A comparative study of renal function in the desert-adapted spiny mouse and the laboratory-adapted C57BL/6 mouse: response to dietary salt load

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Dickinson H, Moritz K, Wintour EM, Walker DW, Kett MM. A comparative study of renal function in the desert-adapted spiny mouse and the laboratory-adapted C57BL/6 mouse: response to dietary salt load. Am J Physiol Renal Physiol 293: F1093–F1098, 2007. First published July 11, 2007; doi:10.1152/ajprenal.00202.2007.—The spiny mouse (Acomys cahirinus) is a nocturnal species native to regions of Egypt and Israel, where it inhabits sandy deserts and rocky terrains (20). In stark contrast to typical rodents in which development of major organs is incomplete at birth and continues well into the neonatal period, organogenesis of the spiny mouse kidney (9), liver (13), lung (16), and various brain regions (4–6) are completed during the 40-day period of fetal life, and thus spiny mice are born relatively mature.

We recently demonstrated that young adult male spiny mice, compared with the similarly sized C57BL/6 mouse, have significantly smaller kidneys with 36% fewer nephrons (9). The glomeruli of the spiny mice are, however, significantly larger than those of C57BL/6 mice, resulting in an equivalent total glomerular volume despite the smaller overall size of the kidney (9). Spiny mice also have a large renal medulla and long renal papillae compared with the C57BL/6 mouse (9), consistent with other desert species (1, 2). Furthermore, conscious mean arterial pressure (MAP) is significantly lower in spiny mice than in C57BL/6 mice (10, 14).

In the present study the functional significance of these renal structural and hemodynamic differences between the spiny mouse and the C57BL/6 mouse were examined. In addition, we chose to examine the effect of a high-salt diet on blood pressure and renal function in these two species. Specifically, we hypothesized that because of the reduced total nephron number and smaller kidney size, the high-salt diet would be a greater hemodynamic challenge to the spiny mouse compared with the C57BL/6 mouse.

MATERIALS AND METHODS

Animals

All experiments were approved in advance by the Monash University Department of Physiology and Department of Anatomy, and Cell Biology Animal Ethics Committees and were conducted in accordance with the Australia Code of Practice for the Care and Use of Animals for Scientific Purposes. The male spiny mice used in this study were obtained from our own laboratory colony as previously described (9). Adult male C57BL/6 mice were obtained from Monash University Animal Services.

Experimental Groups

These experiments involved three separate groups of 13- to 15-wk-old male spiny and C57BL/6 mice to determine basal renal function and examine the effects of high salt. Because the diet of spiny mice in the wild generally contains high levels of salt (18), a salt diet of 10% NaCl was chosen to effectively challenge the spiny mouse kidney.

Experiments performed under anesthesia. Group 1 mice (n = 6/species) acted as controls and remained on a normal salt diet [0.25% (wt/wt) NaCl; Specialty Feeds, Perth, WA, Australia] until they underwent surgical preparation for renal function measurements under anesthesia (7). Group 2 mice (n = 5/species) were placed on a high-salt diet [10% (wt/wt) NaCl] for 1 wk before renal function measurements were taken under anesthesia.

Experiments performed in conscious mice. Group 3 mice (n = 6/species) were exposed to one 24-h urine collection period on the normal salt diet to obtain basal measurements. All mice were then placed on the high-salt diet for 1 wk and exposed to two 24-h urine collection periods taken on days 3 and 7.

Blood Pressure and Renal Function Experiments Under Anesthesia

Mice from groups 1 and 2 were anesthetized (isoflurane mixed with 40% O2-60% N2, 4.5% induction, 2.5–2.8% maintenance; Rhodia Australia, Notting Hill, VIC, Australia) and placed on a servo-controlled heated pad to maintain body temperature at 37.5°C. A...
catheter (tapered SV-35 tubing) was inserted into the left carotid artery for continuous measurement of blood pressure and heart rate (HR) and to obtain a terminal arterial blood sample. The left jugular vein was catheterized (PE-10 tubing) for infusion of a 1% bovine serum albumin solution in saline containing $^{3}$H inulin (5.58 µCi/ml) and $^{14}$C paraaminohippurate (PAH; 1.7 µCi/ml) to maintain normal fluid balance and estimate glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), respectively, by using renal clearance methods (7). The bladder was catheterized to allow the collection of urine, and the mice were allowed to equilibrate for 1 h. Following equilibration, urine was collected over two timed 15-min periods, after which an arterial blood sample (~300 µl) was taken. The amounts (disintegrations per minute) of $^{3}$H inulin and $^{14}$C PAH in the urine and plasma were determined using a liquid scintillation analyzer (Tri-Carb 1900CA; Packard Instrument, Downers Grove, IL) for estimation of GFR and ERPF. Animals were killed at completion of the renal function measurements, and kidney mass was obtained.

