Regulation of amino acid transporters in the rat remnant kidney

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Abstract

**Background.** Partial renal ablation is associated with compensatory renal growth, significant azotaemia, a significant increase in fractional excretion of sodium and changes in solute transport. The present study evaluated the occurrence of adaptations in the remnant kidney, especially in renal amino acid transporters and sodium transporters and their putative role in sodium handling in the early stages (24 h and 1 week) after uninephrectomy.

**Methods.** Wistar rats aged 8 weeks old were submitted to renal ablation of the right kidney—Unx rats (n = 10). 24 hours (n = 5) and 1 week (n = 5) after surgery, rats were anesthetized and the left kidney was removed. Urinary and plasmatic levels of catecholamines, sodium, urea and creatinine were measured. Gene expression of the amino acid and sodium transporters was determined by Real-time reverse transcription PCR. Protein expression was evaluated by Western blot using specific antibodies for the amino acid and sodium transporters.

**Results.** Uninephrectomized (Unx) rats for 24 h showed a lower urinary excretion of L-DOPA, dopamine and DOPAC than the corresponding Sham rats, accompanied by an increase in the expression of the Na\(^{+}\)-K\(^{+}\)-ATPase protein (64% increase). Unx rats for 1 week presented a hypertrophied remnant kidney, higher urine outflow and a ∼2-fold increase in the fractional excretion of sodium. The NHE3 mRNA expression was significantly decreased in Unx rats throughout the study (∼20% decrease). LAT1 transcript and protein were consistently overexpressed at both 24 h and 1 week after uninephrectomy. In contrast, 4F2hc and LAT2 transcript abundance was lower in 24-h Unx rats than in Sham rats (a 36% decrease in both cases).

**Conclusions.** These results provide evidence that the renal expression of the amino acid transporters LAT1, LAT2 and 4F2hc and the sodium transporters Na\(^{+}\)-K\(^{+}\)-ATPase and NHE3 is differently regulated following unilateral nephrectomy. In conclusion, this study allowed us to characterize the renal adaptations in the early stages after uninephrectomy, which showed a combined interaction of multiple mechanisms regulating sodium homeostasis including the renal dopaminergic system, and the abundance of amino acid transporters and sodium transporters.

**Keywords:** LAT1; LAT2; renal dopaminergic system; renal mass reduction

Introduction

The number of renal transplantations with living donor kidneys is progressively increasing world-wide, thus increasing the necessity of detailed knowledge about the short-term and long-term risks involved in this procedure [1]. Increased blood pressure and creatinine levels, hypertension and proteinuria are associated with unilateral nephrectomy in human subjects (reviewed in [2]). However, the factors contributing to the adaptations of the remaining kidney have not been systematically evaluated.

Animal models of reduced renal mass undergo a series of adaptive mechanisms to maintain sodium homeostasis. As the population of surviving nephrons is reduced, the remaining tissue undergoes compensatory hypertrophy with marked alterations in tubular reabsorptive capacities of sodium and water [3–5]. Compensatory changes in the tubular handling of sodium include an increased excretion
of sodium per nephron [6,7]. In this way, sodium balance can be maintained despite a diminishing glomerular filtration rate, when salt intake is unaltered under conditions of reduced renal mass. In rats submitted to uninephrectomy, an increased renal dopamine synthesis was observed in the rat remnant kidney at 2 weeks after surgery. This was accompanied by a dopamine-sensitive enhanced natriuresis with no changes in blood pressure values, suggesting that after uninephrectomy renal dopamine may play an important role in keeping uninephrectomized (Unx) rats within sodium balance [8,9]. The role of the renal dopaminergic system in sodium handling in more early phases after renal mass ablation still remains to be established.

Dopamine of renal origin exerts natriuretic and diuretic effects by activating D1-like receptors located at various regions in the nephron [10,11]. At the level of the proximal tubule, the overall increase in sodium excretion produced by dopamine and D1 receptor agonists results from inhibition of the main sodium transport mechanisms at the basolateral and apical membranes, respectively, Na\(^+\)-K\(^+\)-ATPase and the Na\(^+\)/H\(^+\) exchanger [12–16]. On the other hand, sodium has been found to constitute an important stimulus for the production of dopamine by renal proximal tubule cells [17–19], resulting in increases in the urinary excretion of dopamine and dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) [9,20–23].

