Renal Transplantation Modulates Expression and Function of Receptors and Transporters of Rat Proximal Tubules

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Abstract. Kidney transplantation often leads to disturbances of solute and volume maintenance in humans. To investigate underlying mechanisms, expression and function of renal transporters and receptors of the proximal tubule (PT) were analyzed in an acute rejection model of rat kidney transplantation. Semiquantitative RT-PCR and Western blot, histology, immunohistochemistry, and microfluorometry were performed on whole kidneys and isolated PT. With acute rejection, Na+/H+ -exchanger type-3 (NHE-3) was markedly downregulated. Na+/HCO3–-cotransporter (NBC-1) and Na+/glucose transporter type-2 (SGLT2) were upregulated after transplantation. Expressions of Na+/H+ -exchanger type-1 (NHE-1), Na+/K+ -ATPase (NKA), angiotensin II (AngII) receptor (AT-1), or natriuretic peptide receptor (GC-A) were unaltered. Microfluorometric analyses of intracellular pH, Na+, and Ca2+ demonstrated a decrease in NHE-3 function and AngII-mediated stimulation of NHE-3. AngII-mediated inhibition of NHE-1 and function of all other transporters tested remained unaltered. Function of AT-1 and GC-A were unaffected. Reduced expression of NHE-3 was also confirmed by semiquantitative immunohistochemistry. These findings suggest that expression and function of transmembrane proteins involved in Na+-transport after transplantation and rejection is specifically modulated. The local renin-angiotensin-system is apparently not altered. Downregulation of NHE-3 may be a protective mechanism occurring in the graft.

After renal transplantation, patients often develop tubular disorders. Disturbances of Ca2+ and phosphate metabolism, changes in amino acid transport, and renal tubular acidosis have been described (1–3). Activation of glucose, Na+ and water reabsorption as well as of K+ secretion was demonstrated in the first 5 d after renal homotransplantation (4), and marked diuresis can occur within the first hours after surgery (5). We were interested in the mechanisms involved in these Na+ and water imbalances occurring shortly after transplantation. Possible reasons for this decreased renal function immediately after transplantation are denervation, ischemia, organ rejection, activation of the sympathetic nervous system, or changes in the renin-angiotensin-system (RAS). Long-term effects are mostly due to nephrotoxicity of immunosuppressants or renal artery stenoses (6–9). Posttransplantational disturbances diminish within weeks, while long-term lesions due to rejection and immunosuppression frequently are irreversible (10). Ischemia/reperfusion connected with increased Na+ excretion seems to bias prognosis of the transplant (11). Na+/H+ exchange is involved in regeneration of the transplanted kidney. Non-immunologic factors are increasingly regarded as important for the prognosis of the transplant. In humans, investigations of changes on the tubular level are hardly possible, and only a few functional studies in animals are available (12–20). No direct data exist on expression and function of transporters and receptors involved in transport after renal transplantation.

Transport activity in proximal tubules (PT) is regulated by hormones including angiotensin II (AngII) and atrial natriuretic peptide (ANP) (21,22). AngII affects Na+ transport via G-protein-coupled receptors (22–27). Low concentrations (<1 nM) stimulate Na+ reabsorption through Na+/H+ -exchanger type 3 (NHE-3) involving PKA (21,22) or PKC (28) and modulates NaHCO3 cotransport (NBC-1) (29,30) and Na+/K+ -ATPase (NKA) (31). AngII above 10 nM inhibits Na+/H+ -exchanger type 1 (NHE-1) due to PLA2 -mediated generation of arachidonic acid and P-450-monoxygenase (25,32). ANP inhibits AngII-stimulated Na+-transport (21).

Thus, changes of tubular transport immediately after kidney transplantation could be caused by changes in hormone secretion, expression of receptors or transporters, or variations in transporter activities.

We investigated the effect of transplant rejection on mRNA and protein expression and on functional activity and regulation of transporters involved in Na+-reabsorption in PT after transplantation. We present first data from PT segments isolated from rat kidneys up to 5 d after transplantation, demon-
strating predominant reduction in NHE-3 expression and function after kidney transplantation undergoing acute rejection.

**Materials and Methods**

**Kidney Transplantation**

Male Lewis-Brown-Norway (LBN) and Lewis (LEW) rats (250 to 300 g; Charles River, Sulzfeld, Germany) with free access to standard rat chow (Ssniff, Soest, Germany) and tap water were used. Experiments were approved by a governmental committee on animal welfare and were performed in accordance with national animal protection guidelines. Transplantation was performed as published in detail before (33). In short, the left kidney, including ureter, renal artery, a piece of the aorta, and renal vein, was transferred into the recipient for acute rejection, kidneys of LBN rats were transplanted into uninephrectomized LEW rats. Controls were the second kidney of LBN donors, the second kidney of the recipient, and syngeneically transplanted LBN kidneys.

