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Stimulatory effect of insulin on renal proximal tubule sodium transport is preserved in type 2 diabetes with nephropathy

Motonobu Nakamura [a], Nobuhiko Satoh [a], Masashi Suzuki [a], Haruki Kume [b], Yukio Homma [b], George Seki [a], Shoko Horita [a, *]

[a] Department of Internal Medicine, Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[b] Department of Urology, Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

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ABSTRACT

Our previous study indicates that hyperinsulinemia in metabolic syndrome in the absence of nephropathy may promote hypertension by stimulating renal proximal tubule (PT) sodium transport via insulin receptor substrate (IRS) 2/phosphoinositide 3-kinase pathway. In the present study we showed that the stimulatory effect of insulin on the Na^+-HCO_3 cotransporter NBCe1 in isolated PTs was completely preserved in type 2 diabetic rats with overt nephropathy. Furthermore, the IRS2 expression and insulin-induced Akt phosphorylation in kidney cortex were preserved in these rats. By contrast, the IRS1 expression in kidney cortex was markedly reduced, which might be relevant to enhanced renal gluconeogenesis consistently reported in diabetes. The stimulatory effect of insulin on NBCe1 was preserved also in a human type 2 diabetic patient with advanced nephropathy. These results revealed that insulin can stimulate PT sodium transport even in type 2 diabetes with overt nephropathy. In addition to hypoglycemia, insulin-induced renal sodium retention might also play a role in increased cardiovascular risk associated with intensive glycemic control in type 2 diabetic patients with nephropathy.

1. Introduction

Diabetes mellitus is the worldwide leading cause of end-stage renal disease. Enhancement of renal proximal tubule (PT) absorption found in early phase of diabetes may promote the initiation of diabetic nephropathy via hypertension and/or glomerular hyperfiltration [1,2]. While hyperglycemia may induce hypertrophy and hyperfunction of PTs via a number of metabolic factors [2], insulin may also contribute to the enhancement of PT sodium absorption [3,4].

Defects at the level of insulin receptor (IR) substrates IRSs may underlie selective insulin resistance often found in metabolic syndrome and type 2 diabetes, because the two major substrates IRS1 and IRS2 mediate distinct insulin signaling [5]. Regarding the insulin signaling in PTs, we have previously shown that the IRS2/phosphoinositide 3-kinase (PI3-K) pathway mediates the stimulatory effect of insulin on PT transport [4]. Our recent study has further revealed that while the IRS1-dependent stimulatory effect of insulin on glucose uptake into abdominal adipocytes is severely attenuated, the IRS2-dependent stimulatory effect of insulin on PT sodium transport is completely preserved in insulin resistant rats and humans without nephropathy [6]. These results suggest that hyperinsulinemia in metabolic syndrome may promote hypertension by stimulating PT sodium transport.

Besides serving as a fundamental element in sodium homeostasis, kidney may also play an important role in systemic glucose homeostasis via gluconeogenesis [7]. Renal gluconeogenesis is limited to PTs and suppressed by insulin at physiological conditions, as evidenced by hyperglycemia found in PT-selective IR deficient mice [8]. Several lines of evidence suggest that the kidney may significantly contribute to hyperglycemia in type 1 and type 2 diabetes, at least partially due to the enhanced gluconeogenesis [7,9,10]. Gatica and colleagues proposed that the reduced expression of IR in PTs might be responsible for the enhancement of renal gluconeogenic activities in type 1 and type 2 diabetes [11]. In contrast to this view of attenuated PT insulin signaling in diabetes, however, Mima and colleagues found that the PT insulin signaling, as estimated by phosphorylation of target proteins such as Akt and GSK, is preserved in type 1 diabetic rats [12]. Trevisan and colleagues also found that the stimulatory

* Corresponding author. Fax: +81 3 5800 8806.
E-mail address: shorita-tky@umin.ac.jp (S. Horita).

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effect of insulin on PT sodium transport is preserved in human patients with type 1 diabetes [13]. While these controversial results suggest that the PT insulin signaling may not be uniformly attenuated in diabetes, a central unanswered question is whether the stimulatory effect of insulin on PT transport is preserved in type 2 diabetes with advanced nephropathy. This issue is quite important for understanding the underlying mechanisms for sodium retention, edema, and hypertension in chronic kidney diseases (CKD) associated with type 2 diabetes, the complicated clinical situation that often requires intensive insulin treatment for tight glycemic control [14]. To clarify this issue, we examined the effects of insulin on Na^+–HCO_3^- cotransporter NBCe1 in isolated PTs from rats and humans with overt nephropathy due to type 2 diabetes. NBCe1, a major basolateral exit pathway for sodium and bicarbonate from PTs, is known to be activated by physiological concentrations of insulin [6].

