti-diabetic drug in some countries. Interestingly, GLP-1 receptor (GLP-1R) is expressed not only in β-cells but also in numerous tissues including kidney [8–10]. Also, GLP-1 has been reported to have natriuretic effect in obese human subjects or animal model [11,12]. These findings suggest that exendin-4, a GLP-1 analog, has any extra-islet effects including the regulation of sodium excretion. However, it has been not determined whether exendin-4, which has only 54% homology to GLP-1 [13], really has the extra-islet effects.

We have previously reported that obese type 2 diabetic *db/db* mice showed a significant increase in blood pressure [14,15], although its mechanism has been not elucidated. Previous studies in mice also showed that an increase in intra-renal angiotensin II (angII) levels following an infusion of angII causes salt-sensitive hypertension with impaired salt-handling in the kidney [16] and that the increase of renal angII is a key mediator of hypertension under diabetic condition [17]. Thus, to explore the possibility that exendin-4 has the additional clinical usefulness beyond its blood glucose lowering effect, we examined the effect of exendin-4 on

Abbreviations: AngII, angiotensin II; DPP-IV, dipeptidyl peptidase IV; GLP-1, Glucagon-like peptide 1; GLP-1R, Glucagon-like peptide 1 receptor; MAPKinase, mitogen-activated protein kinase; PKCβ, protein kinase Cβ; RAS, renin-angiotensin system.

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salt-sensitive hypertension using by type 2 diabetic *db/db* mice and an angII-infusion in non-diabetic C57BLK/6J mice.

Materials and methods

Animals and experimental design. Male, 6-week-old, *db/db* mice and non-diabetic *db/m* mice were purchased from CLEA Japan Co. (Osaka, Japan), and maintained on a 12 h-light/dark cycle under a standard laboratory diet and water. The mice were divided into four groups; diabetic *db/db* mice and non-diabetic *db/m* mice were treated intraperitoneally with 20 mg kg body weight exendin-4 (Sigma, St. Louis, MO) or vehicle twice daily for 12 weeks. During experimental periods, blood pressure was measured by the tail cuff method. For the measurement of blood pressure, conscious mice were placed on a hearted pad (37 °C) in temperature-controlled quiet room. After 5 min of rest, systolic blood pressure was measured by a programmable tail-cuff sphygmomanometer (BP98-A; Softron, Tokyo, Japan). The average of 10 consecutive measurements was analyzed in each mouse. All experimental protocols described in this study were approved by the Animal Care Committees of Shiga University of Medical Science.

Urine volume and urinary sodium excretion in response to salt-loading. Evaluation of urinary sodium excretion in response to acute sodium-loading was performed as reported previously [18]. After fasting for 12 h and collection/measurement of excreted urine, 10-week-old, *db/db* and *db/m* mice treated with exendin-4 (20 mg/kg/day) or vehicle were injected intraperitoneally with 1.5 ml of 0.9% saline. During the following 6 h, we collected urine and determined urine volume and urinary sodium excretion.

High salt-induced hypertension. Ten-week-old, *db/db* and *db/m* mice were used. During 2 weeks of drinking 2.0% saline, we inject exendin-4 or vehicle, and then systolic blood pressure was measured and urine was collected intermittently under treatment with exendin-4 or vehicle [19]. Urine samples were analyzed for 24-h urinary sodium excretion.

Measurement of intra-renal angiotensin II (angII) concentration. Intra-renal angII contents were measured by radioimmunoassay as described previously [20].

AngII-induced hypertension. Male, 8-week-old, C57BLK/6J mice (CLEA Japan, Tokyo) were individually housed in box cages. We surgically implanted osmotic minipumps (Alzet, Cupertino, CA) for subcutaneous delivery of angII (1 μg/kg/min) donated by Daiichi Suntory Biomedical Research (Tokyo) [15]. The selected dosage of angII was based on previous studies [16,21]. During the 2-week experimental period, we injected exendin-4 or vehicle and measured blood pressure intermittently. After 2 weeks of angII-infusion, we removed the osmotic minipumps, and finally measured blood pressure at 5 days after removal of pumps.