**RT-PCR and Western Blot**

mRNA-expression was studied in whole kidney and isolated PT lysates. PT segments were enzymatically isolated as described in detail before (34). Total RNA was isolated using RNeasy-kit (Qiagen, Hilden, Germany) incubated with 10 U DNase I (Promega, Heidelberg, Germany) to digest genomic DNA. cDNA first strand synthesis was performed with 5 μg of total RNA, 10 nM dNTP-Mix (Biometra, Gottingen, Germany), 1 nM p(dT)10 nucleotide primer (Boehringer, Mannheim, Germany), and 200 U Moloney murine leukemia virus reverse transcriptase (MMLV-RT; Promega).

cDNA first strand reaction mixture was subjected to a PCR reaction in an UNO II thermocycler (Biometra) using 20 pmol of each primer (Table 1) and 1 U TaqDNA polymerase (Qiagen). Signals were sequenced by SeqLab (Gottingen, Germany). Semiquantification was done by comparing specific signals with an internal standard (GAPDH) amplified in parallel.

Proteins were separated by SDS-polyacrylamide (8%) electrophoresis and transferred to a PVDF membrane incubated with blocking-agent (Amersham, Freiburg, Germany). After primary antibody (Table 1) incubation membranes were covered with SuperSignal (Pierce, Bonn, Germany) before exposure (Kodak, Stuttgart, Germany). Semiquantification was performed by parallel blotting of specific signals and internal standard GAPDH.

**Histology and Immunohistochemistry**

Portions of kidneys were snap-frozen and fixed in 4% formaldehyde in PBS. Histologic changes were examined by light microscopy in paraffin-embedded tissue with specific staining. Glomerular accumulation of matrix proteins was demonstrated by periodic acid-Schiff, peritubular and glomerular fibrosis by Masson-Goldner.

Kryoslices were blocked with blocking agent (Roche, Mannheim, Germany), incubated with primary antibodies (Table 1) and secondary goat-anti-rabbit antibody (Vector, Dianova, Hamburg, Germany), incubated in Streptavidin, Alexa-Flour-594-Conjugate (Mobitec, Gottingen, Germany).

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**Table 1.** PCR primers and antibodies used for Western blot

<table>
<thead>
<tr>
<th>Primer</th>
<th>Base Sequence</th>
<th>Fragment Length</th>
<th>OAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHE-1 sense</td>
<td>GCC CCC TCC TCA TCC AGA CCA AC</td>
<td>365 bp</td>
<td>62°C</td>
</tr>
<tr>
<td>NHE-1 antisense</td>
<td>CCC TCC GTG GTA AAC CCC AGA AA</td>
<td>342 bp</td>
<td>60°C</td>
</tr>
<tr>
<td>NHE-3 sense</td>
<td>AGA AGC GGA GGA ATA GCA GCA T</td>
<td>872 bp</td>
<td>58°C</td>
</tr>
<tr>
<td>NHE-3 antisense</td>
<td>TCA GGC GGC GGA AGT TG</td>
<td>783 bp</td>
<td>57°C</td>
</tr>
<tr>
<td>NBC1 sense</td>
<td>GCC ATC ATC CTG TCC ATT CT</td>
<td>872 bp</td>
<td>58°C</td>
</tr>
<tr>
<td>NBC1 antisense</td>
<td>TTC TTC TTC TTC TCA TCC TCC TT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT1 sense</td>
<td>CAA CTC AAC CCA GAA AAA CAA A</td>
<td>888 bp</td>
<td>64°C</td>
</tr>
<tr>
<td>AT1 antisense</td>
<td>ACC CAG GAA AAG AAG AAA AG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC-A sense</td>
<td>AAA AAT TGT GGA CGG CAC CTG AG</td>
<td>544 bp</td>
<td>61°C</td>
</tr>
<tr>
<td>GC-A antisense</td>
<td>AGG CAG TAT CGG GGC ATC TTG AG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGLT2 sense</td>
<td>CGC CAT CAT TCT CTT CTT CTG CT</td>
<td>320 bp</td>
<td>60°C</td>
</tr>
<tr>
<td>SGLT2 antisense</td>
<td>CCT GCC GTA TTT TTT CCC TTT T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NKA sense</td>
<td>TTC CCC TAC TCC CTG TCC ATC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NKA antisense</td>
<td>CCT TCC CCG CTG TCG T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH sense</td>
<td>CAT CAA CGA CCC CTT CT CAG T</td>
<td>197 bp</td>
<td>56°C</td>
</tr>
<tr>
<td>GAPDH antisense</td>
<td>ACT CCA CGA CAT ACT CAG CAC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Antibody Source**