**Urine Collection and Analysis**

Mice from group 3 were placed in mouse-specific metabolic cages (Scientific Glassware, Faculty of Medicine, University of Melbourne) to obtain urine over a 24-h period. Mice were habituated to the metabolic cages on two occasions, 1 and 3 days before urine collection. Three measurements were taken in each mouse, first under basal conditions with all animals on a normal salt diet and then on days 3 and 7 of the high-salt diet treatment. Body mass, water and food consumption, and feces and urine excretion were also recorded. Data obtained for day 3 of the high-salt diet did not differ from that obtained on day 7, and thus only day 7 values are presented. Urine was collected and frozen for subsequent analysis of urinary electrolytes.

All urine samples were analyzed for Na$, Cl^-$, K$, and urea by spectrophotometry (Synchron CX5CE Delta; Beckman Coulter, Fullerton, CA). Osmolality of urine and plasma samples (taken at completion of renal function measurements) was measured by freezing point depression (Advanced Osmometer 2020; Advanced Instruments, Needham Heights, MA).

**Data Analysis and Statistics**

Data are means ± SE. Two-way ANOVA with diet ($P_{\text{diet}}$) and species ($P_{\text{species}}$) as the fixed factors was used to determine whether the impact of the salt diet on renal variables was different between the species. All statistical tests were performed using the computer-based statistics program SPSS, and $P$ values <0.05 were accepted for statistical significance.

| Table 1. Renal function and mass parameters of anesthetized male spiny and C57BL/6 mice on normal or high-salt diets |
|-----------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Normal-salt diet groups |                      | High-salt diet groups |                      |                   |                 |                   |                 |
|                 | Spiny              | C57BL/6           | Spiny              | C57BL/6           | Spiny              | C57BL/6           | Spiny              | C57BL/6           |
| Prediet BM, g   | 35.8 ± 0.4        | 30.0 ± 1.4        | 35.7 ± 0.9        | 26.5 ± 0.6        |                  |                 |                  |                 |
| Postdiet BM, g  | 36.8 ± 0.6        | 31.1 ± 1.4        | 37.1 ± 0.6        | 26.8 ± 0.5        |                  |                 |                  |                 |
| Change, g       | 0.9 ± 0.3         | 1.1 ± 0.2         | 1.4 ± 0.6         | 0.3 ± 0.3         |                  |                 |                  |                 |
| KM, mg          | 268 ± 11          | 406 ± 17          | 295 ± 10          | 358 ± 12          |                  |                 |                  |                 |
| KM/BW, mg/g     | 7.3 ± 0.2         | 13.1 ± 0.2        | 7.9 ± 0.2         | 13.4 ± 0.5        |                  |                 |                  |                 |
| MAP, mmHg       | 62.0 ± 1.5        | 80.0 ± 2.4        | 67.4 ± 1.7        | 84.0 ± 2.8        |                  |                 |                  |                 |
| HR, beats/min   | 472 ± 8           | 540 ± 8           | 464 ± 16          | 527 ± 20          |                  |                 |                  |                 |
| GFR, µl/min     | 134 ± 4           | 175 ± 21          | 246 ± 15          | 179 ± 14          |                  |                 |                  |                 |
| ERPF, µl/min    | 426 ± 18          | 692 ± 49          | 678 ± 46          | 606 ± 59          |                  |                 |                  |                 |
| RVR, mmHg·µl$^{-1}$·min$^{-1}$ | 0.082 ± 0.003 | 0.065 ± 0.004 | 0.058 ± 0.004 | 0.081 ± 0.009 |                  |                 |                  |                 |
| Filtration Fraction, % | 32 ± 2 | 25 ± 2 | 37 ± 3 | 30 ± 1 |                  |                 |                  |                 |
| Posm, mosmol/kgH2O | 332±5 | 315±6 | 365±12 | 326±5 |                  |                 |                  |                 |

Values are means ± SE; $n = 6$ per species on normal salt diet and 5 per species on high-salt diet; NS, nonsignificant; BM, body mass; KM, kidney mass; MAP, mean arterial pressure; HR, heart rate; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; RVR, renal vascular resistance; $P_{\text{osm}}$, plasma osmolality.

**RESULTS**

**Blood Pressure and Renal Function and the Effect of High Salt**

Renal function and mass parameters of spiny and C57BL/6 mice on normal salt and high-salt diets are shown in Table 1. Spiny mice weighed significantly more but had markedly smaller kidneys than C57BL/6 mice. Thus kidney-to-body mass ratio (KM/BM) of spiny mice was 50% of that of C57BL/6 mice ($P_{\text{species}} < 0.001$; Table 1). C57BL/6 mice on the high-salt diet weighed significantly less than those maintained on the normal salt diet, reflecting a lower body mass at the start of the study with no difference in the change in body mass in the four groups over the 1-wk diet treatment period (Table 1).