The proximal tubules, but not distal segments of the nephron, are endowed with high aromatic L-amino acid decarboxylase (AADC) activity, and epithelial cells of proximal tubules have been demonstrated to synthesize dopamine from circulating or filtered L-3,4-dihydroxyphenylalanine (L-DOPA) [24–26]. Studies on the inward transport of L-DOPA by tubular epithelial cells conducted in rat renal cortical slices [27] and cultured renal cell lines [13,28] demonstrated that uptake of L-DOPA is an active process, mediated through amino acid transporters. The candidate transport systems for L-DOPA may include the Na\(^+\)-dependent systems B\(^0\), B\(^0\),\(+\) and y\(^+\)L, and the Na\(^+\)-independent systems L-amino acid transporters (LAT1 and LAT2) and b\(^0\),\(+\) [13,19,28–32]. Our group has suggested that the L-DOPA transport in immortalized renal proximal tubular epithelial cells of Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) is mediated by LAT2 [33]. Furthermore, we have recently reported the presence of an adaptive regulation of renal amino acid reabsorptive mechanisms in response to acute dietary Na\(^+\) changes [34].

Relative to other amino acid transporters, the expression levels of the Na\(^+\)-dependent amino acid exchanger ASC2 and the heterodimeric transporter LAT1-4F2hc are elevated in a variety of cancer types, suggesting a potential role in cellular growth. This upregulation of ASC2 and LAT1 supports the high levels of protein synthesis necessary for continuous growth and proliferation, via delivery of glutamine and essential amino acids such as leucine, respectively [35].

The hypothesis that we have explored in the present study concerns the occurrence of adaptations in the remnant kidney, especially in renal amino acid transporters and sodium transporters and their putative role in sodium handling in the early stages after uninephrectomy.

**Materials and methods**

**Animal interventions**

All animal interventions were performed in accordance with the European Directive number 86/609, and the rules of the ‘Guide for the Care and Use of Laboratory Animals’, 7th edition, 1996, Institute for Laboratory Animal Research (ILAR), Washington, DC.

Wistar rats aged 8 weeks old and weighing 214–253 g were used in the experiments. The animals were kept under controlled environmental conditions (12 h light/dark cycle and room temperature 22 ± 2°C), and fluid intake and food consumption were monitored daily throughout the study. All animals were fed ad libitum throughout the study with ordinary rat chow.

The rats were anaesthetized with pentobarbital sodium (60 mg/kg, ip), and the surgical ablation of the right kidney was performed according to that previously described [9]—Unx rats (n = 10). The control animals were rats submitted to sham surgery under similar conditions; however, their kidneys remained intact—sham-operated (Sham) rats (n = 10). After total recovery from surgery (4–6 h), the rats returned to the animal facility, where they had free access to food and water. The survival rate was 100%.

Twenty-four hours (24-h Unx rats; n = 5) and 1 week (1-week Unx rats; n = 5) after surgery, the rats were placed in metabolic cages. Twenty-four-hour urine was collected for the quantification of sodium, creatinine, urea and catecholamines. The vials collecting urine for the quantification of catecholamines contained 0.5 ml hydrochloric acid (6 M), to avoid the spontaneous oxidation of the amines and its derivatives.

Afterwards, the rats were anaesthetized with pentobarbital sodium (60 mg/kg, ip) and blood was collected from the heart in tubes containing K\(_2\)EDTA for a later determination of plasma catecholamines and biochemical parameters. The animals were perfused through injection of 50 ml of ice-cold PBS in the left ventriculum. The kidneys were then rapidly removed, decapsulated and weighed. Thereafter, cortices were dissected out, snap-frozen in liquid nitrogen and stored at −80°C until processing for protein and RNA extraction.

**Assay of catecholamines**

The assay of catecholamines and its derivatives in urine and plasma samples (L-DOPA, dopamine, DOPAC, norepinephrine and epinephrine) was performed by HPLC with electrochemical detection, as previously described [19,28]. The lower limit of detection of L-DOPA, dopamine, DOPAC, norepinephrine and epinephrine ranged from 350 to 1000 fmol. Renal delivery of L-DOPA was calculated according to the formula (plasma L-DOPA × creatinine clearance).

