Regulation of renal tubular glucose reabsorption by Akt2/PKB

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1Department of Physiology, University of Tübingen, Tübingen, Germany; 2Department of Physiology and Medical Physics, Medical University of Innsbruck, Innsbruck, Austria; 3Division of Endocrinology, Diabetes and Metabolism, University of Pennsylvania, Philadelphia, Pennsylvania; and 4Department of Medicine (Nephrology), University of California, San Francisco, California

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Kempe DS, Siraskar G, Fröhlich H, Umbach AT, Stübs M, Weiss F, Ackermann TF, Vökl H, Birnbaum MJ, Pearce D, Föller M, Lang F. Regulation of renal tubular glucose reabsorption by Akt2/PKBβ. Am J Physiol Renal Physiol 298:F1113–F1117, 2010. First published February 17, 2010; doi:10.1152/ajprenal.00592.2009.—Akt/PKB is known to regulate the facilitative glucose carrier GLUT4. Nothing is known, however, of the role of Akt/PKB in the regulation of renal epithelial transport. To explore whether Akt/PKB influences the Na+-coupled glucose cotransporter SGLT1, human SGLT1 was expressed in Xenopus laevis oocytes with or without Akt/PKB, and electrolyte glucose transport was determined by dual-electrode voltage clamp. The expression of Akt/PKB in SGLT1-expressing oocytes was followed by an increase in glucose-induced currents. To study the functional significance of Akt/PKB-sensitive renal glucose transport, further experiments were performed in gene-targeted mice lacking functional Akt2/PKBβ (akt2−/−) and in their wild-type littermates (akt2+/+). Plasma glucose concentration was significantly higher in akt2−/− mice than in akt2+/+ mice but was virtually identical to the plasma glucose concentration in fructose-treated akt2+/+ mice. Urinary glucose excretion was significantly higher in akt2−/− mice compared with akt2+/+ mice with or without fructose treatment. Moreover, the glucose-induced depolarization of proximal tubular cells was significantly smaller in isolated, perfused renal tubules from akt2−/− mice than in those from akt2+/+ mice. In conclusion, Akt2/PKBβ plays a role in the regulation of renal glucose transport.

SGLT1; insulin; glucosuria; hyperglycemia

Akt/PKB IS WELL KNOWN to regulate the cellular transport of glucose (12, 15, 34), amino acids (9, 13, 23), Ca2+ (17), H+ (31), Na+ (21), and K+ (24). Akt/PKB participates in the regulation of several renal functions and contributes to a variety of pathophysiological conditions, such as diabetic nephropathy (25, 37), glomerulonephritis (6), vasculitis (35), mesenchymal cell proliferation (32), podocyte cytoskeletal architecture (22), polycystic kidney disease (26), transcription factors (5), and epithelial-to-mesenchymal transition (38). Surprisingly, little is known about a role of Akt/PKB in the regulation of renal tubular transport.

The present study addressed the putative role of Akt/PKB in the regulation of the Na+-linked glucose transporter SGLT1, the major carrier accomplishing renal tubular reabsorption of glucose (36). To this end, in vitro regulation of SGLT1 by Akt/PKB was studied in the Xenopus laevis oocyte expression system. Furthermore, the in vivo significance of Akt/PKB-sensitive regulation of SGLT1 was determined by studying gene-targeted mice lacking functional Akt2/PKBβ (akt2−/−) as well as their wild-type littermates (akt2+/+) (10, 11).

METHODS

In vitro expression. For generation of cRNA, constructs were used encoding wild-type SGLT1 (12) and constitutively active T308D,S473DAkt/PKB (2). The cRNA was generated as described previously (23, 27). Akt/PKB cDNA was kindly provided by Sir Philip Cohen and the cDNA encoding SGLT1 by Hermann Koepsell. For electrophysiology, X. laevis oocytes were prepared as previously described (7, 33). Five nanograms of cRNA encoding SGLT1 were injected on the day of preparation and 7 pmol mRNA encoding constitutively active T308D,S473DAkt/PKB on the consecutive day following preparation of X. laevis oocytes. All recordings were performed at room temperature 3–4 days after the second injection. Two-electrode voltage-clamp recordings (8) were done at a holding potential of −70 mV. The data were filtered at 10 Hz and recorded with a GeneClamp 500 amplifier, a DigiData 1300 A/D-D/A converter, and the pClamp 9.0 software package for data acquisition and analysis (Axon Instruments). The control superfusate (ND-96) contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM HEPES, pH 7.4. Glucose was added to the solutions at the indicated concentrations. The final solutions were titrated to pH 7.4 using NaOH. The flow rate of the superfusion was 20 ml/min, and a complete exchange of the bath solution was reached within 10 s.

Data are provided as means ± SE, and n represents the number of oocytes investigated. All experiments were repeated with at least three batches of oocytes; in all repetitions, qualitatively similar data were obtained.

In vivo function. All animal experiments were conducted according to the guidelines of the American Physiological Society as well as the German law for the welfare of animals and were approved by local authorities.

