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Changes in Sodium or Glucose Filtration Rate Modulate Expression of Glucose Transporters in Renal Proximal Tubular Cells of Rat

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Abstract. Renal glucose reabsorption is mediated by luminal sodium-glucose cotransporters (SGLTs) and basolateral facilitative glucose transporters (GLUTs). The modulators of these transporters are not known, and their substrates glucose and Na^+ are potential candidates. In this study we examined the role of glucose and Na^+ filtration rate on gene expression of glucose transporters in renal proximal tubule. SGLT1, SGLT2, GLUT1 and GLUT2 mRNAs were assessed by Northern blotting; and GLUT1 and GLUT2 proteins were assessed by Western blotting. Renal cortex and medulla samples from control rats (C), diabetic rats (D) with glycosuria, and insulin-resistant 15-month old rats (I) without glycosuria; and from normal (NS), low (LS), and high (HS) Na^+-diet fed rats were studied. Compared to C and I rats, D rats increased ($P < 0.05$) gene expression of SGLT2 by ~36%, SGLT1 by ~20%, and GLUT2 by ~100%, and reduced ($P < 0.05$) gene expression of GLUT1 by more than 50%. Compared to NS rats, HS rats increased ($P < 0.05$) SGLT2, GLUT2, and GLUT1 expression by ~100%, with no change in SGLT1 mRNA expression, and LS rats increased ($P < 0.05$) GLUT1 gene expression by ~150%, with no changes in other transporters. In summary, the results showed that changes in glucose or Na^+ filtrated rate modulate the glucose transporters gene expression in epithelial cells of the renal proximal tubule.

Key words: SGLT2 — SGLT1 — GLUT2 — GLUT1 — Rat kidney

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Introduction

Recently, the relationship between glucose homeostasis and sodium balance in the organism has been the object of several studies, which intend to contribute to the knowledge of morbid disorders such as obesity, diabetes, hypertension, and aging among others [4]. Under these conditions, insulin resistance with hyperinsulinemia seems to be the common factor that may be responsible for increased sodium reabsorption in the kidney [15]. A molecular factor, which may be involved in this relationship, are the sodium-glucose cotransporters (SGLTs) and/or the facilitative glucose transporters (GLUTs) in the renal proximal tubular cells, which may have their gene expression modulated by several factors [9, 10, 11, 20]. The SGLT protein carries the Na^+ down its electrochemical gradient, and glucose against its concentration gradient in the apical membrane of some epithelial cells. Two main isoforms of SGLTs were described: SGLT1 and SGLT2 [9, 10, 11]. They have different tissue distribution as well as kinetic and electrochemical characteristics. The low-affinity and high-capacity glucose transporter SGLT2 is present in the S1 and S2 segments of the proximal tubule, and has a Na^+-to-glucose coupling ratio of 1:1. The high-affinity and low-capacity SGLT1 is present in the S3 segment, and has a Na^+-to-glucose coupling ratio of 2:1 [9, 10, 20]. In the cytoplasm of tubular epithelial cells, glucose in high concentration flows to the peritubular fluid by the basolateral membrane through the facilitative transporters GLUT2 in the S1 and S2 segments, and GLUT1 in the S3 segment [2, 11]. In hyperglycemic diabetic rats, modulation of GLUT1 and GLUT2 has already been reported; however, no changes in SGLT have been described [5].

When glomerular filtration is increased, such as in the extracellular volume expansion, changes in the glucose transporters' gene expression may be achieved to maintain the glucose and Na^+ reabsorption preserved. This regulation may have important physiological and physiopathological consequences. The increased intracellular flux of glucose may stimulate the gene expression of several proteins and growth factors, which have been described as involved in the appearance of some pathological renal processes such as interstitial fibrosis and glomerulosclerosis [3, 14, 23]. In the present study, the glucose transporters SGLTs, GLUT1 and GLUT2 were investigated in kidney from rats submitted to diets with different Na^+ contents, and also in rats in a different glucose homeostasis state, to verify the possible role of filtration rate of glucose and Na^+ on these glucose transporters. The SGLTs and GLUTs mRNAs were assessed by Northern blotting, and the GLUT1 and GLUT2 proteins were assessed by Western blotting. The studies were performed both in cortex and medulla samples, which have been confirmed to be enriched in S1 and S3 proximal tubule segments, respectively.