2. Material and methods

2.1. Animal samples

All animal procedures were in accordance with local institutional guidelines. Male Otsuka Long–Evans Tokushima Fatty (OLETF) rats and Long–Evans Tokushima Otsuka (LETO) rats were supplied by Sankyo Labo Service Corporation. Urine was collected using metabolic cages at 52–54 weeks of age, and urinary protein and creatinine concentrations were determined by the SRL clinical service. Blood was obtained from tail veins after starvation for 12–14 h, and plasma insulin concentrations were determine by a rat insulin ELISA kit (Shibayagi). Kidneys were obtained after the rats were sacrificed by excessive amounts of pentobarbital.

2.2. Human samples

For human PTs and adipocytes, kidney cortex tissues and perirenal fat tissues were obtained during the unilateral nephrectomy for renal carcinoma. The study was approved by the institutional review board of the University of Tokyo School of Medicine and written informed consent was obtained from all the subjects as described [6,15].

2.3. Histological analysis

Formalin-fixed paraffin-embedded renal sections were observed with the periodic acid-Schiff reagent (PAS) or hematoxylin–eosin (HE) staining. For evaluation of rat glomerular damages, the degree of mesangial expansion was graded from 0 to 4 according to the method previously described [16]. Thirty glomeruli were analyzed for each kidney.

2.4. Measurement of NBCe1 activity in isolated PTs

The manually microdissected PT (S2 segment) fragment was transferred to a perfusion chamber mounted on an inverted microscopy, and incubated with acetoxymethylester form of a pH sensitive fluorescence dye 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF/AM; Dojindo) for cell pH measurement with a photometry system (OSP-10; Olympus). Before and 8 min after insulin addition to bath perfusate, NBCe1 activity was determined by the rates of cell pH decrease in response to reduction in bath HCO_3^- concentrations from 25 mM to 12.5 mM as described [6,15].

Table 1
Profiles of LETO and OLETF rats.

	LETO	OLETF	p-Value
Number	4	4	
Fasting plasma glucose (mg/dl)	126 ± 10	222 ± 28	<0.05
Fasting insulin level (ng/ml)	6.7 ± 0.2	4.8 ± 0.9	0.09
Body weight (g)	610 ± 10	570 ± 70	0.56
Urinary protein (mg/mg creatinine)	1.5 ± 0.1	33.8 ± 3.9	<0.001

2.5. Measurement of glucose uptake into isolated human adipocytes

Adipocytes, isolated from perirenal fat tissues by using collagenase digestion, were incubated with glucose-free HEPES-buffer solution containing a fluorescent D-glucose derivative 2-[N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG; Wako Pure Chemical Industries). After washing with glucose-containing HEPES buffer solution, fluorescence retained in the adipocytes was measured with a fluorescence microplate reader as described [6].

2.6. Immunoblotting and immunoprecipitation in rat kidney cortex

Immunoblotting and immunoprecipitation in rat kidney cortex were performed as described [6,15,17]. In brief, thin slices of kidney cortex, divided into pieces of small bundles, were used for detection of insulin-induced Akt phosphorylation. Anti-Akt or anti–phospho-Akt (Ser473) antibodies were from Cell Signaling Technology. For detection of IRS1 and IRS2, kidney cortex tissues were homogenized and subjected to immunoprecipitation using anti-IRS1 or anti-IRS2 antibodies (Cell Signaling Technology) and Protein G–Sepharose (GE Healthcare Bio-Science). An aliquot of tissue lysates was used for immunoblotting with anti-IR (Millipore) or anti-β-actin antibodies (Cell Signaling Technology).