RNA extraction and quantitative real-time PCR. Total RNA was isolated from various tissues by TRIzol protocol (Invitrogen Life Technologies, Carlsbad, CA). Complementary DNA (cDNA) was synthesized using reverse transcript reagents (Takara, Otsu, Japan) after treatment with DNAase (Invitrogen Life Technologies). To determine GLP-1R mRNA expression, these cDNA were amplified by standard polymerase chain reaction (PCR) method [22]. The sequences of the primers of GLP1-R were: (GLP1-R; forward: AT CTTTGCCTTTGTGATGGAC; reverse: CAGCATTTCCGAAACTCCATC).

Urinary cAMP excretion in response to a single injection of exendin-4. Male, 8-week-old, C57BLK/6J mice were housed individually in box

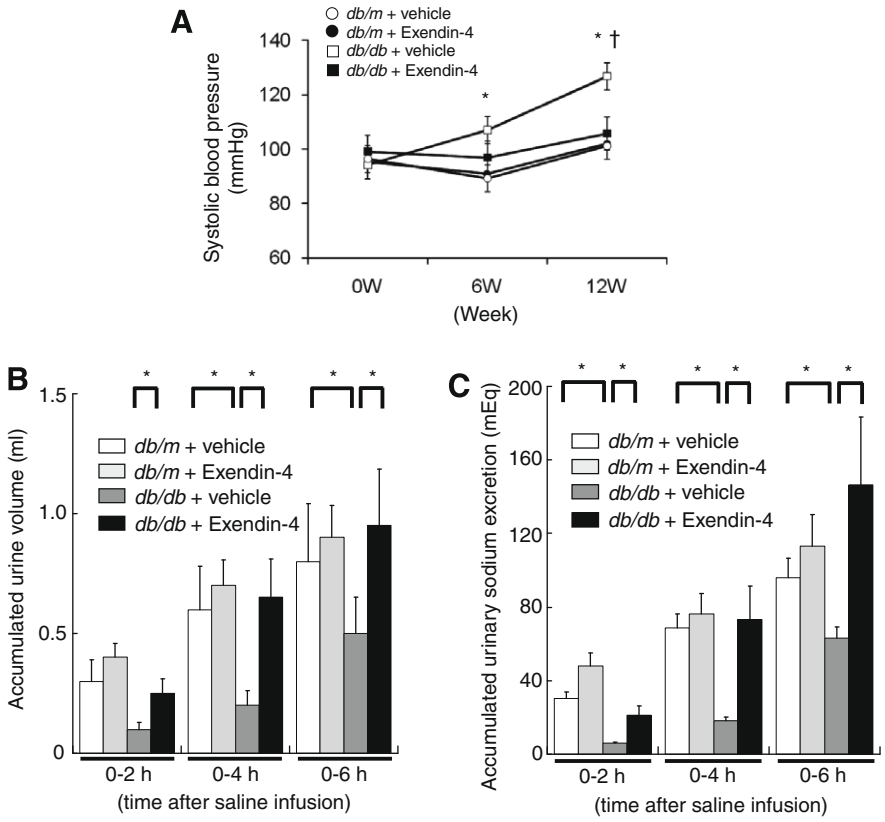

Fig. 1. Effects of exendin-4 on hypertension and salt-sensitivity in *db/db* mice. (A) Systolic blood pressure in each group of mice during 12 weeks of experimental periods. Data are means ± SEM. $n = 12$ for each groups. $^{*}P < 0.05$ vs. *db/m* + vehicle. $^{\dagger}P < 0.05$ vs. *db/db* + exendin-4. (B) Urine volume in each of the indicated four groups of mice over 6 h following intraperitoneal injection of 1.5 ml normal saline. Data are expressed as cumulative urine volume; $n = 6$ for each data point. Values are expressed as means ± SEM. $^{*}P < 0.01$. (C) Urinary sodium excretion in each of the four groups of mice over 6 h following intraperitoneal 1.5 ml normal saline. Data are expressed as cumulative urinary sodium excretion; $n = 6$ for each data point. Data are means ± SEM. $^{*}P < 0.01$.

cages. Each was injected with exendin-4 (20 mg/kg/day) or vehicle and urine samples were collected for 2 h before and after the injections. These urine samples were followed by the measurement of cAMP levels by using ELISA (R&D Systems, Minneapolis, MN).