Material and Methods

Animals

Male Wistar rats were housed under controlled conditions of lighting (lights on from 6:00 AM to 6:00 PM) and temperature (23 ± 1°C). Two protocols of study were conducted, each one with three groups of animals. Protocol 1: 3-month-old control rats (C); 3-month-old diabetic rats (D)—the animals received a single injection of alloxan (40 mg/kg body weight, IV) fifteen days before sacrifice; 15-month-old rats (I)—the animals were spontaneously obese, and developed impaired tolerance to a glucose load as a consequence of insulin resistance [12, 16]. Five days after alloxan injection the rats started to be evaluated every two days, and as diabetic rats were chosen animals that showed polyuria (>25 ml/day), glycosuria > ++, but without ketonuria, significant weight loss, and increased respiratory rate, during a 10-day period. Protocol 2: The rats were fed, from weaning, a normal (NS, 0.5% Na^+), low (LS, 0.06% Na^+), or a high (HS, 3.12% Na^+) salt diet (Harlan Teklad, Madison, WI). The experimental protocol was approved by the Animal Ethics Committee of the Institute of Biomedical Sciences, University of São Paulo, protocol number 084/98.

Renal Sampling

On the day of the experiment, kidneys were removed under sodium pentobarbital anesthesia (50 mg/kg body weight, IP). The abdominal aorta was isolated and catheterized below the renal arteries immediately after clamping the aorta up to the renal arteries and cutting the inferior vena cava up to the renal veins. A blood sample was collected; the kidneys were perfused with Hanks buffer, and immediately removed. Renal cortex and medulla were dissected under ocular control; the tissue fragments of each area were weighed and frozen in liquid nitrogen for further procedures.

Antibodies and Probes

A 15-amino-acid peptide of rat GLUT1, synthesized according to the deduced carboxy-terminal sequence of rat GLUT1, was coupled to keyhole limpet hemocyanin (KLH) and used for immunization of male New Zealand white rabbits. This anti-serum was successfully used for immunoblotting [13]. A 10-amino-acid peptide of rat GLUT2, kindly provided by Dr. M. Saito from the Faculty of Veterinary Medicine, Hokkaido University, Japan, was also coupled to KLH and used for immunization of male New Zealand white rabbits. This antibody was previously checked for immunoblotting suitability in different rat tissues such as skeletal muscle, gut, pancreatic islets, and renal cortex and medulla, evincing immunoreactivity only in tissues which express this transporter isoform [19]. A cDNA probe specific for rat SGLT1, generously provided by Dr. M. Kasahara from the School of Medicine, Teikyo University, Japan, was used for hybridization to SGLT1 in medulla and to SGLT2 in cortex samples, followed by washing at high and low stringency conditions, respectively. Rat GLUT1 and human β-Actin cDNAs were kindly provided by Dr. M. Morimatsu, Faculty of Veterinary Medicine, Hokkaido University, Japan; and human GLUT2 cDNA was kindly provided by Dr. S. Seino, Faculty of Medicine, Chiba University, Japan.

Western Blotting

Tissue samples were homogenized in 10 w/v buffer (in 10 mM Tris-HCl, 1 EDTA, 250 sucrose, pH 7.4, and 5 μg/ml aprotinin), using a Polytron set at 24,000 rpm for 30 sec, and centrifuged at 3,000 × g for 15 min. The supernatant was centrifuged at 12,000 × g for 20 min, and the pellet was resuspended as a plasma membrane fraction (PM), in which the alkaline phosphatase activity has been shown to have increased more than three times compared to the enzyme activity in the supernatant of the first centrifugation. The Western blotting analysis was performed as previously described [13]. Briefly, membrane protein (100 μg from medulla and 150 μg from cortex samples) was solubilized in Laemmli's sample buffer subjected to SDS-PAGE (10%) and electrophoretically transferred to nitrocellulose paper. After blocking with nonfat-milk, the sheets were incubated with the anti-GLUT1 (medulla) or anti-GLUT2 (cortex) antibodies, followed by washing and incubation with [^{125}I]protein-A (Amersham Pharmacia Biotech, Amersham, UK). After the final wash, the nitrocellulose sheets were dried at room temperature, and exposed to an X-ray film for ten days at −70°C. The blots were analyzed by optic densitometry using the software Image Master 1D® (Pharmacia Biotech, Upsalla, Sweden). The results were expressed as arbitrary units (AU) per total amount of protein electrophoresed, or as AU per g of tissue, considering the protein recovery of each sample.

