Renal Function in Mice: Effects of Volume Expansion and Angiotensin II

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Abstract. The present study was performed to validate a simple means for assessing renal function in anesthetized mice and to characterize the renal hemodynamic responses to acute volume expansion and how these responses are altered by concurrent angiotensin II (AngII) infusions. Inulin and para-aminohippurate clearances were used to assess GFR and renal plasma flow (RPF) in three groups of male C57Bl/6 mice anesthetized with inactin (100 mg/kg, intraperitoneally) and ketamine (10 mg/kg). To avoid the hypotension associated with repeated blood sampling, a single blood sample was taken after three timed urine collections. Renal function and mean arterial pressure (MAP) were measured under euvolemic conditions (2.5 μl/min, intravenously, n = 7) during isotonic saline volume expansion (12.5 μl/min, intravenously, n = 5) and during volume expansion with concurrent AngII infusion (5 ng/min · g, n = 5). MAP in the control group was 77 ± 2 mmHg; volume expansion alone did not change MAP significantly (83 ± 2 mmHg), but led to significantly greater values in both GFR and RPF (1.35 ± 0.14 versus 1.01 ± 0.1 ml/min · g and 11.26 ± 1.39 versus 6.29 ± 0.5 ml/min · g, respectively). Infusion of AngII during volume expansion led to significant elevations of MAP (100 ± 3 mmHg, P < 0.05) and prevented the increases in GFR and RPF elicited by volume expansion (0.77 ± 0.08 and 5.35 ± 0.48 ml/min · g, respectively). Volume expansion also elicited marked increases in absolute and fractional sodium excretion (6.1 ± 1.0 versus 0.62 ± 0.2 μEq/min · g and 3.1 ± 0.7 versus 0.4 ± 0.1%, respectively). AngII infusion attenuated the absolute and fractional sodium excretion responses to volume expansion (3.4 ± 1.2 μEq/min · g and 2.5 ± 0.5%, respectively). The present findings demonstrate that anesthetized mice exhibit marked renal hemodynamic and excretory responses to isotonic saline volume expansion. Concomitant AngII infusion attenuates these responses in spite of greater increases in arterial pressure.

Recent advances in transgenic and gene-targeted manipulations of the murine genome have provided new and potentially powerful approaches for studying the structural, biochemical, functional, and pathophysiologic roles of different genes and their products. Indeed, a vast array of gene knockout and functional, and pathophysiologic roles of different genes and their products. Nevertheless, a systematic description of the changes in renal hemodynamics has been infrequent because of the problems associated with assessing renal plasma flow (RPF) and GFR in mice. Recently, Gross et al. (11,12) defined the pressure-natriuresis-diuresis relationships in normotensive and deoxycorticosterone acetate-salt hypertensive mice. However, the absence of data for RPF and GFR prevented a more detailed assessment of the mechanisms responsible. Thus, there are still many uncertainties regarding normal baseline values for renal function in mice and the renal functional responses to experimental manipulations such as volume expansion and angiotensin II (AngII) infusions.

It has been shown that inhibition of endogenous AngII leads to natriuresis after extracellular volume expansion (ECVE) (13) and that maintaining intrarenal AngII levels by infusion of exogenous AngII minimizes natriuretic responses to ECVE (14,15). However, little information is available regarding renal functional responses to ECVE in mice, although Field et al. (16) evaluated the GFR, but not RPF, responses to isotonic volume expansion. In the present study, we determined the renal responses to ECVE with isotonic saline and the responses during concurrent administration of AngII. The small size of mice and their exquisite sensitivity to loss of even minor quantities of blood pose a challenge to clearance studies, which normally require sequential assessment of plasma concentrations of inulin and para-aminohippurate (PAH). In some studies, GFR has been assessed by using radioisotope-labeled inulin (16,17). To use standard colorimetric procedures, however, larger samples are needed, and we evaluated an approach that does not involve blood sampling until the end of the urine collections. Using this approach, we assessed RPF, GFR, and sodium excretory function in three groups of anesthetized mice: euvolemic mice, volume-expanded mice, and volume-expanded mice receiving AngII infusions.