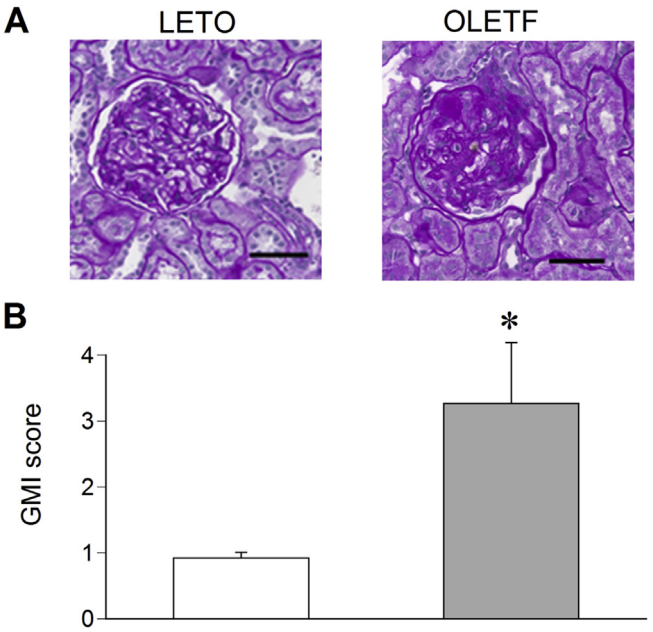

Fig. 1. Diabetic glomerular changes in OLETF rats. (A) Representative PAS staining images of renal sections from OLETF and LETO rats. Bars indicate 50 μm. (B) Glomerular injury (GMI) score was estimated by semiquantitative analysis of mesangial expansion. Open and closed bars represent LETO and OLETF data, respectively. *P < 0.01 vs LETO.