Northern Blotting

Total RNA was extracted from 0.1 g of frozen cortex and medulla samples using Trizol® reagent (Gibco BRL, Grand Island, NY) according to manufacturer's instructions. The RNA (20 μg) was electrophoresed on 1.2% formaldehyde-agarose gel, blotted, and fixed onto nylon membranes and then hybridized to cDNA probe specific to rat SGLT1 (at 37°C, 40% formamide, overnight). Filters were washed at high-stringency conditions (the low-stringency protocol, plus one wash at 1% SDS, 0.1 × SSC, 50°C, 15 min) for SGLT1 mRNA detection, and at low-stringency conditions (two washes at 0.1% SDS, 2 × SSC, 25°C, 5 min) for SGLT2 detection, and exposed to autoradiography. After that, the filters were stripped by washing two times at high-stringency conditions and hybridized to cDNA probe for β-actin. The same

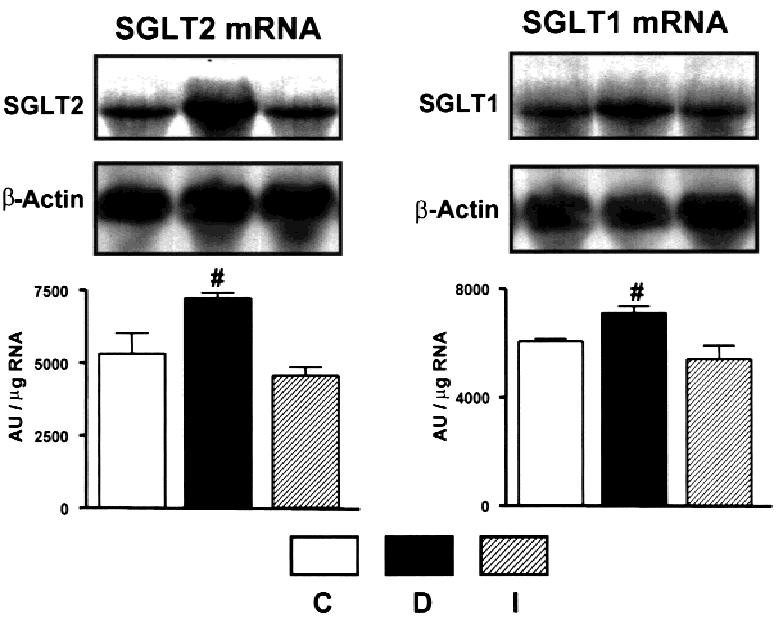

Fig. 1. Renal cortical SGLT2 (left) and medullar SGLT2 (right) mRNAs from 3-month-old control (*C*) and diabetic (*D*) rats, and from 12-month-old insulin resistant (*I*) rats. *Top:* typical autoradiograms of SGLT1, SGLT2 and β-actin mRNAs were shown. *Bottom:* mRNA content, expressed as arbitrary units (AU) / μg of total RNA, after normalization to the β-actin mRNA value is shown. Data are means ± SEM of at least 4 different experiments. #$P < 0.05$ *vs. C* and *I*

samples of cortex and medulla total RNA were additionally probed, in different experiments, at high-stringency conditions, to GLUT1 and GLUT2 respectively, and thereafter to β-actin. The blots were analyzed by scanner densitometry as described for Western analysis, and the results were expressed as arbitrary units (AU) per μg of total RNA, after normalization to the respective β-actin value, and corrected considering that in each experiment the control value represents 20 μg of total RNA.

Analytical Procedures

Plasma samples were assayed for glucose by the glucose oxidase method (Analisa Diagnóstica, São Paulo, Brasil), and for insulin by RIA (Coat-A-Count, Diagnostic Products, Los Angeles, CA). The total protein concentration of membrane samples was assayed by Lowry's method.

Data Analysis

All values are reported as means ± SEM. The transporters were quantified in at least four different experiments, using different rats from all animal groups. Statistical comparisons were made by ANOVA (Student-Newman-Keuls as a post-test).