The gene-targeted mice lacking functional Akt2/PKBβ (akt2−/−) as well as their wild-type littermates (akt2+/+) were described previously (10, 11). The mice (44 females, aged 5–8 mo) were fed a control diet (C1314, Altromin, Lage, Germany) for 24-h urine collection as described previously (10, 11). The mice had free access to tap drinking water.

For evaluation of renal excretion, both, akt2−/− and akt2+/+ mice were placed individually in metabolic cages (Techniplast, Hohenpeissenberg, Germany) for 24-h urine collection as described previously (28). They were allowed a 3-day habituation period, during which food and water intake, urinary flow rate, urinary excretion of glucose, and body weight were recorded every day to ascertain that the mice were adapting to the new environment. Subsequently, a 24-h collection of urine was performed for 3 consecutive days to obtain urinary parameters. To ensure quantitative urine collection, the metabolic cages were siliconized and urine was collected under water-saturated oil. As dietary fructose induces hyperglycemia, a subset of akt2+/+ mice was treated with 10% fructose in the drinking water for 3 wk.
transport is expected to depolarize the basolateral cell membrane. The magnitude of the depolarization depends on the magnitude of the induced current on the one hand and on the resistances of cell membranes and shunt on the other.

Statistical analysis. Data are provided as means ± SE, and n represents the number of independent experiments. All data were tested for significance using Student’s t-test or ANOVA with Tukey’s test as a post hoc test, as appropriate. Only results with P < 0.05 were considered statistically significant.

RESULTS

To explore whether Akt/PKB influences the Na⁺-coupled glucose carrier SGLT1, mRNA encoding the carrier was expressed in X. laevis oocytes with or without mRNA encoding constitutively active Akt/PKB. When X. laevis oocytes were injected with RNA-free water, glucose (10 mM) did not induce an appreciable current, indicating that X. laevis oocytes do not express significant levels of electrolytic glucose carriers (Fig. 1). Moreover, injection of mRNA encoding constitutively active T308D,S473DPKB did not result in an appreciable glucose-induced current (Fig. 1). In oocytes injected with cRNA encoding SGLT1, the addition of glucose (10 mM) to the bath solution induced an inward current (Iₒ), which was significantly increased by the additional expression of constitutively active T308D,S473DPKB. The observations demonstrate that T308D,S473DPKB stimulates SGLT1.

Additional experiments were performed to determine whether Akt2/PKBβ significantly contributes to the in vivo regulation of renal tubular glucose transport. To this end, experiments were performed in gene-targeted mice lacking functional Akt2/PKBβ (akt2−/−) and their wild type littermates (akt2+/+). The plasma glucose concentration under non-fasting conditions was significantly higher in akt2−/− mice (133.2 ± 5.0 mg/dl, n = 18) than in akt2+/+ mice (117.0 ± 2.3 mg/dl, n = 19). Following injection of glucose, the plasma glucose concentration increased to significantly higher values and declined more slowly in akt2−/− mice than in akt2+/+ mice (Fig. 2). Following treatment of akt2+/+ mice with fructose (10% added to the drinking water), however, the plasma glucose concentration increased to similarly high values (134.0 ± 4.5 mg/dl, n = 15) as those of akt2−/− mice.

The insulin plasma concentration was significantly higher in akt2−/− mice (5.0 ± 2.2 ng/ml, n = 15) than in akt2+/+ mice.

**Fig. 1.** Coexpression of Akt/PKB stimulates electrogenic glucose transport in SGLT1-expressing X. laevis oocytes. Representative original tracings (top) and arithmetic means ± SE (n = 7–11) of glucose (10 mM)-induced currents (Iₒ) in X. laevis oocytes injected with water without (H₂O) or with SGLT1 (SGLT1) without or with additional coexpression of constitutively active T308D,S473DPKB (PKB) are shown. ***Statistically significant difference (P < 0.001) from absence of SGLT1. #Significant difference (P < 0.05) from absence of PKB.

**Fig. 2.** Plasma glucose concentrations following intraperitoneal glucose injection into akt2−/− and akt2+/+ mice. Arithmetic means ± SE of plasma glucose concentrations following intraperitoneal injection of glucose (3 g/kg body wt) into akt2−/− (n = 7) and akt2+/+ mice (n = 6) are shown. *Significant difference (P < 0.05) between akt2−/− and akt2+/+ mice.
(2.5 ± 0.7 ng/ml, n = 8) and in fructose-treated akt2/+ mice (3.5 ± 1.9 ng/ml, n = 5).

Urinary flow rate was significantly larger in akt2−/− mice (1.7 ± 0.2 ml/day, n = 18) and in fructose-treated akt2/+ mice (2.2 ± 0.3 ml/day, n = 15) than in untreated akt2+/- mice (0.5 ± 0.1 ml/day, n = 11). The creatinine clearance was not different between the genotypes (akt2+/- mice: 11.9 ± 2.4 µl·min⁻¹·g body wt, n = 7; akt2−/−: 12.2 ± 2.4 µl·min⁻¹·g body wt, n = 8). Urinary creatinine excretion was again similar in akt2−/− mice (0.44 ± 0.03 mg/day, n = 18), in untreated akt2+/- mice (0.32 ± 0.04 mg/day, n = 11), and in fructose-treated akt2+/- mice (0.48 ± 0.05 mg/day, n = 15).