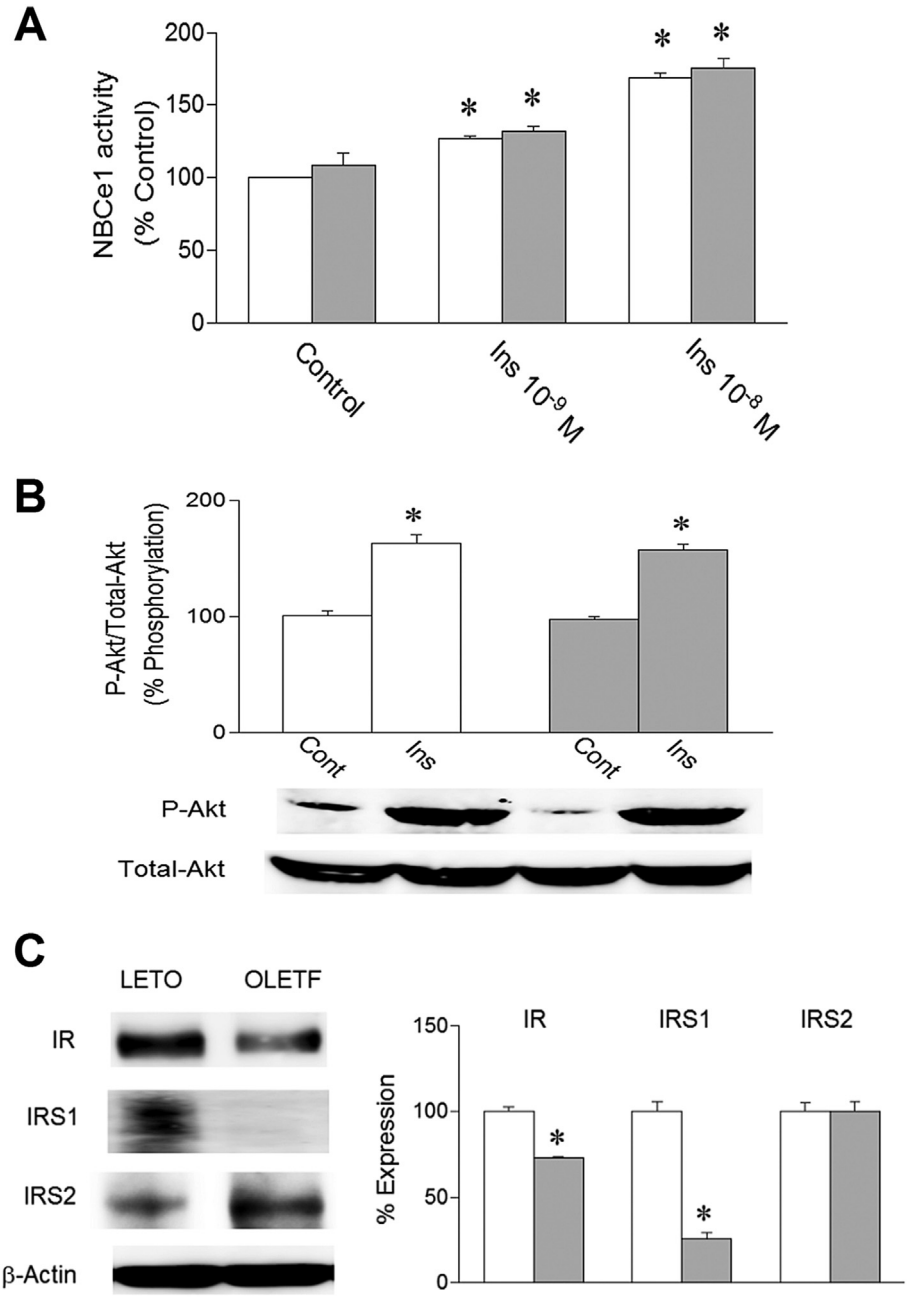

Fig. 2. Renal effects of insulin in LETO and OLETF rats. (A) Effects of insulin on NBCe1 activities. Open and closed bars represent LETO and OLETF data, respectively. n = 4–12 for each. *P < 0.01 vs Basal data (Control). (B) Insulin-induced Akt phosphorylation in kidney cortex samples. Open and closed bars represent LETO and OLETF data, respectively. Intensity data for phosphorylated Akt (P-Akt)/Total Akt (T-Akt) were quantified by densitometry. n = 4 for each. *P < 0.01 vs. Control. (C) Expression of IR, IRS1, and IRS2 proteins in renal cortex. Open and closed bars represent LETO and OLETF data, respectively. Intensity data for IR, IRS1, or IRS2/β-actin were quantified by densitometry. n = 4 for each. *P < 0.01 vs. LETO.

2.7. Statistical analysis

The data were represented as mean ± SEM. Significant differences were determined by applying Student's t test or ANOVA with Bonferroni's correction, as appropriate. Statistical significance was set at $p < 0.05$.

3. Results and discussion

Unlike control LETO rats, hyperphagic OLETF rats are known to display obesity, hyperinsulinemia, and insulin resistance at ages around 20 weeks, but subsequently develop overt type 2 diabetes with proteinuria at ages around 30–40 weeks [6,16,18,19]. Therefore, OLETF rats are considered to be a good model for human type 2 diabetes with nephropathy. Consistent with this view, OLETF rats at ages 52–54 weeks no longer displayed obesity or hyperinsulinemia, but did show frank fasting hyperglycemia and massive proteinuria (Table 1). As previously reported [16,18], renal histological analysis revealed typical diabetic glomerular damages as represented by extensive mesangial expansion in OLETF rats (Fig. 1).

We compared the effects of insulin on NBCe1 in OLETF and LETO rats. The basal NBCe1 activities were comparable in these rats. Moreover, the stimulatory effects of physiological concentrations of

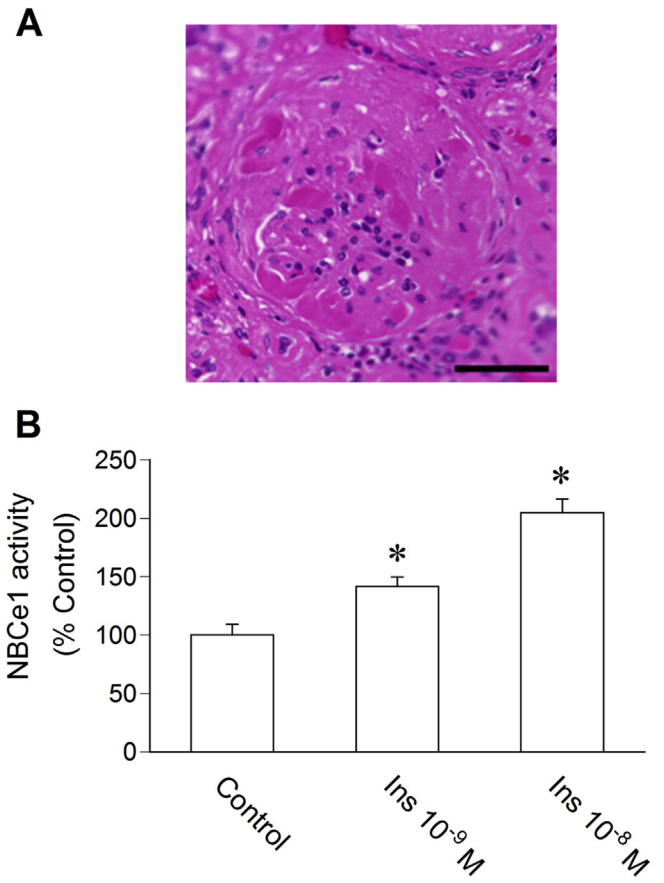

Fig. 3. Effects of insulin in a human type 2 diabetic patient with nephropathy. (A) A representative HE staining image of renal sections. Bar indicates 50 μm. (B) Effects of insulin on NBCe1 activities. n = 4 for each. **P < 0.01 vs. Control.

insulin on NBCe1 were indistinguishable in these rats (Fig. 2A). These results indicate that the stimulatory effect of insulin of PT sodium transport is preserved in type 2 diabetic rats with nephropathy. Insulin-induced Akt phosphorylation in kidney cortex samples was also comparable in OLETF and LETO rats (Fig. 2B).