Results

The body weight of uncontrolled alloxan-induced diabetic rats remained stable during the 15 days of evolution; therefore, their final body weight was lower ($P < 0.05$) than the controls (230.0 ± 19.3 *vs.* 313.3 ± 25.8 g). On the day of the experiment, compared to C rats, the D rats showed higher ($P < 0.01$) plasma glucose levels (290.0 ± 11.7 *vs.* 114.2 ± 8.0 mg/dl) and lower ($P < 0.01$) plasma insulin levels (28.0 ± 1.9 *vs.* 47.9 ± 3.8 μU/ml). The 15-month-old obese rats (I) had a higher ($P < 0.001$) body weight (500.0 ± 23.6 g) compared to both control and diabetic rats. They were judged obese not only by their increased body weight, but also because they showed increased ($P < 0.05$) Lee Index (31.3 ± 0.2 *vs.* 30.1 ± 0.1) and epididymal fat mass (8.18 ± 1.03 *vs.* 0.94 ± 0.06 g). The Lee Index [body weight (g)$^{1/3}$ / nasoanal length (cm)] represents for rats the body mass index (BMI) used to evaluate obesity in humans. On the day of the experiment, compared to C rats, I rats showed unchanged glycemia (178.5 ± 15.3 *vs.* 168.1 ± 10.3 mg/dl) with increased ($P < 0.001$) insulinemia (111.7 ± 10.1 *vs.* 47.9 ± 3.8 μU/ml). The hyperinsulinemia of aged obese rats indicates their insulin resistance, which has already been clearly reported [11, 15]. On the other hand, the animals in salt-diet protocol did not change their body weights (NS, 364.3 ± 15.3; LS, 357.4 ± 11.9; HS, 324.8 ± 13.5 g) significantly.

In preliminary studies, the efficiency of the sampling procedure of renal cortex and medulla as expression of mainly S1 and S3 tubular segments, respectively, was confirmed. The GLUT2 protein and mRNA were not detected in medulla samples, and the GLUT1 protein and mRNA were detected in a very low amount in cortex, as expected according to the kidney topology of these transporters.

The mRNAs of SGLTs from C, D and I rats are shown in Fig. 1. Compared to C and I rats, the abundance of both SGLT1 and SGLT2 mRNAs of D rats was increased ($P < 0.05$) by 20% and 30% respectively. The GLUT2 mRNA in D rats (Fig. 2) showed an increase ($P < 0.01$) of ~100% compared to C and I rats, similar to the GLUT2 protein increase ($P < 0.05$) observed in a per-g-of-tissue basis. On the other hand, in renal medulla (Fig. 3), compared to C rats, the GLUT1 mRNA

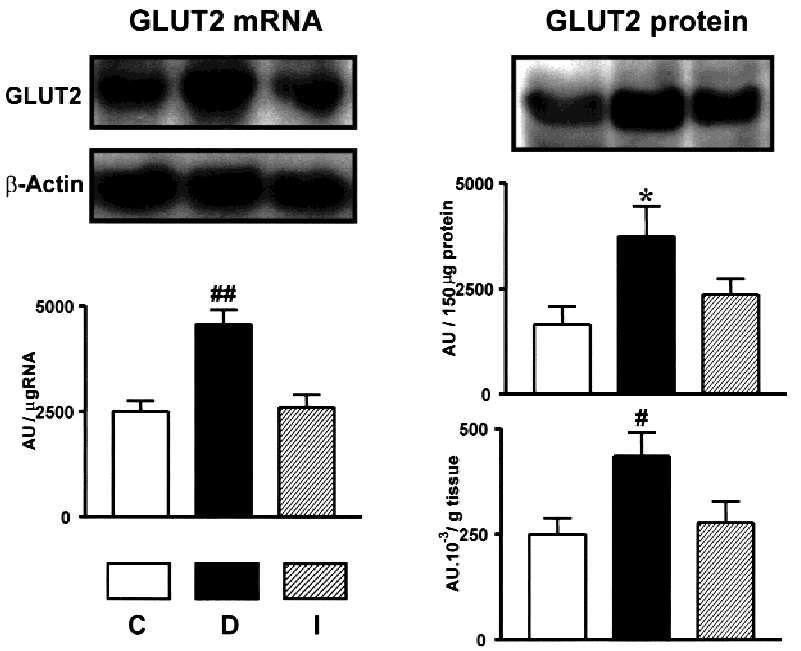

Fig. 2. GLUT2 gene expression in renal cortex from 3-month-old control (*C*) and diabetic (*D*) rats, and from 12-month-old insulin resistant (*I*) rats. *Left:* GLUT2 mRNA analysis: typical autoradiograms of GLUT2 and β-actin (*top*); and mRNA content, expressed as arbitrary units (AU) / μg of total RNA, after normalization to the β-actin mRNA value (*bottom*). *Right:* GLUT2 protein analysis: typical autoradiogram (*top*); the GLUT2 content, expressed as AU / 150 μg of protein (*middle*); and the GLUT2 content, expressed as AU / g of tissue weight (*bottom*). Data are means ± SEM of at least 4 different experiments.
*$P < 0.05$ *vs. C;* #$P < 0.05$ *vs. C* and *I;* ##$P < 0.001$ *vs. C* and *I*