- **NHE-1**: BD Life Science Research (Heidelberg, Germany)
- **NHE-3**: Chemicon International (Hofheim, Germany)
- **NBC-1**: Alpha Diagnostic International (San Antonio, TX)
- **AT1**: Santa Cruz Biotechnology (Santa Cruz, CA)
- **NKA**: Upstate Technology (Lake Placid, NY)
- **GAPDH**: Trevigen Product Data (Gaithersburg, MD)

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*PCR-reaction conditions were as follows: 3 min at 94°C, 30 s at the optimal annealing temperature (OAT) and 1 min at 72°C, 1 cycle; 30 s at 94°C, 30 s at OAT and 1 min at 72°C, 30 cycles; 30 s at 94°C, 30 s at OAT and 10 min at 72°C, 1 cycle.*

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Isolation of PT Segments for Microfluorometry

PT (S1 to S2 segments) were mechanically isolated in MEM-EARLE medium (Biochrom, Berlin, Germany), transferred to a perfusion chamber, and fixed by two holding pipettes for microflurometry at the open end of tubules.

General Functional Data

Total kidney function, body weight, and BP (tail cuff plethysmography) were recorded daily. Twenty-four hours before surgery, animals were housed in metabolic cages. Urine and blood samples were analyzed for protein (Bradford Blue), creatinine (photometric kit, Enzym-Pap; Roche Diagnostics, Mannheim, Germany) and electrolytes by flame photometry (Instrumentation Laboratory 943, Kirchheim, Germany).

Chemicals and Solutions

During fluorescence measurements, PT were superfused at 10 ml/min with standard solution (37°C; 145 mM NaCl, 1.6 mM K2HPO4, 0.4 mM KH2PO4, 5 mM D-glucose, 1 mM MgCl2, 1.3 mM Ca2+-gluconate, pH 7.4) or a HCO3–/CO2-containing solution (110 mM NaCl, 25 mM NaHCO3, 3.6 mM KCl, 5 mM D-glucose, 1 mM MgCl2, 1.3 mM Ca2+-gluconate, pH 7.4) gassed with 5% CO2/95% air. To estimate activity of Na+/H+ exchange, the NH4+/NH3-pulse technique was used with 20 mM NaCl replaced by 20 mM NH4Cl.

2',7'-bis(2-carboxy-ethyl)-5(6)-carboxyfluorescein-acetoxymethyl ester (BCECF-AM), fura2-acetoxylmethyl ester (fura2-AM), ionomycin, and carbonylcyanid-m-chlorophenyl hydrazon (CCCP) were obtained from Sigma. ANP was provided by Niedersächsisches Institut für Peptid-Forschung, Hannover, Germany, and NHE-1–specific and NHE-3–specific inhibitors (HOE694 and S0100669) kindly provided by J. Puenter, Aventis Pharma Deutschland GmbH, Frankfurt.

Measurements of pHi, [Ca2+]i, and [Na+]i

PT were separately loaded with BCECF-AM (2 μM, 15 min), fura2-AM (5 μM, 30 min), or SBFI-AM (10 μM, 45 min) and excited at 488 and 436 nm (BCECF) or 340 and 380 nm (fura2, SBFI), respectively, as described before (34,35). Fluorescence was detected at 520 to 560 nm (BCECF) or 500 to 530 nm (fura2, SBFI) with a single photon-counting-tube (H3460–04; Hamamatsu, Herrsching, Germany). Calibration of pHi and [Na+]i, were performed with CCCP (1 μM) or nystatin (160 μM) (35), respectively, in separate experiments. Calibration of [Ca2+]i was attempted with the Ca2+-ionophore ionomycin (1 μM) (36).

Statistical Analyses

Functional experiments were performed with averaged pre- and post-control measurements for each experimental maneuver. Data were compared with two-sided unpaired, paired t test or ANOVA variance analysis for multiple comparisons where appropriate. Data are presented as mean values ± SEM (n = number of tubules, kidneys, or lysates). A P-value < 5% was considered statistically significant.

Results

General Functional Data

Blood and urine samples were collected 24 h before surgery/sacrifice. Serum values for Na+, K+, protein, creatinine clearance, BP, and body weight did not differ between the groups (Table 2). Urinary Na+ and K+ excretion decreased, and proteinuria was observed only on day 2 after transplantation, and urine volume increased up to day 4.