Cell culture. Opossum kidney (OK) proximal tubular cells were purchased (American Type Culture Collection, Manassas, VA) and grown in Dulbecco's modified Eagle medium culture media supplemented with 10% serum under constant flow of 5% carbon dioxide at 37 °C. To examine the effect of exendin-4 in angII-induced phosphorylation of extracellular signal-regulated kinase (pERK1/2), OK cells were pre-incubated with exendin4 for 4 h and followed by stimulation with angII at the concentration of 10^{-9} M. After 5 min of stimulation with angII, the cells were harvested and ERK1/2 phosphorylation was determined by immunoblot analysis using anti-ERK2 (Santa Cruz Biotechnology, Santa Cruz, CA) and anti-phospho-p42/p44 ERK (Cell Signaling Technology, Beverly, MA). The results were expressed as the ratio of pERK1/2 to ERK2.

Statistical analysis. Data were expressed as means ± SEM, with *n* denoting the number of animals. Differences between groups were examined for statistical significance using analysis of variance (ANOVA) followed by Scheffe's test. A *P* value less than 0.05 denoted the presence of a statistically significant difference.

Results

Effects of exendin-4 on impaired salt-sensitivity in db/db mice

Firstly, we confirmed the significant increase in systolic blood pressure in *db/db* mice at 6 and 12 weeks of observation periods (Fig. 1A). This increase at 12 week was significantly attenuated by daily injection of exendin-4 (Fig. 1A). We thus examined the effect of exendin-4 on impaired salt-sensitivity in *db/db* mice. In an acute salt-loading study, the urine volume of *db/db* mice tended to be smaller during the first 2-h after infusion of saline than that of *db/m* mice, and it was significantly less than *db/m* mice during the next 4 h (Fig. 1B). The sum of urinary sodium excretion in *db/db* mice was significantly less than in the other groups throughout a total of 6 h (Fig. 1C). The delays in urine excretion and urinary sodium excretion in response to acute salt-loading were significantly attenuated in *db/db* mice treated with exendin-4 (Fig. 1B and C).

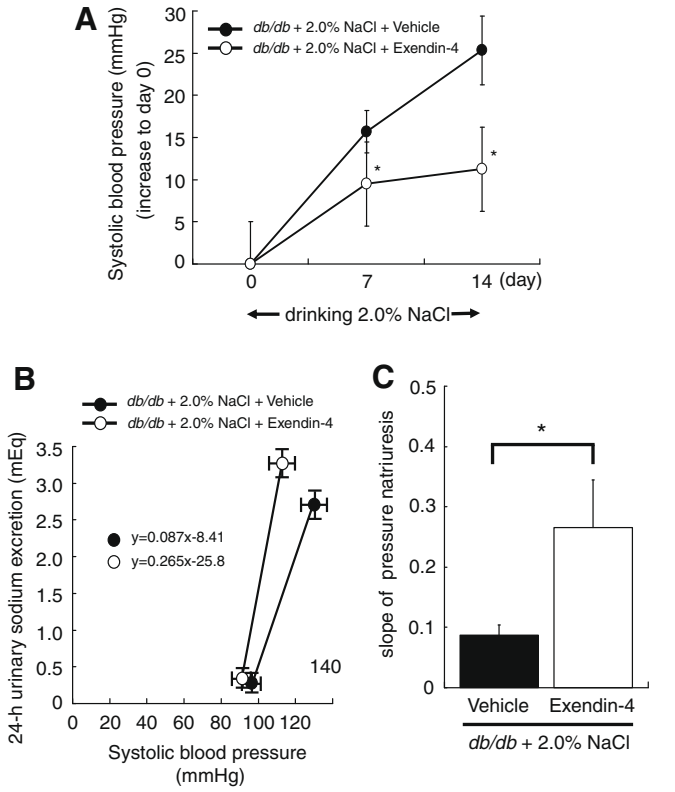

Fig. 2. Effects of exendin-4 on high salt-induced hypertension in *db/db* mice. (A) Systolic blood pressure in *db/db* mice provided with water containing 2.0% NaCl treated with vehicle or exendin-4 for 2 weeks. Values are expressed as means ± SEM. $^{*}P < 0.01$ vs. *db/db* mice + 2.0% NaCl + vehicle. $n = 6$ for each data point. $^{*}P < 0.01$. (B) Pressure-natriuresis curves in *db/db* mice provided with water containing 2.0% NaCl treated with vehicle or exendin-4 for 2 weeks. (C) Slopes of pressure-natriuresis curves shown in (B). Data are means ± SEM. $n = 6$ for each data point. $^{*}P < 0.01$ vs. *db/db* mice with exendin-4.