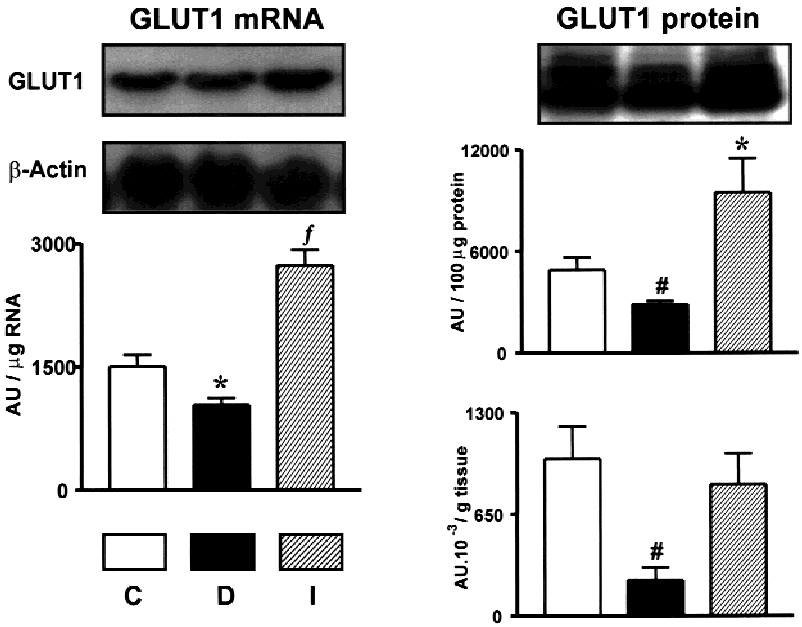

Figure 3. GLUT1 gene expression in renal medulla from 3-month-old control (*C*) and diabetic (*D*) rats, and from 12-month-old insulin-resistant (*I*) rats. *Left:* GLUT1 mRNA analysis: typical autoradiograms of GLUT1 and β-actin (*top*); and mRNA content, expressed as arbitrary units (AU) / μg of total RNA, after normalization to the β-actin mRNA value (*bottom*). *Right:* GLUT1 protein analysis: typical autoradiogram (*top*); the GLUT1 content, expressed as AU / 150 μg of protein (*middle*); and the GLUT1 content, expressed as AU / g of tissue weight (*bottom*). Data are means ± SEM of at least 4 different experiments.
*$P < 0.05$ *vs. C;* #$P < 0.05$ *vs. C* and *I;* $^{f}P < 0.001$ *vs. C* and *D*

and protein of D rats were similarly decreased ($P < 0.05$) by ~40%. However, the GLUT1 gene expression in I rats was differently modulated: the mRNA increased ($P < 0.001$) compared to C and D rats; and the protein on a per-100-μg-of-protein basis was increased ($P < 0.05$), compared to C and D rats, whereas on a per-g-of-tissue basis, it was increased ($P < 0.05$) only compared to D rats.

The salt diets also modulated the glucose transporters. Figure 4 shows that the SGLT2 mRNA in HS rats increased ($P < 0.001$) by more than 200% compared to NS and LS rats, whereas the SGLT1 mRNA was similar in all groups. The GLUT2 expression (Fig. 5) showed that the mRNA and protein, expressed per-g-of-tissue, were increased by ~150% ($P < 0.05$) in HS rats compared to NS and LS rats. Per 150 μg of protein electrophoresed, the GLUT2 protein of HS rats was increased by 80% ($P < 0.05$) compared to NS rats. On the other hand, in renal medulla (Fig. 6), the GLUT1 mRNA ($P < 0.001$) and protein ($P < 0.05$) were similarly increased in both HS and LS rats, when compared to NS rats.