Materials and Methods

The studies described were performed in accordance with the guidelines and practices established by the Tulane University Animal Care and Use Committee. Because the C57Bl/6 mice are commonly

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used as the genetic background for gene-targeted mutations, this strain was used to establish our baseline values. Male C57Bl/6 mice (Charles River, Wilmington, MA) were housed in a temperature- and light-controlled room and allowed free access to standard diet (Ralston-Purina, St. Louis, MO) and tap water. On the day of the experiment, mice weighing 22 to 29 g were anesthetized with a combination of Inactin (thiobutabarbital sodium, 100 mg/kg, intraperitoneally) and Ketalar (ketamine, 10 mg/kg). Suplemental doses of anesthesia (ketamine, 5 mg/kg, intramuscularly) were administrated as required. The mice were placed on a servo-controlled surgical table that maintained body temperature at 37°C, and a tracheostomy was performed. The animals were allowed to breath air enriched with O2 by placing the exterior end of the tracheal cannula inside a small plastic chamber into which humidified 95% O2/5% CO2 was continuously passed. We have found that this procedure, which has been shown to improve the stability of arterial BP of anesthetized mice, also improves the stability of arterial pressure of anesthetized rats. Systemic arterial pressure did not change significantly in any experiment, decreased the hematocrit. The rest of the values were not significantly different among the groups.

In our study, a single arterial blood sample was taken at the end of the third clearance period to avoid BP decreases during the urine collections due to sampling. Thus, it was necessary to establish that the urine samples used for our clearance measurements were collected under steady-state conditions. This was evaluated by comparison of infusion and excretion rates for inulin and PAH. For both inulin and PAH, there were no significant differences between the urine excretion rates and intravenous infusion rates during any of the periods. Under conditions in which excretion and infusion rates are in equilibrium, one can assume that steady state has been achieved and that plasma concentrations are remaining steady.

As shown in Figure 1, the ECVE group had a slightly but not significantly higher MAP than the euveleomic group (83 ± 2 versus 77 ± 2). However, the group with ECVE and concurrent AngII infusion exhibited significantly higher MAP compared with euveleomic and ECVE groups (100 ± 3 mmHg). Systemic arterial pressure did not change significantly in any

### Analytical Procedures

Blood and urine samples were analyzed for inulin, PAH, sodium, and potassium concentrations as reported previously (19). Inulin and PAH concentrations were measured using standard colorimetric techniques with 90 μl of plasma. Sodium and potassium concentrations were determined by flame photometry. Plasma osmolality was determined using vapor pressure osmometry. GFR was calculated from urine inulin and plasma inulin concentrations and urine flow. Similarly, PAH clearance was used as an index of glomerular filtration rate.

### Statistical Analyses

Results are expressed as mean ± SEM. Statistical comparisons among groups were performed using one-way ANOVA. Statistical significance was defined as P < 0.05.

### Results

Baseline values for body weight, kidney weight, hematocrit, plasma sodium and potassium concentrations, and plasma osmolalities for all three experimental groups are summarized in Table 1. As expected, the volume expansion, which averaged approximately 7 to 8% body weight during the course of the experiment, decreased the hematocrit. The rest of the values were not significantly different among the groups.

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### Table 1. Basal values for three groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n = 7)</th>
<th>Group 2 (n = 5)</th>
<th>Group 3 (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>24.8 ± 0.47</td>
<td>23.5 ± 0.48</td>
<td>26.1 ± 0.8</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>0.30 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47 ± 1</td>
<td>39 ± 1</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>Plasma sodium concentration (mEq/L)</td>
<td>149 ± 3</td>
<td>151 ± 2</td>
<td>144 ± 3</td>
</tr>
<tr>
<td>Plasma potassium concentration (mEq/L)</td>
<td>4.6 ± 0.8</td>
<td>3.4 ± 0.6</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>Plasma osmolality (mosmol/kg)</td>
<td>292 ± 4</td>
<td>293 ± 3</td>
<td>290 ± 5</td>
</tr>
</tbody>
</table>

*Group 1 is the euveleomic group, group 2 is the volume-expanded group, and group 3 is the volume-expanded Ang II-infused group.

*P* < 0.05 compared with group 1.
of the groups during the experimental periods, indicating cardiovascular stability of the anesthetized mice prepared for clearance experiments.

As shown in Figure 2, the ECVE group exhibited significantly higher GFR values compared with the euvolemic group and the AngII infusion group (1.35 ± 0.14 versus 1.01 ± 0.1 and 0.77 ± 0.08 ml/min · g). The ECVE group also had significantly higher RPF values based on PAH clearances (Figure 3) than the euvolemic or AngII infusion groups (11.26 ± 1.39 versus 6.29 ± 0.5 and 5.35 ± 0.48 ml/min · g). For both GFR and PAH clearances, there were progressive increases in these values during the three periods, indicating that the vasodilation elicited by the volume expansion continued during the time course of the study. The AngII infusions prevented the volume expansion-induced increases in RPF and GFR.