Effects of exendin-4 on salt-sensitive hypertension in db/db mice

We next examined the effect of exendin-4 on the development of 2-week high salt-induced hypertension in *db/db* mice. In *db/db* mice, systolic blood pressure gradually increased during the period of 2% saline loading. This increase was significantly inhibited by daily injection of exendin-4 during experimental periods (Fig. 2A). Fig. 2B shows the pressure natriuresis curve, i.e., the relationship between blood pressure and sodium intake (sodium excretion) in each group of mice. Exendin-4 significantly increased the slope of pressure natriuresis curve but had no effect on the *x*-intercept, indicating that exendin-4 attenuated high salt-sensitivity in *db/db* mice (Fig. 2B and C).

Functional expression of GLP-R in the kidney

We confirmed the expression of GLP-1R in various tissues including kidney by a series of RT-PCR assay (Fig. 3A). Exendin-4 is also reported to increase intracellular cAMP levels after its binding to the receptor, which is required for the induction of its physiological actions in several tissues [23]. A single injection of exendin-4 resulted in a significant increase in urinary cAMP excretion (Fig. 3B), suggesting that exendin-4 functionally interacts with its receptor in the kidney of mice and may have any direct effects in the kidney.

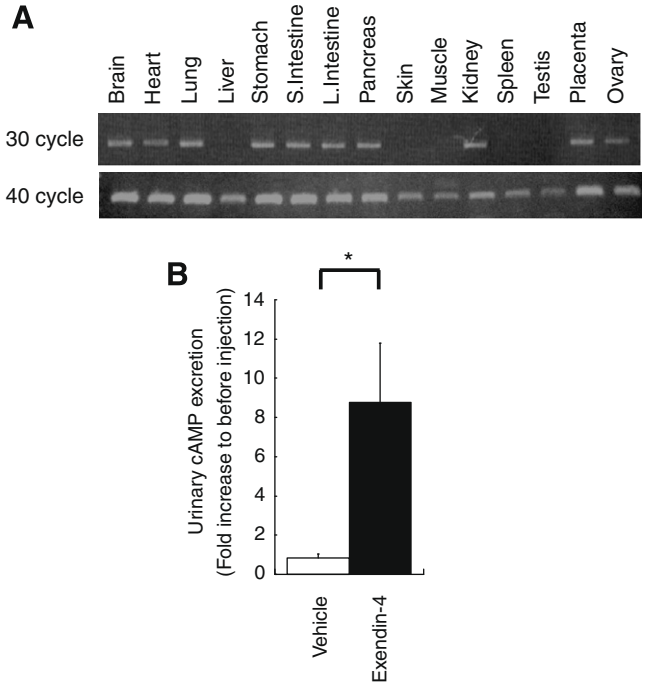

Fig. 3. Functional expression of GLP-1 receptor in the kidney. (A) Expression of GLP-1 receptor in various tissues determined by RT-PCR. (B) Urinary cAMP excretion in response to a single injection of exendin-4 or vehicle. Data were expressed as fold increases of cAMP levels. Data are means ± SEM. $n = 8$ for each groups. $^{*}P < 0.01$.

Effects of exendin-4 on angII-induced hypertension

Next, we confirmed that intra-renal angII levels in *db/db* mice were significantly higher than in *db/m* mice (Fig. 4A). Then, we investigated the anti-hypertensive effect of exendin-4 against angII-induced hypertension. Infusion of angII through implanted osmotic mini-pump in lean and non-diabetic C57BLK6/J mice resulted in a gradual rise in systolic blood pressure and increase in body weight, but these two parameters returned immediately to baseline levels after the removal of the implanted mini-pump (Fig. 4B). Treatment with exendin-4 significantly inhibited these angII-induced increases in systolic blood pressure and body weight (Fig. 4B). Next, we determined the possible mechanism of the above effect of exendin-4 by investigating ang-II-induced phosphorylation of ERK1/2, which is an important mediator of intracellular angII signaling in renal cells. AngII significantly increased the phosphorylation of ERK1/2, whereas exendin-4 significantly inhibited its phosphorylation in a dose–dependent manner (Fig. 4C and D).

