Acute sodium overload produces renal tubulointerstitial inflammation in normal rats

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Introduction

- Tubular sodium reabsorption is a major determinant of renal oxygen consumption
- Renal tubular epithelial cell hypoxia associated with increased transport activity plays an important role in the development of structural damage in tubular cells

- The increase in either tubular reabsorption or glomerular filtration rate may impair the adequate renal oxygenation, favoring tubulointerstitial (TI) inflammation development
- Renal hypoxia upregulates adhesion molecules, cytokines, chemokines, leukocyte infiltration, and the production of reactive oxygen species (ROS)

- ROS act as second messengers in angiotensin II (ANG II) and transcription factor nuclear factor-kappa B (NF-kB) activation
- Recent reports indicate the importance of NF-kB activation in renal pathophysiology

- NF-kB stimulates the angiotensinogen gene, which is the precursor for local ANG II production
- Local ANG II is produced by tubular epithelial cells or macrophages at concentrations of 100- to 1000-fold higher than that of plasmatic ANG II
Local ANG II may induce the expression of other proinflammatory genes, chemokines, cytokines, adhesion molecules and angiotensinogen.

Local ANG II produces major TI inflammatory activity, enhances superoxide anion production.

**Aim of study**

- The present study was to determine whether acute sodium overload could trigger an inflammatory reaction in the tubulointerstitial (TI) compartment in normal rats.

**Materials and methods**

- Tracheostomy
- Urethane 10%
- Polyethylene-75 cannular
- T4 tube
### Methods

<table>
<thead>
<tr>
<th>Na+ Concentration</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15 M</td>
<td>G1</td>
</tr>
<tr>
<td>0.5 M</td>
<td>G2</td>
</tr>
<tr>
<td>1.0 M</td>
<td>G3</td>
</tr>
<tr>
<td>1.5 M</td>
<td></td>
</tr>
</tbody>
</table>

- **Infusion**: ISS
- **MAP 30 min**: NaCl
- **Blood sample**: MAP 30 min
- **Urine**: MAP 30 min

### Urine and blood measurements

- **Urine volume** was determined by gravimetry (urine density as 1.0 g/ml).
- **Na⁺, K⁺, and creatinine** (ml/min) in urine and plasma samples were measured by standard methods.
- **Plasma osmolality** (mOsmol/kg) was determined by freezing-point depression.

### Kidney processing and examination

- Left kidney was perfused with ISS through the abdominal aorta until the blood was washed out.
- The parenchyma presented a pale appearance.
- Harvested for light microscopy and immunohistochemical studies.

### Urine and blood measurements

#### Light microscopy and immunolabeling

- Tissues were fixed in phosphate-buffered 10% formaldehyde (pH 7.2)
- Embedded in paraffin
- Cut 3 µm
- Stained with hematoxylin-eosin and Masson's Trichrome
- Deparaffinized
- Rehydrated

#### Light microscopy and immunolabeling

- Endogenous peroxidase activity was blocked by treating with 0.5% H₂O₂ in methanol for 30 min.
- Local ANG II was detected using an anti-human antibody dilution of 1:200.
- Immunostaining using a commercially modified avidin–biotin–peroxidase complex technique, Vectastain ABC kit.
- Counterstained with hematoxylin.
Morphological analysis
- using a light microscope
- Ten consecutive microscopic fields (X 400)
- analyzed to evaluate morphological changes in glomeruli and tubules
- image analyzer Image-Pro Plus version 4.5 for Windows

Statistical analysis
- Kolmogorov and Smirnov method
- Newman–Keuls test
- mean ± s.e.m.
- Kruskal–Wallis test and Dunn’s multiple comparison test
- mean of % of positive staining area/mm² ± s.d.
- Static signifiant P < 0.05

Results

Table 1 Urine and blood measurements

<table>
<thead>
<tr>
<th></th>
<th>C (n=7)</th>
<th>G1 (n=6)</th>
<th>G2 (n=7)</th>
<th>G3 (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Flow (ml/min)</td>
<td>6.8±0.8</td>
<td>33.7±4.2*</td>
<td>39.1±5.3**</td>
<td>68.4±7.1***</td>
</tr>
<tr>
<td>Urinary Na+ (mEq/l)</td>
<td>51±1</td>
<td>309±19**</td>
<td>286±13**</td>
<td>29±16**</td>
</tr>
<tr>
<td>Plasmatic Na+ (mEq/l)</td>
<td>144±1</td>
<td>147±1</td>
<td>151±2**</td>
<td>159±2**</td>
</tr>
<tr>
<td>Urinary K+ (mEq/l)</td>
<td>246±16</td>
<td>129±19**</td>
<td>45±5**</td>
<td>30±2**</td>
</tr>
<tr>
<td>Plasmatic K+ (mEq/l)</td>
<td>2.9±0.1</td>
<td>2.7±0.1</td>
<td>2.8±0.1</td>
<td>3.3±0.1</td>
</tr>
<tr>
<td>Plasma Osmolality (mOsmol /kg)</td>
<td>303±2</td>
<td>301±4</td>
<td>307±5</td>
<td>320±5***</td>
</tr>
<tr>
<td>Fractional excretion Na (%)</td>
<td>8.1±6.03</td>
<td>3.3±6.1**</td>
<td>10.0±6.09***</td>
<td>16.3±1.4***</td>
</tr>
<tr>
<td>Degree of intracellular dehydration (%)</td>
<td>0.4±0.3</td>
<td>1.9±0.0**</td>
<td>4.1±0.5**</td>
<td>6.5±0.2***</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>8.5±1.5</td>
<td>9.3±1.5</td>
<td>8.4±2.8</td>
<td>8.0±2.8</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>77±2</td>
<td>76±5</td>
<td>77±3</td>
<td>81±3</td>
</tr>
</tbody>
</table>
Figure 1 | Control (C): Na⁺ 0.15 M (ISS); G1: Na⁺ 0.3M, G2: Na⁺ 1.0 M, G3: Na⁺ 1.5 M. *P < 0.05 vs basal period. Values are expressed as mean±s.e.m.