The ECVE group exhibited significantly higher total sodium excretion and urine flow (Figure 4) compared with the euvolemic and AngII infusion groups (6.1 ± 1.0 versus 0.61 ± 0.2 and 3.41 ± 1.22 μEq/min · g, P < 0.05). The sodium excretion did not change significantly during the course of the experiment in the euvolemic group (0.42 ± 0.17 to 0.73 ± 0.23 μEq/min · g). In addition to the increased sodium excretion occurring during the equilibration period, the ECVE group exhibited additional increases in sodium excretion during the course of the three urine collections (3.8 ± 0.6 to 9.2 ± 1.5 μEq/min · g, P < 0.05). The volume-expanded mice infused with AngII also exhibited significantly higher sodium excretions than the euvolemic group (3.41 ± 1.22 versus 0.61 ± 0.2 μEq/min · g); however, the concurrent AngII infusion prevented the progressive increases in sodium excretion that were observed in the volume expansion group during the three periods of urine collections (3.8 ± 0.4 versus 3.1 ± 0.4 μEq/min · g, NS).

As shown in Figure 5, the changes in fractional sodium excretion that occurred were similar to the changes in absolute sodium excretion. The ECVE group exhibited significantly higher fractional sodium excretion than the euvolemic group (3.13 ± 0.7 versus 0.37 ± 0.09%). Also, the volume-expanded mice infused with AngII had significantly higher fractional

![Figure 1. Mean arterial pressures during experimental periods under euvolemic conditions (■), volume-expanded conditions (▲), and volume-expanded plus AngII infusion (●). *P < 0.05 compared with other groups.](image1)

![Figure 2. GFR under euvolemic conditions (■), volume-expanded conditions (▲), and volume expansion with concurrent infusion of AngII (●). *P < 0.05 compared with other groups.](image2)

![Figure 3. Para-aminohippurate clearances under euvolemic conditions (■), volume-expanded conditions (▲), and volume expansion with concurrent infusion of AngII (●). *P < 0.05 compared with other groups.](image3)
sodium excretion than the euvolemic mice (2.47 ± 0.49 versus 0.37 ± 0.09%). In the euvolemic group, the fractional sodium excretion did not change during the course of the experiment (0.25 ± 0.09 to 0.47 ± 0.11%, NS). However, the ECVE group exhibited progressive increases in fractional sodium excretion during the three clearance periods (2.12 ± 0.44 to 4.26 ± 0.7%, P < 0.05). This increase in the fractional sodium excretion elicited by ECVE was prevented by AngII (2.73 ± 0.53 to 2.31 ± 0.47%, NS).

Absolute and fractional potassium excretion rates were also determined in the three groups. During euvolemic conditions, absolute potassium excretion rates ranged from 1.03 to 1.72 μEq/min * g, while the average fractional potassium excretion rates ranged from 22 to 24% of the filtered load. Fractional potassium excretion rates were not significantly altered by the volume expansion without or with the AngII infusions.

Discussion

The aim of present study was to evaluate a relatively simple approach to the measurement of renal hemodynamics in the anesthetized mouse and the responses to isotonic ECVE with and without concomitant infusion of AngII. For the evaluation of renal function in mice, it seemed particularly important to avoid blood sampling during the course of the urine collections needed for the clearance measurements. In initial pilot studies, we observed that there was often a hypotensive response after withdrawal of even small samples in the range of 50 to 100 μl. This hypotensive response thus raised the possibility of further activation of the sympathetic nervous system and pressor hormonal mechanisms that could reduce renal function. To minimize further activation of these compensatory vasoconstrictor and pressor mechanisms, the blood sampling was deferred until after completion of the urine collections. While the plasma concentrations can most accurately be used for the clearance calculations during the final urine collection period, we feel that the finding that the inulin and PAH excretion rates were equal to the corresponding infusion rates indicates steady-state conditions. This methodologic approach is simple, does not require modification of the analytical methods, and does not require the use of radioisotopes, which have been used in some
previous studies (16). This approach allows routine compar-
isation of renal function among groups of mice, which is often the main requirement when comparing genetically manipulated animals with their corresponding wild-type controls.

It is well known that AngII plays a key role in the regulation of renal hemodynamics and tubular function (20). It has also been shown that volume expansion leads to suppression of plasma and kidney AngII (21) and that inhibition of endoge-

ous AngII participates in the natriuresis after acute volume expansion (13). Furthermore, it has been shown that main-
tained AngII concentrations by infusion of exogenous AngII reduces the responses to volume expansion (14,15). Because these results have been obtained in rats, it seemed important to characterize the renal functional responses to ECVE in mice and to determine whether AngII also exerts a major influence on these responses. In the present study, ECVE led to increases in GFR and PAH clearance and proportionally greater in-

creases in sodium excretion. In our study, the isotonic solution used for expansion contained only 1% albu-
moin, so it is likely that decreases in colloid osmotic pressure contributed to the increases in osmolality and renal abnormalities in angiotensinogen-deficient mice by the human renin and human angiotensinogen genes. J Clin Invest 99: 1258–1264, 1997


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