Discussion

In this study, we demonstrated that obese type 2 diabetic *db/db* mice show high salt-sensitivity, and that exendin-4 has an anti-hypertensive effect in *db/db* mice and angII-infused mice, which is related to attenuation of high salt-sensitivity. These results indicate the first finding that exendin-4, a GLP-1 analog, has extra-islet effect including the regulation of salt-handling.

Several previous reports have shown that intravenous infusion of GLP-1 enhanced sodium excretion in obese men [11] and inhibited the development of hypertension in Dahl salt-sensitive (Dahl S) rats [12]. However, by now, the effect of exendin-4, aGLP-1 analog, on salt-sensitivity or hypertension remains unclear. Here, we can show that exendin-4 also has anti-hypertensive effect through the attenuation of high salt-sensitivity, although exendin-4 has only 53% amino acid homology to GLP-1 [13]. This finding is first evidence that new anti-diabetic agent, exendin-4, improves high salt-sensitivity and hypertension. Recent reports suggest that high

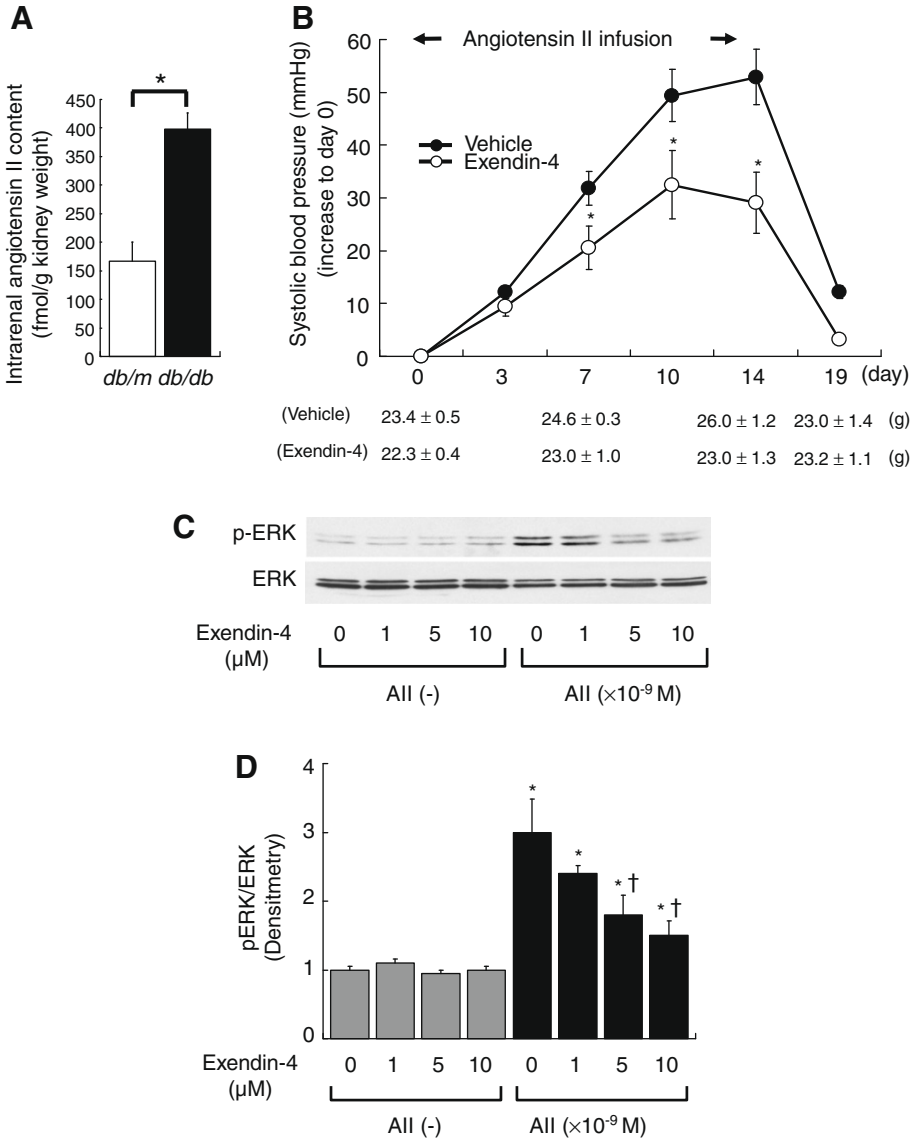

Fig. 4. Intra-renal angiotensin II content and effects of exendin-4 on angiotensin II-induced hypertension. (A) Intra-renal angiotensin II content in the kidney of *db/m* and *db/db* mice. Data are means ± SEM. $n = 4$ for each group. $^{*}P < 0.01$. (B) Systolic blood pressure and body weight (numerical data only) in C57BLK6/J mice after angiotensin II treatment combined with vehicle or exendin-4. Data are means ± SEM. $n = 4$ for each data point. $^{*}P < 0.01$. (C) Representative blots showing exendin-4-related dose–dependent inhibition of angII-induced phosphorylation of ERK. (D) Quantitative results of three independent experiments are shown. Data are means ± SEM; $^{*}P < 0.05$ vs. angII (0 M) + exendin-4 (0 μM), $^{\dagger}P < 0.05$ vs. angII ($\times 10^{-9}$ M) + exendin-4 (0 μM).

salt-sensitivity largely contributes to the development of hypertension and subsequent micro- and macro-vascular diseases [24]. Taken together, these findings suggest that exendin-4 has additional clinical usefulness for the prevention of cardi- or renal-vascular diseases associated with salt-sensitivity beyond its blood glucose lowering effect.