Figure 2 | Filtered load. Control (C): Na⁺ 0.15 M (ISS); G1: Na⁺ 0.5M, G2: Na⁺ 1.0M; and G3: Na⁺ 1.5M. *P<0.05; **P<0.01 vs basal period. Values are expressed as mean±s.e.m.

Figure 3 | Absolute sodium tubular reabsorption (NaTR). Control (C): Na⁺ 0.15 M (ISS); G1: Na⁺ 0.3 M, G2: Na⁺ 1.0 M, and G3: Na⁺ 1.5 M. *P<0.05; **P<0.01 vs basal period. Values are expressed as mean±s.e.m.

Figure 4 | Immunostaining of z-SMA in renal interstitium. (a) Quantitative representation of positive staining/mm², expressed as percentage ± s.d. Control (C): Na⁺ 0.15 M (ISS); G2: Na⁺ 1.0 M; and G3: Na⁺ 1.5 M. *P<0.05; **P<0.01 vs control; #P<0.01 vs G2.

Figure 5 | Immunostaining of TGF-β1 in TI renal tissue. (a) Quantitative representation of positive staining / mm², expressed as percentage ± s.d. Control (C): Na⁺ 0.15 M (ISS); G2: Na⁺ 1.0 M, and G3: Na⁺ 1.5 M. *P<0.05; **P<0.01 vs control; #P<0.05 vs G2.

Figure 6 | Immunostaining of RANTES in renal tissue. (a) Quantitative representation of positive staining/mm², expressed as percentage ± s.d. Control (C): Na⁺ 0.15 M (ISS); G2: Na⁺ 1.0 M, and G3: Na⁺ 1.5 M. *P<0.05; **P<0.01 vs control; #P<0.01 vs G2.
Discussion

- The experimental model characterized by saline overload showed undamaged glomerular function evidenced by normal or increased CC with no alterations in MAP and RBF.

- Hypertonic saline overload expands the extracellular fluid space by extracting water from the cells.

- The diagnosis of salt poisoning is usually based on an increase in serum sodium concentration above 160 mEq/l.

- The presence of immunostaining for inflammatory markers in our study was in agreement with the progressive sodium overload and increasing FE$_{Na}$ (G3 > G2 > G1).

- TGF-$
\beta$1 is a member of a family of five polypeptides that exert complex effects on organ development, cell growth and differentiation.

- TGF-$
\beta$1 associated with locally enhanced ANG II and extracellular matrix proteins that diminished by angiotensin-converting enzyme inhibition.
RANTES is a chemokine synthesized by epithelial and endothelial cells, as well as macrophages that contribute to massive recruitment of neutrophils and eosinophils to the inflammatory site.

RANTES, detected in tubular epithelium and glomerular and peritubular endothelium at high NaCl overload.

α-SMA is a smooth muscle cell cytoskeleton protein and a marker of trans-differentiation from fibroblast to myofibroblast.

Express α-SMA leading to extracellular matrix expansion, constitutes and early event in interstitial fibrogenesis.

Renal epithelial cells hypoxia

Increase in ROS, NF-kB and ANG II

Inflammatory process in tubular epithelium

Cytokines, chemokines and angiotensinogen gene

In the present study

We have observed a positive staining for ANG II and transcription factor NF-kB in rats with acute saline overload, a model well characterized by a depressed plasmatic renin activity.

Recent evidence has implicated NF-kB as an important osmosignaling molecule that is activated in response to hyperosmolarity in both renal medullar interstitial and endothelial cell.

The injury observed in G3 may not specifically be only a consequence of the excessive tubular sodium reabsorption but also due to cellular dehydration from cellular hyperosmolarity.

Further studies

The model of hypertonic expansion could be useful for the analysis of the therapeutic effects of anti-inflammatory agents.

Strategies for salt-sensitive hypertension treatment should be considered, not only for the use of drugs that lower blood pressure but also for the control of renal interstitial inflammation.
Our data provide insight into the inflammatory process during acute sodium overload not associated with hemodynamic alterations.

- **Filtered load** = จำนวนสารที่ถูกกรองออกที่glomerulus
  = GFR × concentration of plasma sodium

- **Fractional excretion** = จำนวนสารที่ขับทิ้งในปัสสาวะ/filtered load (0.5-1)

**RANTES**: Regulated upon Activation, Normal T-cell expressed and secreted