**Plasma and urine ionogram and biochemistry**

The quantification of sodium, urea and creatinine in plasma and urine samples was performed by Cobas Mira Plus analyser (HORIBA ABX, France) as previously described [8].

**RNA extraction**

Kidney cortices were homogenized (Dixax, Heidelberg—Sigma-Aldrich, USA) in Trizol reagent (75 mg/ml; Invitrogen, USA), and total RNA was extracted according to the manufacturer’s instructions. The RNA obtained was dissolved in diethylpyrocarbonate (DEPC)-treated water and quantified by spectrophotometry at 260 nm.

**Reverse transcription**

The total RNA extracted from individual rat kidney cortices was treated with DNase (Ambion, USA), to eliminate potential genomic DNA contamination. cDNA was synthesized from 1 µg of total RNA in a total volume of 20 µl. Reverse transcription was performed with the SuperScript First Strand System for RT-PCR (Invitrogen, USA), using 250 ng/µl random hexamers as primers at 50°C, according to the manufacturer’s instructions. For real-time quantitative PCR, 2 µl out of the 20 µl reverse transcription reaction mixture was used.
Renal function of Unx rats

Table 1 summarizes the physiological parameters of the 8-week-old Unx and sham-operated Wistar rats for 24 h and 1 week. Uninephrectomy had no effect on body growth, as Unx rats attained the same weight as did Sham rats. Twenty-four-hour uninephrectomy showed no differences in the remnant kidney weight between Unx and Sham rats. Kidney weight 1 week after surgery, however, was significantly altered; the Unx rats presented a hypertrophied remnant kidney weighing 20.1 ± 6.3% more than the corresponding kidney in Sham rats. Urine volume showed no differences in 24-h Sham and Unx rats, but increased significantly in 1-week Unx versus Sham rats. Plasma urea was greater in Unx rats 24 h and 1 week after surgery, while plasma creatinine was similar between Unx and Sham rats. Creatinine clearance decreased in 24-h and 1-week Unx rats, although this difference did not achieve statistical significance. Uninephrectomy had no influence in the fractional excretion of sodium of 24-h Unx rats, but led to an ~2-fold increase in 1-week Unx rats.

The urinary excretion of L-DOPA, dopamine, DOPAC, noradrenaline and adrenaline were decreased in Unx rats at 24 h, but showed no differences 1 week after uninephrectomy. In addition, the levels of plasma L-DOPA were similar between Unx and Sham rats throughout the study, while the renal delivery of L-DOPA was significantly decreased in both 24-h and 1-week Unx rats. Urinary dopamine plus DOPAC divided by L-DOPA delivery showed a significant increase 1 week after surgery (Figure 1).

Effect of uninephrectomy on mRNA transcript abundance

Transcript abundance of amino acid transporters and sodium transporters was measured by quantitative RT-PCR. As shown in Figure 2A, renal LAT1 transcript levels in Unx rats were significantly higher than in Sham rats at both 24 h and 1 week after uninephrectomy (36 ± 11% and 43 ± 12% increase, respectively). On the other hand, uninephrectomy led to a significant decrease in both LAT2 and 4F2hc mRNA levels of 24-h Unx rats (36 ± 4% lower in both cases), while 1 week after surgery this difference was no longer observed (Figure 2B and C). No significant changes were found in either group in the mRNA expression of the Na^+ dependent amino acid transporter ASCT2.
Regulation of amino acid transporters in the rat

Table 1. Body weight (BW) and left kidney weight (LKW), increase in renal mass, metabolic balance and renal function in sham-operated (Sham) and uninephrectomized (Unx) rats 24 h and 1 week after surgery