While our data support the preserved PT sensitivity to insulin in type 2 diabetes, several data suggest the impaired PT insulin signaling in diabetes [7]. In particular, the attenuated expression of IR in PTs was proposed to be at least partially responsible for the enhanced renal gluconeogenesis in diabetes [11]. We therefore examined the expression of IR, IRS1, and IRS2 in rat kidney cortex tissues. The expression of IR was indeed moderately reduced, but the expression of IRS1 was more severely reduced in OLETF rats. By contrast, the expression of IRS2 was completely preserved in OLETF rats (Fig. 2C). These results suggest that the residual expression of IR in diabetes might be sufficient for mediating the IRS2-dependent signaling in PTs.

We next examined the effects of insulin in a human patient with type 2 diabetic nephropathy. The patient was a 59 year-old male, who was diagnosed as having type 2 diabetes more than 20 years ago. His glycemic control was poor as reflected by high hemoglobin A1c values that often exceeded 10%, and treatment with insulin was started 4 years ago. His renal function was slowly decreased, and his estimated glomerular filtration rate was 39.2 ml/min per 1.73 m^2 at nephrectomy for renal carcinoma. He had massive proteinuria (2.5 g/g creatinine), and extensive nodular and global glomerulosclerosis confirmed the presence of advanced diabetic nephropathy (Fig. 3A). The basal NBCe1 activity, as estimated by the rates of cell pH decrease in response to half reduction of bath HCO_3^- concentrations, was 0.55 ± 0.05 pH unit/min (n = 4). This value was comparable to the mean value obtained from 11 control patients who underwent nephrectomy but did not have insulin resistance, diabetes, or renal diseases (0.60 ± 0.05 pH unit/min). More importantly, insulin markedly stimulated NBCe1 in this diabetic patient (Fig. 3B), and the degree of NBCe1 stimulation was comparable to that found in the control patients [6]. We also examined the effect of insulin on glucose uptake into perirenal adipocytes in this patient. The basal glucose uptake was significantly reduced to the value corresponding to 29 ± 7% (n = 3) of that in the control patients. Moreover, the glucose uptake stimulated by 10^{-8} M insulin was also markedly reduced to the value corresponding to 24 ± 7% (n = 3) of that in the control patients. These values were very similar to those found in 5 patients with insulin resistance [6], indicating that insulin signaling in adipocytes was significantly blunted in this patient.

We have previously found that the IRS2-dependent stimulation of PT transport by insulin is preserved in insulin resistance in the absence of nephropathy [6]. The present study now showed that the stimulatory effect of insulin on PT transport is preserved even in type 2 diabetes with overt nephropathy. Our previous study revealed that the regulatory mechanisms of IRS2 expression are quite distinct in liver and kidney [6]. In the normoglycemic environment, bone morphogenetic protein 7 may enhance the IRS2 expression in PTs [20]. Nevertheless, the exact mechanism by which the IRS2 expression in PTs is exceptionally preserved in insulin resistance and type 2 diabetes remains to be clarified. By contrast, the IRS1 expression in kidney cortex was severely reduced in diabetic OLETF rats. Given the augmented renal gluconeogenesis in diabetes [7,9,10], it is tempting to speculate that the insulin-mediated inhibition of renal gluconeogenesis is dependent on IRS1 but not IRS2. Future studies are required to confirm whether this hypothesis can explain the diabetes-related, seemingly selective insulin resistance in PTs.

Our present findings may have important implications for understanding risks and benefits of intensive glucose lowering in type 2 diabetic patients. Thus, intensive glycemic control failed to show clear benefits on cardiovascular events and/or mortality rates in several large clinical trials [21–23]. Tight glycemic control in CKD patients with type 2 diabetes, which often required higher doses of insulin than conventional glycemic control, was reported to even increase cardiovascular risk [14]. Hypoglycemia inevitably associated with intensive glucose lowering may have a strong influence on overall mortality [14,24]. Because PT transport has significant impacts on whole-body sodium homeostasis and blood pressure regulation [15,25,26], however, we should also take into account the risk of renal sodium retention when higher doses of insulin were to be used to achieve tight glycemic control.

Conflict of interest

The authors have no conflict of interest to disclose.

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