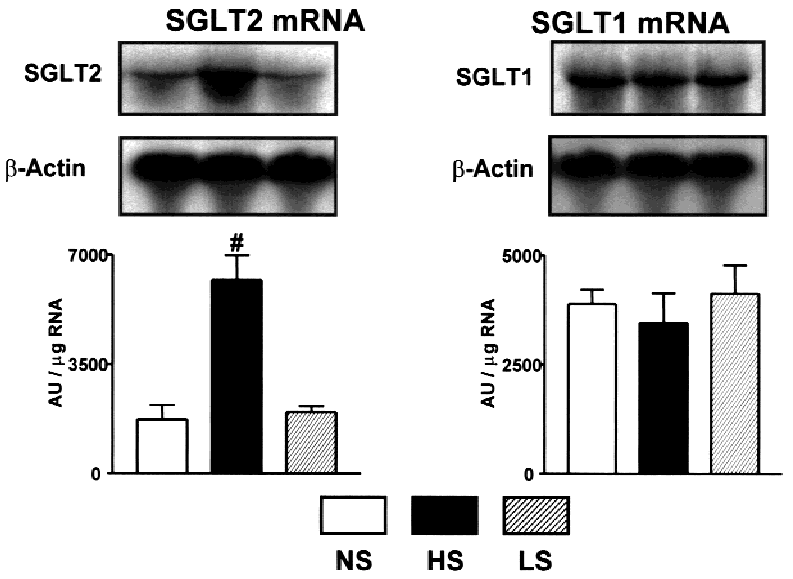

Fig. 4. Renal cortical SGLT2 (*left*) and medullar SGLT2 (*right*) mRNAs from 3-month-old normal (*NS*), high (*HS*) and low (*LS*) Na^+ diet fed rats. *Top:* typical autoradiograms of SGLT1, SGLT2 and β-actin mRNAs were shown. *Bottom:* mRNA content, expressed as arbitrary units (AU) / μg of total RNA, after normalization to the β-actin mRNA value was shown. Data are means ± SEM of at least 4 different experiments.
#$P < 0.001$ *vs. NS* and *LS*

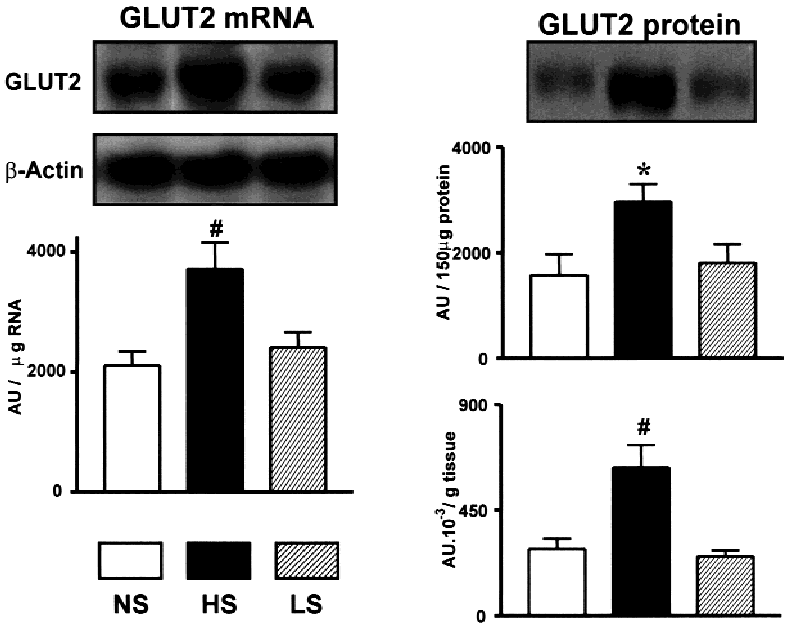

Fig. 5. GLUT2 gene expression in renal cortex from 3-month-old normal (*NS*), high (*HS*) and low (*LS*) Na^+ diet fed rats. *Left:* GLUT2 mRNA analysis; typical autoradiograms of GLUT2 and β-actin (*top*); and mRNA content, expressed as arbitrary units (AU) / μg of total RNA, after normalization to the β-actin mRNA value (*bottom*). *Right:* GLUT2 protein analysis; typical autoradiogram (*top*); the GLUT2 content, expressed as AU / 150 μg of protein (*middle*); and the GLUT2 content, expressed as AU / g of tissue weight (*bottom*). Data are means ± SEM of at least 4 different experiments.
*$P < 0.05$ *vs. NS;* #$P < 0.05$ *vs. NS* and *LS*

Discussion

The renal glucose reabsorption is mediated by two classes of glucose transporters: the luminal SGLTs and the basolateral GLUTs [9, 10]. Previous studies have focused on GLUT1 and GLUT2 proteins in kidney from diabetic rats, but the modulation of SGLTs has not yet been known under this condition [5, 7, 21, 22]. Technical trouble to assess SGLT proteins may include the production of good antibodies. The very high hydrophoby of the COOH-terminus of SGLT proteins may be a reason why the antibodies do not easily bind to their immunogenic COOH-epitope. We tried to assess SGLT1 using a commercial antibody made with the extracellular loop peptide of rabbit gut SGLT1 sequence, but were not successful. We also tried to obtain a cDNA based on the sequence of mRNA SGLT2 extracted from the Gene Bank (accession number U29881 gi 1098606). We used constructed primers (Gibco BRL) sense (1261-1282) and antisense (2157-2178) to obtain a fragment of ~923 bp, similarly described by others (1), but no cDNA from renal cortex purified poly(A)$^+$RNA samples could be amplified. Therefore, considering the high homology between SGLT1 and SGLT2 mRNAs, using a cDNA