<table>
<thead>
<tr>
<th></th>
<th>24 h</th>
<th>1 Week</th>
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<tr>
<td></td>
<td>Sham</td>
<td>Unx</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>215.4 ± 2.7</td>
<td>220.4 ± 5.6</td>
</tr>
<tr>
<td>Left kidney weight (g)</td>
<td>1081.5 ± 41.2</td>
<td>1157.7 ± 31.6</td>
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<tr>
<td>LKW/BW</td>
<td>5.0 ± 0.2</td>
<td>5.3 ± 0.2</td>
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<tr>
<td>Increase in renal mass (%)</td>
<td>5.6 ± 3.2</td>
<td>7.6 ± 4.5</td>
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<tr>
<td>Plasma urea (mg/dl)</td>
<td>25.8 ± 1.7</td>
<td>37.9 ± 1.7a</td>
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<tr>
<td>Urinary urea (mg/dl)</td>
<td>2644.0 ± 628.2</td>
<td>2603.8 ± 455.5</td>
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<tr>
<td>Plasma creatinine (mg/dl)</td>
<td>0.31 ± 0.04</td>
<td>0.37 ± 0.02</td>
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<tr>
<td>Urinary creatinine (mg/dl)</td>
<td>91.0 ± 26.8</td>
<td>80.3 ± 17.5</td>
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<tr>
<td>Urine volume (ml/day)</td>
<td>11.4 ± 2.8</td>
<td>10.0 ± 1.7</td>
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<tr>
<td>Creatinine (ml/min)</td>
<td>2.07 ± 0.6</td>
<td>1.32 ± 0.1</td>
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<td>FENa+ (%)</td>
<td>0.11 ± 0.03</td>
<td>0.12 ± 0.03</td>
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Values are mean ± SEM; n = 5 experiments/group.

Ccreatinine = creatinine clearance; FENa+ = fractional excretion of Na+.

*Significantly different from corresponding values in sham-operated rats (P < 0.05).

(Figure 2D). Concerning the sodium transporters, NHE3 transcript quantification showed a significant decrease in Unx rats for 24 h (23 ± 5% lower) and for 1 week (19 ± 5% lower), while the Na+-K+-ATPase expression remained unchanged throughout the study (Figure 2E and F).

Effect of uninephrectomy on protein abundance

The protein expression of amino acid transporters and sodium transporters was assessed by means of immunoblotting. The protein expression levels of LAT1 were significantly higher in Unx rats than in Sham rats at 24 h (67 ± 19%) and 1 week (60 ± 20%) after nephrectomy (Figure 3A). When compared to Sham rats, 4F2hc protein abundance was 37 ± 5% lower in Unx rats at 24 h, with no changes 1 week after surgery (Figure 3C). There were no significant differences in the expressions of the Na+-independent transporter LAT2 and the Na+-dependent transporter ASCT2 (Figure 3B and D). Since 4F2hc is required to bring LAT1 and LAT2 to the cell membrane [36,37], which is essential for the latter to be operational [34,38], the LAT1/4F2hc and LAT2/4F2hc kidney ratios are assumed to reveal the functionality of the transporters. These ratios were obtained by the simultaneous quantification of the LAT2 and 4F2hc protein expression from the same blots. Twenty-four hours after uninephrectomy, the LAT1/4F2hc ratio was significantly higher in Unx rats (1.7 ± 0.18) than in Sham rats (1.0 ± 0.12). This increased expression of LAT1 over 4F2hc does not necessarily mean that a significant number of LAT1 units are not functional, but may alternatively suggest an enhanced turnover of LAT1 transport units. A higher LAT1/4F2hc ratio was still present 1 week after uninephrectomy (1.3 ± 0.12 and 1.0 ± 0.11 in Unx and Sham rats, respectively), although in this case it did not represent a statistically significant difference. No significant differences occurred at either 24 h or 1 week post-nephrectomy on LAT2/4F2hc ratios (data not shown). The protein expression of the apical sodium transporter NHE3 was ~20% lower both at 24-h and 1-week Unx rats, although this did not attain a significant difference (Figure 3E). In contrast, the expression of the basolateral transporter Na+-K+-ATPase showed a significant increase of 64 ± 18% at 24 h, with no differences 1 week after surgery (Figure 3F).

Discussion

This study provides evidence that the renal expression of the amino acid transporters LAT1, LAT2 and 4F2hc and the sodium transporters Na+-K+-ATPase and NHE3 is differently regulated following unilateral nephrectomy, suggesting a potential role in the functional adaptations of the remnant kidney.