Table 2. Effects of transplantation on whole animal functional data

<table>
<thead>
<tr>
<th></th>
<th>LEW Rats Control</th>
<th>LBN Rats Control</th>
<th>LEW Rats 2 d.a.t.</th>
<th>LEW rats 4 d.a.t.</th>
<th>LBN rats 4 d.a.t.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>272 ± 7 (15)</td>
<td>258 ± 4 (21)</td>
<td>267 ± 6 (15)</td>
<td>265 ± 6 (15)</td>
<td>235 ± 2c (6)</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>107 ± 2 (15)</td>
<td>121 ± 6 (22)</td>
<td>101 ± 5 (7)</td>
<td>112 ± 6 (8)</td>
<td>119 ± 13 (6)</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>2.2 ± 0.3 (27)</td>
<td>2.5 ± 0.4 (26)</td>
<td>2.2 ± 0.5 (16)</td>
<td>2.0 ± 0.2 (15)</td>
<td>1.7 ± 0.3 (5)</td>
</tr>
<tr>
<td>Na+ in serum (mM)</td>
<td>141 ± 2 (12)</td>
<td>136 ± 1 (24)</td>
<td>137 ± 2 (16)</td>
<td>140 ± 1 (16)</td>
<td>142 ± 1c (6)</td>
</tr>
<tr>
<td>Na+ in urine (mM)</td>
<td>141 ± 13 (31)</td>
<td>122 ± 6 (32)</td>
<td>16 ± 3b (16)</td>
<td>88 ± 7b (15)</td>
<td>105 ± 15 (5)</td>
</tr>
<tr>
<td>FE Na (%)</td>
<td>0.33 ± 0.03 (12)</td>
<td>0.40 ± 0.05 (10)</td>
<td>0.07 ± 0.02b (10)</td>
<td>0.79 ± 0.3 (10)</td>
<td>0.50 ± 0.09 (5)</td>
</tr>
<tr>
<td>K+ in serum (mM)</td>
<td>8.2 ± 0.6 (12)</td>
<td>6.4 ± 0.4 (22)</td>
<td>6.2 ± 0.4 (15)</td>
<td>5.7 ± 0.2 (15)</td>
<td>6.7 ± 0.4 (6)</td>
</tr>
<tr>
<td>K+ in urine (mM)</td>
<td>290 ± 17 (30)</td>
<td>286 ± 12 (30)</td>
<td>57 ± 5b (15)</td>
<td>157 ± 18b (17)</td>
<td>238 ± 23 (5)</td>
</tr>
<tr>
<td>FE K (%)</td>
<td>19.2 ± 5.5 (10)</td>
<td>20.7 ± 2.4 (10)</td>
<td>9.73 ± 1.8b (10)</td>
<td>27.0 ± 3.5 (10)</td>
<td>25.5 ± 4 (5)</td>
</tr>
<tr>
<td>Protein in serum (mg/ml)</td>
<td>64 ± 3 (13)</td>
<td>56 ± 3 (7)</td>
<td>55 ± 2 (14)</td>
<td>54 ± 4 (13)</td>
<td>57 ± 0.6 (6)</td>
</tr>
<tr>
<td>Protein excretion (mg/24 h)</td>
<td>16.7 ± 1.3 (30)</td>
<td>16.4 ± 1.0 (29)</td>
<td>25.7 ± 5.0b (17)</td>
<td>14.4 ± 1.7 (17)</td>
<td>13.4 ± 1.3 (4)</td>
</tr>
<tr>
<td>Urine volume (ml/24 h)</td>
<td>14.2 ± 1.0 (13)</td>
<td>14.4 ± 0.6 (32)</td>
<td>22.4 ± 2.5b (17)</td>
<td>27.1 ± 4.0b (17)</td>
<td>15.4 ± 1.1 (5)</td>
</tr>
</tbody>
</table>

a Mean values ± SEM with the number of animals in brackets. Urine samples were collected from animals housed in metabolic cages for 24 h before transplantation (controls) or sacrifice 2 or 4 days after transplantation (d.a.t.). FENa and FEK indicate fractional excretion of Na+ and K+, respectively.

b Significantly different to LEW control rats.

c Significantly different to LBN control rats (P < 0.05).

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mRNA and protein expression was tested in whole kidney 1, 2, 4, and 5 d after transplantation, and mRNA expression in isolated PT 2 and 4 d after transplantation. Figure 1 summarizes the observed decreases in NHE-3 mRNA and protein expression and an early increase in NBC-1 mRNA expression with delayed increase in protein expression. mRNA expression for Na\(^+\)/H\(^+\)-glucose cotransporter type 2 (SGLT2) was unaltered except for a slight increase on day 1 (control: 0.71 ± 0.12; n = 5; day 2: 1.70 ± 0.17, n = 6). Due to lack of commercially available antibodies against rat SGLT2, Western blot experiments were not performed. Expression of NHE-1, NKA, AngII receptor (AT1), and guanylate cyclase receptor type A (GC-A) remained unaltered (Table 3).

To study whether changes in expression were restricted to transplanted kidneys or rejection, expression was also examined in the remaining second kidney of recipients and syngeneically transplanted kidneys. Figure 2 shows mRNA expression in right own kidneys of LEW recipients of LBN (acute rejection) and of syngeneically transplanted LBN kidneys (no rejection). Neither NHE-1 nor NHE-3 expression was significantly changed in both groups.