We have previously reported that *db*/*db* mice showed significant increase in blood pressure, although its mechanism has been not elucidated. In this study, we showed first evidence that *db*/*db* mice has high-salt-sensitivity, suggesting that high salt-sensitivity might partially contribute to the increase in blood pressure in *db*/*db* mice. Several metabolic factors such as obesity, hyperglycemia and hyperinsulinemia contribute to the impairment of salt-sensitivity in obese type 2 diabetes [24]. A recent study reported that activation of intra-renal RAS largely contributed to the development of salt-sensitive hypertension in mice treated with angII [16]. In our study, we showed significantly high intra-renal angII contents in *db*/*db* mice. This finding is first evidence showing that enhanced RAS activity exists in the kidney of *db*/*db* mice. AngII-infusion into lean and non-diabetic C57BLK/6J mice increased systolic blood pressure and body weight gain and these increases were immediately reversed after cessation of angII-infusion. These results confirmed the findings of previous studies [16,25], which also indicated that the angII-induced increase in systolic blood pressure was caused by fluid retention via excess sodium reabsorption. Our study demonstrated that GLP-1R was expressed in the kidney as previous report, and that exendin-4 antagonized this angII-induced hypertension and fluid retention. Considered together, these results suggest that exendin-4 can regulate renal salt-handling regardless of metabolic condition and prevent salt-sensitive hypertension. A previous report concluded that anti-hypertensive effect of GLP-1 infusion in Dahl S rats was totally dependent on the attenuation of salt-sensitivity but not insulin resistance. In addition this report, our result showing that exendin-4 inhibited the development of hypertension in angII-infused mice and that single injection of exendin-4 increased urinary cAMP excretion can provide the further evidence that the stimulating GLP-1 signaling in the kidney directly regulates salt-handling in the kidney.

Intra-renal RAS activation is reported in various renal pathological conditions, such as inflammation, apoptosis, oxidative stress, fibrosis and impaired salt-sensitivity [26]. There is ample evidence that the actions of angII in the kidney are mainly mediated by the activation of ERK and are antagonized by increased cellular cAMP levels [27,28]. In this study, injection of exendin-4 in non-diabetic mice increased urinary cAMP excretion and pre-incubation of cultured renal cells with exendin-4 inhibited angII-induced phosphorylation of ERK1/2. Therefore, it is possible that exendin-4 could potentially result in improvement of renal pathology associated with angII activation such as inflammation, fibrosis and hypertension.