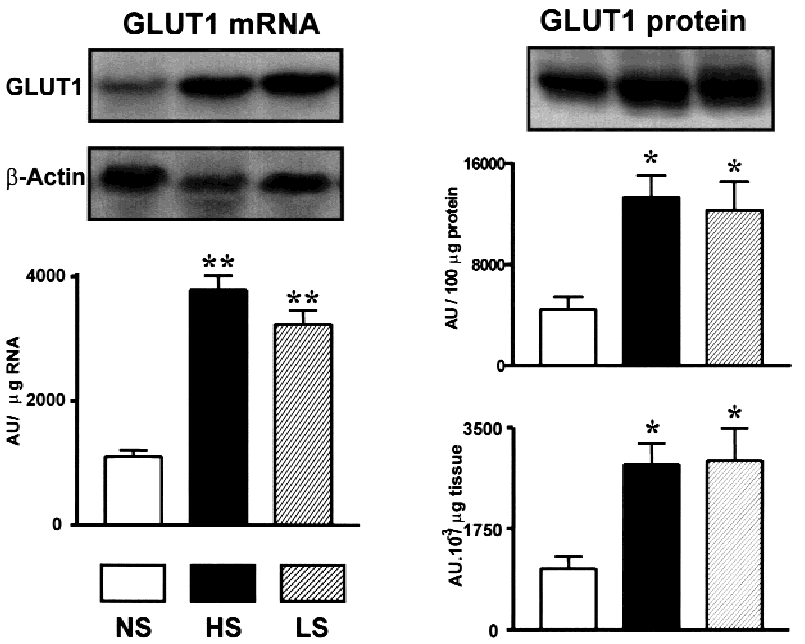

Fig. 6. GLUT1 gene expression in renal medulla from 3-month-old normal (*NS*), high (*HS*) and low (*LS*) Na^+ diet fed rats. *Left:* GLUT1 mRNA analysis; typical autoradiograms of GLUT1 and β-actin (*top*); and mRNA content, expressed as arbitrary units (AU) / µg of total RNA, after normalization by the β-actin mRNA value (*bottom*). *Right:* GLUT1 protein analysis: typical autoradiogram (*top*); the GLUT1 content, expressed as AU / 150 µg of protein (*middle*); and the GLUT1 content, expressed as AU / g of tissue weight (*bottom*). Data are means ± SEM of at least 4 different experiments. $^*P < 0.01$ *vs. NS;* $^{**}P < 0.001$ *vs. NS*

probe specific to rat SGLT1, we could successfully detect SGLT2 mRNA in cortex by washing the filters in low stringency conditions. Under these conditions, the SGLT1 probe hybridized to an ~4.0-kb band in medulla and to an ~2.5-kb band in cortex, in agreement with the predicted molecular weight of SGLT2 and SGLT1 mRNAs, respectively. The observation that high-stringency washing eliminates the signal in cortex samples, but not in medulla samples, reinforces the fact that the SGLT1 probe is detecting SGLT2 mRNA.

The main cellular mechanism for GLUT1 and GLUT2 modulation in kidney seems to be the intracellular concentration of glucose. This is supported by studies of cultured renal NRK cells, which showed that GLUT1 protein levels were lowered by glucose feeding and increased by glucose deprivation [8], whereas GLUT2 protein seems to be inversely regulated. Concerning GLUT1 and GLUT2 gene expression, our results showed similar variations of mRNA and protein, suggesting that transcriptional modulations are being reflected in protein tissue content changes.

In diabetic rats, the filtration rate of glucose is strongly increased, and that increases the glucose transport at the luminal membrane. On the other hand, hyperglycemia decreases the efflux gradient at the basolateral membrane. These effects together increase the intracellular glucose concentration in the proximal tubular cells. Since the diabetic animals have a marked glycosuria, these mechanisms are affecting not only the S1 segment, where the bulk of glucose reabsorption occurs under physiological conditions, but also the S3 segments. In fact, we observed that GLUT2 and GLUT1 expression are respectively increased and decreased, as previously reported [5]. In addition, we are showing that both SGLT1 and SGLT2 mRNAs have increased, suggesting that the SGLTs are modulated by glucose the same way GLUT2 is.