**Histology**

With acute rejection, perivascular edema with sparse cortical and medullary initial infiltrates of lymphocytes (day 1) and of immunocompetent cells (day 2) were found, without changes in vessels, glomeruli, and tubules (Figure 3). Glomerulitis, endothelialitis in larger vessels, and spots of peritubular interstitial infiltrates of blastoid lymphatic cells were diagnosed on day 4; on day 5, the whole renal parenchyma was excessively infiltrated by lymphatic cells, immunocompetent cells (tubulitis), severe glomerulitis, and endovasculitis were found. In syngeneically transplanted animals, no pathologic findings were diagnosed.

**Immunohistochemistry**

In immunohistochemical stainings, intensity of NHE-1 and NHE-3 paralleled the Western blot results. Figure 4 demonstrates unchanged NHE-1 expression restricted to the basolateral membrane at day 4, while NHE-3 expression was clearly reduced but restricted to the luminal membrane already at day 2 after transplantation. In five kidneys, significant decreases in stained tubules from 85 ± 3% to 70 ± 3% (day 2) and 67 ± 4% (day 4) after transplantation were seen.

**pH\(_i\) Measurements**

Posttransplantational basal pH\(_i\) in PT remained unchanged. Possible changes in basal activities of transporters and influence of GC-A on pH\(_i\) were studied in control and PT isolated from kidneys 2 and 4 d after transplantation (Table 4). The

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**Table 3. Changes in expression levels of mRNA and protein in the acute rejection model**

<table>
<thead>
<tr>
<th>Transmembrane Protein</th>
<th>PCR PT</th>
<th>PCR Kidney</th>
<th>WB Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHE-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NHE-3</td>
<td>↓ (D2 + 4)</td>
<td>↓ (D2–5)</td>
<td>↓ (D1–5)</td>
</tr>
<tr>
<td>NBC-1</td>
<td>—</td>
<td>↑ (D1–2)</td>
<td>↑ (D5)</td>
</tr>
<tr>
<td>SGLT2</td>
<td>0</td>
<td>↑ (D1)</td>
<td>—</td>
</tr>
<tr>
<td>AT1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GC-A</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>NKA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* PT, proximal tubule; WB, Western blot; 0, no effect; —, not tested; D1–5, day 1 to 5 after transplantation; ↓, decrease compared to control; ↑, increase compared to control.
NHE1-inhibitor HOE694 (1 μM) and the NHE3-inhibitor S0100669 (10 μM) caused only small decreases of resting pHᵢ, which were unaltered by transplantation. Inhibition of NBC-1 with DIDS (0.5 mM) increased basal pHᵢ with no effect of transplantation. Removal of HCO₃⁻/CO₂ rapidly acidified PT similarly in controls and after transplantation. The small acidification caused by ANP (10 nM) in controls was also not altered by transplantation.

AngII at 10 nM decreased pHᵢ similarly between the groups (Figure 5). AngII at 10 pM increased pHᵢ in PT from control kidneys, which was reversed to decreases at days 2 and 4. When acidifying PT (NH₄⁺-pulse) Na⁺/H⁺ exchange was activated.

Transplantation had no effect on pHᵢ recovery rates in the presence of the NHE-1-inhibitor HOE694 (Table 5), while it was reduced in the presence of the NHE-3-inhibitor S0100669 (10 μM, Figure 6). ANP (10 nM)–induced reduction in pHᵢ recovery rate was unaltered by transplantation (Table 5).

pHᵢ recovery increased in the presence of 10 pM AngII and decreased at 10 nM AngII (Figure 7). Comparable to effects on basal pHᵢ (Figure 5), stimulation of pHᵢ recovery by 10 pM AngII was decreased after 2 d and reversed to an inhibition after 4 d, with qualitatively identical effects in the presence (data not shown) or absence of HCO₃⁻/CO₂.

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Figure 2. mRNA expression of NHE-3 and NHE-1 in remaining own kidneys of Lewis recipients and syngeneically transplanted LBN kidneys. Two and four days after transplantation, neither NHE-3 (A) nor NHE-1 mRNA expression (C) in whole kidney lysates was significantly changed in right kidneys of Lewis recipients of LBN kidneys that underwent acute rejection. C = kidneys obtained from LBN donor rats. 2 and 4 = remaining own kidney of Lewis recipients of LBN kidneys obtained 2 or 4 d after transplantation (d.a.t.). mRNA and protein expression of NHE3 (B) and NHE-1 (D) of syngeneically transplanted LBN kidneys. C = kidneys obtained from LBN donor rats. 4 = LBN kidneys transplanted into LBN rats obtained 2 or 4 d after transplantation. Mean values ± SEM with number of animals examined in parenthesis. * Significantly different from controls, P < 0.05.

Figure 3. Composite photomicrograph demonstrating representative histologic lesions of control rats and acute rejection after transplantation of LBN kidneys into LEW-rats (PAS; magnification, ×200): Control kidney (A). Increased number of infiltrating cells 2 (B) and 4 d (C) after transplantation mediating the immunologic process of interstitial and vascular rejection.