Based on our present results indicating the effect of exendin-4 on hypertension in two salt-sensitive mice model, we conclude that exendin-4, a new anti-diabetic agent, has anti-hypertensive properties, which may be associated with improvement of high salt-sensitivity and angII-induced abnormalities. Collectively, our results suggest that exendin-4 is a potentially useful and multipotent therapeutic agent for vascular diseases associated with high salt-sensitivity.

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References

[1] B. Isomaa, P. Almgren, T. Tuomi, B. Forsen, K. Lahti, M. Nissen, M.R. Taskinen, L. Groop, Cardiovascular morbidity and mortality associated with the metabolic syndrome, Diabetes Care 24 (2001) 683–689.
[2] R. Kahn, J. Buse, E. Ferrannini, M. Stern, The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes, Diabetes Care 28 (2005) 2289–2304.
[3] H.A. Koomans, J.C. Roos, P. Boer, G.G. Geyskes, E.J. Mees, Salt sensitivity of blood pressure in chronic renal failure. Evidence for renal control of body fluid distribution in man, Hypertension 4 (1982) 190–197.
[4] A. Morimoto, T. Uzu, T. Fujii, M. Nishimura, S. Kuroda, S. Nakamura, T. Inenaga, G. Kimura, Sodium sensitivity and cardiovascular events in patients with essential hypertension, Lancet 350 (1997) 1734–1737.
[5] T. Uzu, G. Kimura, A. Yamauchi, M. Kanasaki, K. Isshiki, S. Araki, T. Sugiomoto, Y. Nishio, H. Maegawa, D. Koya, M. Haneda, A. Kashiwagi, Enhanced sodium sensitivity and disturbed circadian rhythm of blood pressure in essential hypertension, J. Hypertens. 24 (2006) 1627–1632.
[6] D.J. Drucker, The biology of incretin hormones, Cell Metab. 3 (2006) 153–165.
[7] R. Perfetti, P. Merkel, Glucagon-like peptide-1: a major regulator of pancreatic beta-cell function, Eur. J. Endocrinol. 143 (2000) 717–725.
[8] B.P. Bullock, R.S. Heller, J.F. Habener, Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor, Endocrinology 137 (1996) 2968–2978.
[9] Y. Wei, S. Mojsov, Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences, FEBS Lett. 358 (1995) 219–224.
[10] P. Schlatter, C. Beglinger, J. Drewe, H. Gutmann, Glucagon-like peptide 1 receptor expression in primary porcine proximal tubular cells, Regul. Pept. 141 (2007) 120–128.
[11] J.P. Gutzwiller, S. Tschopp, A. Bock, C.E. Zehnder, A.R. Huber, M. Kreyenbuehl, H. Gutmann, J. Drewe, C. Henzen, B. Goeke, C. Beglinger, Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men, J. Clin. Endocrinol. Metab. 89 (2004) 3055–3061.
[12] M. Yu, C. Moreno, K.M. Hoagland, A. Dahly, K. Ditter, M. Mistry, R.J. Roman, Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats, J. Hypertens. 21 (2003) 1125–1135.
[13] R. Goke, H.C. Fehmann, T. Linn, H. Schmidt, M. Krause, J. Eng, B. Goke, 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 (1993) 19650–19655.
[14] M. Chin, M. Isono, K. Isshiki, S. Araki, T. Sugimoto, B. Guo, H. Sato, M. Haneda, A. Kashiwagi, D. Koya, Estrogen and raloxifene, a selective estrogen receptor modulator, ameliorate renal damage in db/db mice, Am. J. Pathol. 166 (2005) 1629–1636.
[15] K. Shibuya, K. Kanasaki, M. Isono, H. Sato, M. Omata, T. Sugimoto, S. Araki, K. Isshiki, A. Kashiwagi, M. Haneda, D. Koya, *N*-acetyl-seryl-aspartyl-lysyl-proline prevents renal insufficiency and mesangial matrix expansion in diabetic db/db mice, Diabetes 54 (2005) 838–845.