The different Na^+ diets showed important changes in the glucose transporters. If Na^+ filtration rate was a very important direct modulator, a similar regulation of the transporters would be expected in both S1 and S3 segments, but that was not observed in the high-Na^+ diet-fed rats. However, intracellular glucose concentration may be again the main modulator. In cortex from HS rats, the very high Na^+ filtration rate improves the cellular glucose influx in the early S1 segment. Most of the filtrated glucose is rapidly influxed in this segment, and the high-capacity but low-affinity GLUT2 transporter can not proportionally increase the cellular efflux of glucose. Therefore, the S1 epithelial cells may be temporarily submitted to a high glucose concentration, high enough to develop the SGLT2 and GLUT2 modulation observed. On the other hand, although the luminal Na^+ concentration is also very high in S3 segments of HS rats, the modulation of the glucose transporters was not the same as that in S1 segments. The SGLT1 mRNA was unchanged, whereas the GLUT1 protein was strongly increased. This may still be explained by intracellular glucose concentration [8], which could be very low in the S3 epithelial cells. Since the high filtrated rate of Na^+ provided almost a complete glucose reabsorption in S1 segments, no substrate would be available for reabsorption in S3 territory. That may occur despite high Na^+ concentration; and consequently, the intracellular glucose may decrease.

In the low-Na^+ diet-fed rats, a low or a normal in-

tracellular glucose concentration may be expected, and the SGLT2 and GLUT2 were shown to be unchanged in the S1 segment. However, in the S3 segment of LS rats, the low Na^+ electrochemical gradient may reduce the glucose influx through the SGLT1, which has a Na^+:glucose ratio of 2:1, and, as a consequence, the intracellular glucose concentration is very low. This mechanism may be responsible for the increased GLUT1 protein without detectable changes in SGLT1 mRNA.

Insulin-resistant 15-month-old rats had no important changes in glucose filtration rate, and SGLT2 and SGLT1 mRNAs were unchanged. These data reinforce the idea that high glucose filtration rate is an important modulator of SGLTs in diabetic rats. The I rats have impaired glucose tolerance [12] which could provide intermittent glycemic increases at post-prandial periods, a potential modulator of glucose transporters, especially in the basolateral membrane [5]. Although it was not statistically significant, the GLUT2 protein was increased by ~40%, and that may represent some biological modulation. On the other hand, the I rats showed a very high increase in GLUT1 mRNA and protein contents, showing an important gene expression modulation. Alternatively, when the results were expressed per-g-of-tissue weight, the GLUT1 increase disappeared because the I rats had a significant reduction in the total protein yield of medulla samples. This pointed out that morphostructural changes occurred during the development of the rat. This may be due mainly to a hypertrophic process of medullar structures, which, by reducing the relative total tissue protein content, could explain the results observed. Since a reduction in intracellular glucose concentration cannot be expected, the mechanism of the GLUT1 gene modulation in I rats is not clear. However, a severe hyperinsulinemia is present in this model [12], which may be causing modulation in glucose transporters, differently from the other models in which the insulin concentration was normal or low [5].

As first reported for GLUT1 in cultured kidney NRK cells [8], intracellular glucose concentration may be the main modulator of other glucose transporters in kidney, as observed in the present study. However, in the in vivo modulations, some other systemic or local mechanisms cannot be excluded. The insulin sensitivity has been shown as a modulator of GLUT1 [5] and GLUT4 [13, 19] gene expression, and it may also modulate kidney glucose transporters as observed in the 15-month-old obese rats. The renin-angiotensin, which stimulates the RAS activation or even the water balance, may be acting locally in the tubular cells, modulating the glucose transporter gene expression [17, 18]. In addition, angiotensin, an inhibitor of insulin transduction signal, modulates the hormone sensitivity [16] and may act on glucose transporter gene expression. Finally, metabolic local changes, such as cellular acidosis that stimulates renal ammoniogenesis and gluconeogenesis, both of which are able to stimulate GLUT2 gene expression [2, 6], must be considered.

The changes in gene expression of glucose transporters observed in the proximal renal tubule in the present study may be a very important mechanism, still to be extensively investigated. They not only represent responses designed to adjust the transtubular glucose flux in different glucose or Na^+ filtration rate conditions, but also play an active role in kidney injury, such as in diabetic nephropathy.

Taken together, these results indicate that changes in glucose or Na^+ filtration rate affect the gene expression of SGLTs and GLUTs in the proximal tubule. Although the cellular mechanisms are not clearly known at present, the intracellular glucose concentration seems to play a key role in these glucose transporter modulations.

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