In PT from recipient’s own kidneys and in syngeneically transplanted kidneys 2 and 4 d after transplantation, both pHᵢ recovery in the presence of the NHE-3-inhibitor S0100669 (10 μM) or the stimulatory effect of 10 pM AngII did not differ from controls (Figure 8).

[Ca²⁺]ᵢ and [Na⁺]ᵢ Measurements

Figure 9 summarizes concentration dependence curves of AngII effects on [Ca²⁺]ᵢ in PT before and 2 and 4 d after...
transplantation. Curves for peak increases or plateau values of 
\([\mathrm{Ca}^{2+}]_i\) after AngII did not differ between the groups.

Ouabain (NKA-inhibitor, 1 mM) and phlorizin (SGLT2-
inhibitor, 1 mM) caused reversible increases or decreases in 
\([\mathrm{Na}^+]_i\), respectively, which were not altered by transplantation.
Basal \([\mathrm{Na}^+]_i\) in control PT was 20 mM ± 1 (n = 53), which 
was unchanged after transplantation.

**Discussion**

The kidney has a major role in the regulation of salt and 
water balance; therefore, specific studies of renal transport 
function after transplantation are of principal relevance. In the 
human situation, identification of mechanisms involved in 
functional changes after kidney transplantation is complicated 
by the fact that these patients receive immunosuppression 
therapies. Certain immunosuppressants like cyclosporin A alter 
transport along the nephron. We have chosen animal models 
that allow to differentiate the effects of transplantation with 
and without rejection on tubular transport from those of an 
immunosuppression therapy. Furthermore, severe uremia, 
which occurs within a few days if both kidneys are removed 
before transplantation of an allogeneic kidney in the absence of 
immunosuppression, would again complicate the differentiation 
of the various factors involved in transplantation-related 
alterations in renal function. The present study, summarizing 
results from renal transplantations without any immunosuppres-
sion, needs to be compared in a further step with renal 
transplantations, including immunosuppression and bilateral 
nephrectomy.

In the acute rejection model of rat renal transplantation 
without immunosuppression, we observed specific changes in 
mRNA and protein expression as well as function of transport-
ers and hormone receptors, partially already occurring within 
24 h. In detail, with acute rejection mRNA and protein levels 
for NHE-3 were downregulated, while those for NBC-1 were 
upregulated. Other transporters (NHE-1, SGLT2, NKA) and 
hormone receptors (AT1, GC-A) showed unaltered expression.
Decreased NHE-3 expression was paralleled by decreased ac-
tivity and lack of AngII-mediated stimulation. These results 
favor reduced reabsorption of \([\mathrm{Na}^+]_i\) and H2O acutely after 
transplantation. Apparently changes in the RAS system are not 
involved, because AT1-expression and AngII-mediated in-
creases in \([\mathrm{Ca}^{2+}]_i\), were unaltered.

These findings differ from those for ischemia/reperfusion 
models (37) and suggest that changes in expression of proteins 
involved in tubular transport after transplantation are subjected 
to rejection-associated mechanisms. Marked histologic 
changes typical for rejection occurred in the grafts; therefore, 
decreased tubular function due to nonspecific degenerative 
changes must be considered. This however, is highly unlikely, 
because expression, activity, and regulation of transporters was 
selectively unaffected, reduced, or augmented. In another syn-
geneic rat kidney transplantation model without rejection, lim-
ited urinary concentrating ability and tubular damage was also 
reported (14). Ischemia after transplantation could be consid-
ered as a cause of delayed graft function (19,38), but ischemia/ 
reperfusion results in a different pattern of changes in expres-
sion levels of renal transporters and receptors compared with 
the present findings. Furthermore, the ischemic period was 
only 40 min in this model; therefore, the described functional
changes after transplantation are probably not primarily due to ischemia/reperfusion.

AngII regulates transepithelial Na\(^+\)/H\(^+\) reabsorption via Na\(^+\)/H\(^+\)\(-\)exchange and NaHCO\(_3\)\(-\)cotransport in PT (22,23,27–29,39). Na\(^+\)/H\(^+\)\(-\)exchange across the luminal membrane is mediated by NHE-3 (40,41), whereas NHE-1 in the basolateral membrane serves pHi regulation (42). With acute rejection, no changes of AT1 expression were found, indicating that cellular components of the RAS were unaltered. This unchanged expression was paralleled by unaltered AngII-mediated increases in [Ca\(^{2+}\)]\(_i\). The signaling pathway of AngII concentrations above 10 nM involves an increase in [Ca\(^{2+}\)]\(_i\) (43). This does not exclude changes in circulating AngII levels, which still could modify RAS-mediated functions.