Furthermore, uninephrectomy was associated with the known consequences of partial renal ablation, namely, compensatory renal growth, significant azotaemia, significant increase in fractional excretion of sodium and dopamine synthesis and changes in solute transport [6,24,39], these effects being particularly evident in 1-week Unx rats rather than 24 h after surgery. In fact, Unx rats for 24 h presented a decrease in the renal delivery of l-DOPA and decreased urinary excretion of dopamine and its deaminated metabolite DOPAC, which suggest a reduced renal synthesis of dopamine. At this stage, Unx rats presented no renal hypertrophy and no changes in FENa+, but showed an increase in the expression of the Na+-K+-ATPase protein. The discrepancy between mRNA levels and protein expression observed in Na+-K+-ATPase might be related to post-transcriptional regulation, such as the control of splicing efficiency, precursor RNA stability, polyadenilation or RNA transport [40]. Several studies have indicated an enhancement of sodium transport early after uninephrectomy, evidenced by an increase in Na+-K+-ATPase activity, which disappears with progressive renal growth [41–43]. Two weeks after uninephrectomy, Na+-K+-ATPase activity in renal proximal tubules has been found to be similar to that in corresponding controls [9,44]. These findings are in agreement with the results reported here at 1 week after surgery, since at this stage, the early increase in the
Fig. 1. Urinary levels of l-3,4-dihydroxyphenylalanine (l-DOPA, A), dopamine (B), 3,4-dihydroxyphenylacetic acid (DOPAC, C), noradrenaline (D), adrenaline (E), plasmatic l-DOPA (F), l-DOPA delivery (G) and urinary dopamine plus DOPAC divided by l-DOPA delivery (H) in sham-operated (Sham) and uninephrectomized (Unx) rats 24 h and 1 week after surgery. Bars represent means of five determinations per group and error bars represent SEM. The absolute levels (in nanomoles per day) of urinary l-DOPA, dopamine, DOPAC, noradrenaline and adrenaline in sham-operated rats 24 h and 1 week after surgery were as follows: (l-DOPA; 0.75 ± 0.04 and 1.82 ± 0.2, respectively), (dopamine; 12.6 ± 1.8 and 18.2 ± 1.6, respectively), (DOPAC; 25.7 ± 3.1 and 47.7 ± 1.8, respectively), (noradrenaline; 6.3 ± 0.8 and 6.1 ± 0.5, respectively) and (adrenaline; 0.42 ± 0.03 and 0.42 ± 0.04, respectively). The absolute levels of plasmatic l-DOPA (in pmoles/ml) and of l-DOPA delivery (in pmoles/min) in sham-operated rats 24 h and 1 week after surgery were as follows: (plasmatic l-DOPA; 5.01 ± 0.1 and 5.30 ± 0.2, respectively) and (l-DOPA delivery; 10.4 ± 0.3 and 25.8 ± 1.2, respectively). *Significantly different from corresponding values in sham-operated rats (P < 0.05).

Na⁺-K⁺-ATPase protein expression and reduced dopamine synthesis are no longer present in the hypertrophied kidney of Unx rats. The 1.8-fold increase in [FE_{Na⁺}] in 1-week Unx rats contrasts with the results on the Na⁺-K⁺-ATPase protein expression, unless one assumes that the slightly decreased protein abundance of the sodium transporter NHE3 may contribute to the enhanced Na⁺ excretion in 1-week Unx rats. This possibility, however, has to be considered with caution since increases in FE_{Na⁺} in Unx rats are usually associated with post-proximal sodium excretion mechanisms [45,46].

