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

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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

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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).

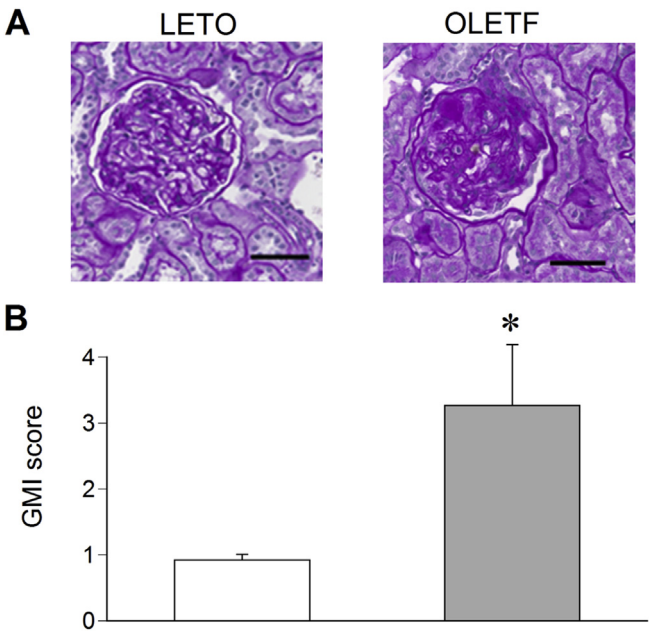

Fig. 1. Diabetic glomerular changes in OLETF rats. (A) Representative PAS staining images of renal sections from OLETF and LETO rats. Bars indicate 50 μm. (B) Glomerular injury (GMI) score was estimated by semiquantitative analysis of mesangial expansion. Open and closed bars represent LETO and OLETF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

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