Primary tubular effects of ANP and GC-A in the PT are inhibition of reabsorption of substrates, electrolytes, and volume (21,25,44,45). In the present study also, no significant changes in GC-A expression were found. Thus, the receptors of the two counterpart systems, AT1 and GC-A, were not primarily modified after kidney transplantation.

Due to unchanged expression levels of GC-A and AT1 in PT of transplanted kidneys, we were interested whether there is

### Table 4. Effects of HOE694 (NHE-1 specific inhibitor), S0100669 (NHE-3 specific inhibitor), DIDS (NBC-1 specific inhibitor), HCO\(_3\)/CO\(_2\) removal, and ANP on resting pHi of isolated PT

<table>
<thead>
<tr>
<th></th>
<th>HOE694 (1 (\mu)M)</th>
<th>S0100669 (10 (\mu)M)</th>
<th>DIDS (0.5 mM)</th>
<th>HCO(_3)/CO(_2) Removal</th>
<th>ANP (10 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−0.11 ± 0.02 (8)</td>
<td>−0.13 ± 0.03 (8)</td>
<td>0.26 ± 0.04 (10)</td>
<td>−0.86 ± 0.19 (7)</td>
<td>−0.07 ± 0.02 (11)</td>
</tr>
<tr>
<td>Day 2</td>
<td>−0.10 ± 0.01 (6)</td>
<td>−0.11 ± 0.01 (5)</td>
<td>0.31 ± 0.03 (11)</td>
<td>−0.92 ± 0.15 (12)</td>
<td>−0.11 ± 0.04 (5)</td>
</tr>
<tr>
<td>Day 4</td>
<td>−0.12 ± 0.02 (6)</td>
<td>−0.09 ± 0.02 (7)</td>
<td>0.37 ± 0.04 (11)</td>
<td>−0.88 ± 0.15 (12)</td>
<td>−0.11 ± 0.02 (7)</td>
</tr>
</tbody>
</table>

* Data represent changes in pHi as mean values ± SEM with the number of observations given in brackets. There were no statistical differences between the groups (ANOVA with posthoc Tukey test).

### Table 5. Effects of transplantation on pH\(_i\) recovery from NH\(_4\)+-induced acidification in the presence of the NHE-1 inhibitor or of ANP

<table>
<thead>
<tr>
<th></th>
<th>HOE694 (1 (\mu)M)</th>
<th>ANP (10 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−27 ± 5 (7)</td>
<td>−29 ± 8 (10)</td>
</tr>
<tr>
<td>Day 2</td>
<td>−21 ± 3 (7)</td>
<td>−21 ± 5 (9)</td>
</tr>
<tr>
<td>Day 4</td>
<td>−22 ± 7 (8)</td>
<td>−29 ± 6 (8)</td>
</tr>
</tbody>
</table>

* Data represent changes in pH\(_i\) recovery rates after NH\(_4\)+-induced acidification in % of control rates. Mean values ± SEM with the number of observations given in brackets. There were no statistical differences between the groups (ANOVA with posthoc Tukey test).

Figure 5. Effects of low and high concentrations of angiotensin II (AngII) on basal pH\(_i\) of proximal tubules (PT) isolated from kidneys of control rats and of kidneys 2 or 4 d after transplantation. While the inhibitory effect of 10 nM AngII (black bars) was unaltered after transplantation, the stimulatory effect of 10 pM AngII (open bars) was lost after transplantation. Mean values ± SEM with the number of observations given in brackets. * Statistical difference to the effects in PT from control kidneys (\(P < 0.05\)).

Figure 6. Changes in Na\(^+\)/H\(^+\) exchange activity (% inhibition) induced by the NHE-3 inhibitor S0100669 (10 \(\mu\)M) in PT isolated from kidneys of control rats and from transplanted kidneys 2 or 4 d after transplantation. Data represent effects on pH\(_i\) recovery rates after NH\(_4\)+-induced (20 mM) acidification. Mean values ± SEM with the number of observations given in brackets. * Statistical difference to the effects in PT from control kidneys (\(P < 0.05\)).
any change in AngII- or ANP-mediated regulation of Na\(^+\)/H\(^+\)-exchange in isolated PT after transplantation. ANP caused a small acidification of PT, which did not differ between tubules from control and transplanted kidneys. The inhibition of Na\(^+\)/H\(^+\)-exchange by ANP also remained unmodified. AngII displays different effects on renal Na\(^+\) and fluid retention (22,43,46). Concentrations of AngII below 1 nM activate several transporters including NHE-3, whereas concentrations of AngII above 10 nM have opposite effects. Ischemia/reperfusion in rat kidneys led to a decline in mRNA-expression of NHE-3 and an increase of NHE-1 (11,47). In the present study, NHE-3 expression was downregulated during acute rejection. By contrast to intense ischemia/reperfusion, NHE-1 expression was not changed with acute rejection, indicating a subtype-specific downregulation of Na\(^+\)/H\(^+\)-exchange. Finally, AngII-mediated stimulation (low concentration) of Na\(^+\)/H\(^+\)-exchange, due to NHE-3-activation, was lost after transplantation, whereas AngII-mediated inhibition (high concentration) of Na\(^+\)/H\(^+\)-exchange, probably reflecting an action on basolateral NHE-1 (22), remained unaltered. These findings suggest that the observed changes in AngII-mediated regulation of Na\(^+\)/H\(^+\)-exchange are due to a reduction in NHE-3 expression only and not in AT1, as this should have modified regulation of NHE-1 as well. Again, decreased NHE-3 activity was not observed in the recipients’ own kidney and only minor in syngeneically transplanted kidneys. This small decrease in NHE-3 expression probably reflects the small ischemia-in-
produced effect reported before (11,47), which significantly differs from the marked decrease seen after transplantation with acute rejection. These observations clearly indicate that reduced NHE-3 expression and function is not due to either systemic factors or ischemia/reperfusion damage. Interestingly, changed activity of NHE-3 has been shown to participate in acute and chronic hypertension in rats (48), making this transporter a prime target for changes in expression and function under pathophysiological conditions.