Renal LAT1 transcript and protein quantification revealed a sustained overexpression throughout the study. As discussed previously, this amino acid transporter is involved
in continuous cell growth and proliferation. Recent studies have demonstrated that LAT1 is a major L-type amino acid transporter in a variety of cancer cells including hepatic, oral, breast, bladder and colon [47]. LAT1 preferentially mediates the sodium-independent transport of large neutral amino acids such as leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine and histidine and is a major route for providing tumour cells with branched and aromatic amino acids [48]. Originally identified in activated lymphocytes and in rat hepatomas [49], the primary tissues displaying high expression of LAT1 thus either contain many proliferating cells, such as bone marrow, or contain endothelial and/or epithelial barriers that display vectorial, secretory amino acid transport from the extracellular space into other compartments, such as placenta, blood-brain barrier, ovary, testes and mammary glands [50,51]. Furthermore, LAT1 is also expressed in regenerating liver after partial hepatectomy [50]. At the kidney level, LAT1 has a very limited tissue distribution and, to our knowledge, there is no report on the exact localization of LAT1 in the nephron [34]. Nevertheless, Soares-da-Silva and Serrao described the transport of leucine by LAT1 in pig LLC-PK1 renal cells [31]. Although LAT1 has been shown to be present in brain capillary endothelial cells, its presence in the renal vasculature has not been defined. It is well established that shortly after unilateral nephrectomy, enhanced cell proliferation is demonstrable in the remaining contralateral kidney [52]. Compensatory renal growth is characterized by cell-cycle arrest in the G1 phase. As compensation for the nephron loss and in order to augment work capacity and maintain normal renal function, the remaining nephrons increase their RNA and protein synthesis with minimal alterations in DNA replication. Additionally, there is an inhibition of protein degradation and turnover contributing to cell enlargement and increased total cellular protein content [53–55]. These changes increase residual kidney size, but not the number of existing renal cells. While the bulk of compensatory organ growth can be attributed primarily to the hypertrophy of the proximal tubular epithelial cells, other kidney cells may actively participate in the process [52]. Morphological and stereological analysis performed by Pfaller et al. show that the distal convoluted tubules and the cortical collecting duct undergo ‘length’ hypertrophy (increase of tubular length
without thickening of the tubular wall and without an increase in number of cells, participating in compensatory kidney enlargement 10 and 21 days after unilateral nephrectomy [56]. Four and 24 days after uninephrectomy, Seyer-Hansen et al. also detected an increase in total glomerular volume and glomerular filtration surface, although this glomerular hypertrophy was less pronounced than tubular growth [57]. Other studies detected an increase in interstitial blood vessel volume and an overall remodelling and proliferation in renal cortical peritubular capillaries after experimental nephron reduction [58,59]. Therefore, LAT1 should be expected to be increased in the different cell types and tissues along the nephron (epithelial, interstitial and vascular cells) in order to provide cells with essential amino acids for high levels of protein synthesis associated with cell activation or hormonal stimulation and also to
sustain kidney growth associated with renal mass reduction [60].

4F2hc is a glycoprotein heavy chain that associates with the catalytic light chain LAT1 forming a heterodimeric transporter and is thought to be involved in the trafficking of the complex to the plasma membrane. Even so, the increased LAT1/4F2hc ratio in 1-week Unx rats does not necessarily compromise the transport activity or surface expression of LAT1, but may alternatively suggest an enhanced turnover of LAT1 transport units. Studies on the function and structure of heterodimeric amino acid transporters using truncated mutants of 4F2hc demonstrated that LAT1 retains its antiport mechanism when the extracellular domain of 4F2hc, responsible for the recognition of light chains, is completely removed and that the disulfide bridge connecting the dimers is not necessary for the surface expression of LAT1 [61,62]. Furthermore, Würtz et al. reported that rBAT, the other known heavy chain, is able to translocate LAT1, LAT2 and γLAT1 to the plasma membrane when coexpressed with these light chains in *Xenopus laevis* oocytes [63].

In contrast to LAT1, the Na+-dependent ASCT2 expression, another transporter associated with cellular growth, remained unchanged throughout the study, suggesting that the mechanisms involved in compensatory hypertrophy may differ from that described for tumoural cells.