[16] S.D. Crowley, S.B. Gurley, M.I. Oliverio, A.K. Pazmino, R. Griffiths, P.J. Flannery, R.F. Spurney, H.S. Kim, O. Smithies, T.H. Le, T.M. Coffman, Distinct roles for the kidney and systemic tissues in blood pressure regulation by the renin-angiotensin system, J. Clin. Invest. 115 (2005) 1092–1099.
[17] G.F. Strippoli, M. Craig, F.P. Schena, J.C. Craig, Antihypertensive agents for primary prevention of diabetic nephropathy, J. Am. Soc. Nephrol. 16 (2005) 3081–3091.
[18] Y. Ge, A. Bagnall, P.K. Stricklett, K. Strait, D.J. Webb, Y. Kotelevtsev, D.E. Kohan, Collecting duct-specific knockout of the endothelin B receptor causes hypertension and sodium retention, Am. J. Physiol. Renal Physiol. 291 (2006) F1274–F1280.
[19] D.Y. Huang, K.M. Boini, H. Osswald, B. Friedrich, F. Artunc, S. Ullrich, J. Rajamanickam, M. Palmada, P. Wulff, D. Kuhl, V. Vallon, F. Lang, Resistance of mice lacking the serum- and glucocorticoid-inducible kinase SGK1 against salt-sensitive hypertension induced by a high-fat diet, Am. J. Physiol. Renal Physiol. 291 (2006) F1264–F1273.
[20] A. Nishiyama, D.M. Seth, L.G. Navar, Renal interstitial fluid concentrations of angiotensins I and II in anesthetized rats, Hypertension 39 (2002) 129–134.
[21] N. Kawada, E. Imai, A. Karber, W.J. Welch, C.S. Wilcox, A mouse model of angiotensin II slow pressor response: role of oxidative stress, J. Am. Soc. Nephrol. 13 (2002) 2860–2868.
[22] S. Kume, T. Uzu, S. Araki, T. Sugimoto, K. Isshiki, M. Chin-Kanasaki, M. Sakaguchi, N. Kubota, Y. Terauchi, T. Kadowaki, M. Haneda, A. Kashiwagi, D. Koya, Role of altered renal lipid metabolism in the development of renal injury induced by a high-fat diet, J. Am. Soc. Nephrol. 18 (2007) 2715–2723.
[23] S. Park, X. Dong, T.L. Fisher, S. Dunn, A.K. Omer, G. Weir, M.F. White, Exendin-4 uses Irs2 signaling to mediate pancreatic beta cell growth and function, J. Biol. Chem. 281 (2006) 1159–1168.
[24] T. Uzu, M. Nishimura, T. Fujii, M. Sakaguchi, M. Kanasaki, K. Isshiki, S. Araki, T. Sugiomoto, A. Kashiwagi, G. Kimura, Benidipine attenuates glomerular hypertension and reduces albuminuria in patients with metabolic syndrome, Hypertens. Res. 30 (2007) 161–165.
[25] S.D. Crowley, S.B. Gurley, M.J. Herrera, P. Ruiz, R. Griffiths, A.P. Kumar, H.S. Kim, O. Smithies, T.H. Le, T.M. Coffman, Angiotensin II causes hypertension and

cardiac hypertrophy through its receptors in the kidney, Proc. Natl. Acad. Sci. USA 103 (2006) 17985–17990.
[26] Y. Huang, N.A. Noble, J. Zhang, C. Xu, W.A. Border, Renin-stimulated TGF-beta1 expression is regulated by a mitogen-activated protein kinase in mesangial cells, Kidney Int. 72 (2007) 45–52.
[27] E. Giasson, M.J. Servant, S. Meloche, Cyclic AMP-mediated inhibition of angiotensin II-induced protein synthesis is associated with suppression of tyrosine phosphorylation signaling in vascular smooth muscle cells, J. Biol. Chem. 272 (1997) 26879–26886.
[28] X.C. Li, O.A. Carretero, J.L. Zhuo, Cross-talk between angiotensin II and glucagon receptor signaling mediates phosphorylation of mitogen-activated protein kinases ERK 1/2 in rat glomerular mesangial cells, Biochem. Pharmacol. 71 (2006) 1711–1719.