Conflicting results exist regarding NKA expression after ischemia/reperfusion. In rats after severe ischemic damage or reperfusion, NKA expression was significantly reduced (11,37,49); after mild ischemia, cortical NKA expression was unaltered, but the protein was also expressed in luminal membranes (50,51). The fact that neither NKA expression in whole kidney nor expression or function in PT were altered in the rejection model studied here supports again that ischemia/reperfusion was at best only of minor importance, probably due to the short cold and warm ischemic periods in these models. In contrast to the downregulation of NHE-3, NBC-1 was upregulated with acute rejection, suggesting that the observed changes are due to specific mechanisms and not to general damage. These slightly delayed changes in HCO3− transport in the PT after transplantation might hint at secondary changes due to possible transplantation-associated disturbances in the acid-base status of the animals. Such differential regulation of NBC-1 and NHE-3 will lead to reduction in energy-consuming Na+-transport and still helps to keep intracellular Na+ concentration low. Thus, this regulation could be a protective response of the traumatized kidney.

In rabbits, reduced mRNA expression of Na+-glucose transporters as a consequence of ischemia/reperfusion had been reported (52). We showed unchanged SGLT2 expression. Phlorizin-induced inhibition of SGLT, mostly represented by SGLT2 in S1/S2-segments (51), similarly increased [Na+]i in PTs from control and transplanted kidneys suggesting unaltered activity. The observed changes in our model, partially observed already 24 h after transplantation, are most likely not due to infiltration or histologic derangements, as these changes need more time to fully develop. Therefore, immediately after transplantation, multiple in part non-immunologic factors, like changes of the RAS and of the endothelins, may contribute to the regulation of the transporters observed in the present study.

Nevertheless, because, in contrast to findings in kidneys undergoing rejection, none of the observed changes in expression or function were significantly altered in the remaining second autochthonous kidney of the recipients nor in syngeneically transplanted kidneys, regulation of transmembrane proteins found in kidneys undergoing rejection are certainly triggered by rejection and most likely not consequences of systemic effects.

In conclusion, we demonstrate first, that expression of membrane proteins is specifically modulated early after transplantation, indicating that changes in expression and function in these models are not due to general necrosis or apoptosis. Second, our results differ from data obtained after ischemia/reperfusion. Third, the regulatory pattern seems not to be the consequence of a systemic effect. We suppose that changes in expression of transporters and receptors after transplantation and rejection are continuously modulated, which may influence the prognosis of the graft. These data suggest that transplantation with acute rejection leads to ischemia-independent downregulation of Na+ and H2O reabsorption in PT. Such a downregulation of the most important transport system primarily involved in Na+ reabsorption and consecutively solute and volume reabsorption within 24 h after transplantation leads to a significant decrease in energy consumption in this sensitive nephron segment. This mechanism could be an important step for the highly traumatized transplanted kidney to save energy and avoid further functional and structural damages. Thus, inhibition of NHE-3 in the donor organ before transplantation and immediately after transplantation may be advantageous for the recovery of renal function and, therefore, for the prognosis of the renal transplant.

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References


Table 6. Effects of transplantation on Na+-K+-ATPase and Na+-glucose cotransport

<table>
<thead>
<tr>
<th>Ouabain (1 mM)</th>
<th>Phlorizin (1 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31 ± 5 (7)</td>
</tr>
<tr>
<td>Day 2</td>
<td>22 ± 2 (9)</td>
</tr>
<tr>
<td>Day 4</td>
<td>30 ± 3 (7)</td>
</tr>
</tbody>
</table>

* Data represent changes in [Na+]i, as mean values ± SEM with the number of observations given in brackets. There were no statistical differences between the groups (ANOVA with posthoc Tukey test).