As shown in Fig. 3, urinary glucose excretion was significantly larger in akt2−/− mice (n = 18) than in untreated akt2+/- mice (n = 11) or in fructose-treated akt2+/- mice (n = 15). The difference between the genotypes was still significantly higher after correction for the urinary excretion of creatinine (Fig. 3). Blotting the urinary glucose excretion vs. the plasma glucose concentration confirmed that the difference in urinary glucose excretion was not due to the differences in plasma glucose concentration (Fig. 4). The calculation of linear regressions yielded significantly different slopes and thus confirmed the significant difference between akt2−/− and akt2+/- mice.

To determine whether the enhanced renal glucose excretion is secondary to decreased electrogenic glucose transport in proximal renal tubules, the basolateral cell membrane potential was determined in isolated, perfused tubules in the absence and presence of glucose in the luminal perfusate. As a result, in the absence of luminal glucose, the basolateral cell membrane potential was similar in akt2−/− mice (−62.6 ± 1.5 mV, 10 tubules from 6 animals) and in akt2+/- mice (−61.1 ± 2.0 mV, 8 tubules from 5 animals). The application of 20 mM glucose to the luminal perfusate was followed by a significantly more pronounced depolarization in akt2+/- mice than in akt2−/− mice (Fig. 5).
DISCUSSION

The present study reveals a role of Akt2/PKBβ in the regulation of renal tubular glucose transport. Similar to what was observed earlier (12), the coexpression of constitutively active T308D,S473D-PKB was followed by a marked increase in SGLT1 activity. Along those lines, the glucose-induced depolarization was significantly smaller and the renal excretion of glucose was significantly larger in Akt2/PKBβ knockout mice (akt2−/−) than in their wild-type littermates (akt2+/+).

In X. laevis oocytes, the injection of Akt/PKB alone did not trigger a glucose-induced current, indicating that the increased current in SGLT1-expressing X. laevis oocytes following additional expression of Akt/PKB was not due to the upregulation of an endogenous electrogenic glucose carrier but due to upregulation of SGLT1.

The observations in X. laevis oocytes were confirmed by the glucosuria in mice deficient in Akt2/PKBβ. As described earlier (10, 11), the plasma glucose concentration in nonfasted animals was significantly higher in akt2−/− mice than in akt2+/+ mice. Since plasma insulin concentrations were significantly higher in akt2−/− mice than in akt2+/+ mice, the hyperglycemia was apparently due to insulin resistance and not the result of impaired insulin release.

At least in theory, the glucosuria could have resulted from the slight but significant increase in plasma glucose concentration. Thus additional experiments were performed to elucidate whether the increased plasma concentration could have accounted for the glucosuria of akt2−/− mice. To this end, an advantage was taken from the effect of dietary fructose, which is known to generate hyperglycemia (14). As illustrated in Figs. 3 and 4, the hyperglycemia in fructose-treated mice was not followed by glucosuria in akt2−/− mice compared to that of akt2−/− mice. Thus it is safe to conclude that decreased renal tubular transport rather than increased filtered load accounted for the glucosuria in akt2−/− mice. It should be kept in mind, however, that the glucosuria could at least in part have been due to decreased activity of SGLT2 in akt2−/− mice. It is feasible that SGLT2 is similarly sensitive to Akt2 as SGLT1. An influence of Akt/PKB on SGLT2 activity, however, has not been tested yet.

The ability of Akt/PKB to stimulate SGLT1 is shared by the serum- and glucocorticoid-inducible kinase SGK1 (12). However, under physiological conditions, SGK1 does not contribute to the regulation of renal tubular glucose transport, as SGK1 is usually not expressed in the proximal nephron (18). In diabetic nephropathy, however, the hyperglycemia stimulates the renal tubular expression of SGK1 (19), which in turn stimulates renal tubular glucose transport, thus blunting glucosuria (1). In contrast to SGK1, Akt2/PKBβ contributes to the regulation of renal tubular glucose transport under physiological conditions.

Both SGK1 and Akt2/PKBβ are stimulated by the phosphoinositide-dependent kinase PDK1 (3, 16). Complete lack of PDK1 is not compatible with survival (20). However, hypomorphic mice expressing only some 20% of normal PDK1 levels (20) suffer from a subtle but significant decrease in intestinal glucose absorption and renal tubular glucose reabsorption (4). According to the present observations, PDK1 influences epithelial glucose transport at least in part by activating Akt2/PKBβ.

In conclusion, Akt2/PKBβ stimulates renal tubular glucose transport and thus participates in the regulation of SGLT1.

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GRANTS

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES

Akt2/PKBβ REGULATES RENAL GLUCOSE TRANSPORT