LAT2 is a major Na+-independent amino acid transporter with marked expression in transporting epithelia, namely in the kidney and intestine, and its functionality is dependent on the abundance of 4F2hc. The heterodimerization of LAT2 with 4F2hc is a prerequisite for the transporter to reach the cell surface. Moreover, the glycoprotein may regulate the functional surface expression of the transporter [36–38,64]. Therefore, parallel decreases in renal 4F2hc protein and transcript and LAT2 transcript in Unx rats for 24 h may actually translate into decreases in LAT2 functionality. These results correlate well with the decreased synthesis of dopamine, since LAT2 is suggested to mediate the inward transport of L-DOPA [33]. This correlation is further evident when dividing urinary dopamine plus DOPAC/L-DOPA delivery showed a significant increase in Unx rats, which may indicate an increased dopamine synthesis 1 week following uninephrectomy. Furthermore, 1 week after surgery, Unx rats showed no differences in the LAT2/4F2hc expression and the urinary levels of catecholamines parallel to the ones in Sham-operated animals, suggesting a compensatory mechanism of the remaining kidney to overcome the renal mass reduction and increase the synthesis of dopamine. Recently, in an uninephrectomy experimental model in rats, the role of the renal dopaminergic system in the control of sodium homeostasis showed time-dependent variations, suggesting that natriuretic dopamine assumes a particular importance 2 weeks after uninephrectomy, but not in later phases (10 and 26 weeks) [66]. In fact, there is a general consensus regarding the renal adaptations beyond 2 weeks after left kidney ablation, characterized by an increased renal dopaminergic activity, enhanced diuresis and natriuresis and renal hypertrophy [8,9,67,68]. Unlike these reports, 24 h after surgery, we observed a diminished dopamine urinary excretion with no differences in natriuresis or renal growth. However, 1 week after surgery, Unx rats showed increased dopamine synthesis and fractional excretion of sodium as well as compensatory kidney growth, suggesting a tendency to match the alterations observed 2 weeks after nephrectomy.

In conclusion, this study allowed us to characterize the renal adaptations in the early stages after uninephrectomy, which showed a combined interaction of multiple mechanisms regulating sodium homeostasis including the renal dopaminergic system, and the abundance of amino acid transporters and sodium transporters.

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**Conflict of interest statement.** None declared.

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Treatement with PPAR-γ agonists in IgAN


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Protective effect of peroxisome proliferator-activated receptor-gamma agonists on activated renal proximal tubular epithelial cells in IgA nephropathy

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Abstract

Background. We have previously demonstrated a glomerulo-tubular ‘crosstalk’ operating in the pathogenesis of tubulointerstitial injury in IgA nephropathy (IgAN). The present study aims to explore any possible beneficial effect of a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist in alleviating the tubulointerstitial inflammation in IgAN.

Methods. Human proximal tubular epithelial cells (PTEC) were pre-treated with increasing concentration of a PPAR-γ agonist rosiglitazone or troglitazone (0–5 µM) followed by further incubation with the conditioned medium (IgA-HMC) collected from human mesangial cells (HMC) incubated with polymeric IgA isolated from IgAN patients. Gene expression of interleukin-6 (IL-6) and angiotensin II type 1 receptor (ATR1) were determined by real-time reverse transcription–polymerase chain reaction (RT–PCR); protein expression of IL-6 and ATR1 was determined by ELISA and western blot, respectively. The mitogen-activated protein kinase extracellular signal-related kinase (ERK1/2) activation was examined by western blot.

Results. An IgA-HMC conditioned medium prepared from IgAN patients increased gene expression and protein synthesis of IL-6 and ATR1 in PTEC when compared with a conditioned medium prepared from healthy controls. The upregulated gene expression and protein synthesis of IL-6 and ATR1 in PTEC induced by the IgA-HMC conditioned medium were readily attenuated following pre-treatment with a PPAR-γ agonist, thiazolidinedione (TZD). The ATR1-downregulating effect exerted by the PPAR-γ agonist occurred through the inhibition of ERK1/2 activation. The PPAR-γ antagonist, GW9662, significantly attenuated the inhibitory action of rosiglitazone on the increased synthesis of IL-6 and ATR1 protein.

Conclusion. Our current findings suggest that the PPAR-γ agonist attenuates excessive inflammatory response in activated PTEC in IgAN through suppressing ATR1 expression. This ATR1-downregulating effect is likely through the inhibition of ERK1/2 activation and is found to be PPAR-γ dependent. TZDs may possibly be new therapeutic additives to established treatment regime for renin–angiotensin system (RAS) blockade in IgAN.