Irrespective of the level of dietary salt, anesthetized spiny mice had a significantly lower MAP and HR than C57BL/6 mice ($P_{\text{species}} < 0.001$; Table 1). On a high-salt diet, both spiny and C57BL/6 mice had a significantly higher MAP (8 and 5%, respectively) than mice on a normal salt diet ($P_{\text{diet}} = 0.05$; Table 1). Spiny mice had a slight but significantly lower hematocrit (normal salt: 43.71 ± 0.01%; high salt: 43.55 ± 0.01%) than C57BL/6 mice (normal salt: 45.50 ± 0.01%; high salt: 44.58 ± 0.01%; $P_{\text{species}} < 0.05$). There was no difference between the normal and high-salt groups. Plasma osmolality was greater in spiny mice than in C57BL/6 mice ($P_{\text{species}} < 0.001$) irrespective of the level of dietary salt. Both species had higher plasma osmolalities following the high-salt diet ($P_{\text{diet}} < 0.01$; Table 1), with the effect similar in both species (Table 1).

Spiny and C57BL/6 mice had a similar GFR on the normal salt diet, which was significantly higher for both following the high-salt diet ($P_{\text{diet}} < 0.001$). The difference between normal salt and high-salt values was, however, greatest in spiny mice (183 vs. 103% in C57BL/6 mice, $P_{\text{int}} < 0.01$, where $P_{\text{int}}$ is the $P$ value for the interaction of species and diet; Table 1). On normal salt diet, ERPF and effective renal blood flow (data not shown) were lower in spiny mice than in C57BL/6 mice ($P_{\text{species}} < 0.05$). Spiny mice had a much higher ERPF on the high-salt diet compared with those on the normal salt diet ($P_{\text{int}} < 0.01$; Table 1). Irrespective of the level of dietary salt,
spiny mice had a significantly greater filtration fraction (FF) than C57BL/6 mice ($P_{\text{species}} < 0.01$; Table 1). Both species had greater FF on the high-salt diet ($P_{\text{diet}} < 0.05$), with the effect similar in both species (Table 1).

Renal vascular resistance (RVR) was similar between the groups; however, spiny mice on the high-salt diet had a significantly lower RVR compared with those maintained on the normal salt diet, consistent with the higher ERPF ($P_{\text{int}} < 0.01$; Table 1).

**Urinary Excretion Profiles of Conscious Animals Before and After 7 Days of High Salt**

There was no effect of the 7-day high-salt diet on the body mass of either spiny (pre-salt: $34.9 \pm 0.3$ g; after high salt: $35.2 \pm 0.4$ g) or C57BL/6 mice (pre-salt: $29.6 \pm 0.9$ g; after high salt: $28.7 \pm 0.9$ g). Food intake was similar between species and did not change with the high-salt diet (Fig. 1A). Both species showed a reduction in feces weight after 7 days on the high-salt diet with a greater reduction in C57BL/6 mice ($P_{\text{int}} < 0.01$; Fig. 1B). This effect was due to reduced water content of the feces (data not shown). C57BL/6 mice consumed significantly more water than spiny mice ($P_{\text{species}} < 0.001$; Fig. 1C). On the high-salt diet, spiny and C57BL/6 mice increased their water consumption ($P_{\text{diet}} < 0.001$), but the effect was greater in C57BL/6 mice with a 3.5-fold increase compared with only a 2.5-fold increase in spiny mice ($P_{\text{int}} < 0.01$; Fig. 1C). C57BL/6 mice produced more urine than spiny mice, particularly in response to the high-salt diet, where C57BL/6 mice showed a sixfold increase compared with only a fourfold increase in spiny mice ($P_{\text{int}} < 0.01$; Fig. 1D).

The high-salt diet led to significant and similar increases in 24-h Na$^+$ and Cl$^-$ excretions in both spiny and C57BL/6 mice ($P_{\text{diet}} < 0.001$; Table 2). However, urinary concentration of Na$^+$ and Cl$^-$ increased more markedly in the spiny mice such that the concentrations of these electrolytes reached were almost twice those in C57BL/6 mice ($P_{\text{int}} < 0.01$ and 0.001 for Na$^+$ and Cl$^-$, respectively; Table 2). Urinary K$^+$ concentrations and 24-h excretions were similar between species, and both species showed a reduction in K$^+$ concentrations following 7 days of the high-salt diet ($P_{\text{diet}} < 0.001$). Urinary urea concentrations were significantly higher in the spiny mice irrespective of the level of dietary salt ($P_{\text{species}} < 0.001$). Both species showed a similar, marked decrease ($\sim 70 – 80\%$) in urinary urea concentrations after 7 days of the high-salt diet ($P_{\text{diet}} < 0.001$). Urinary urea excretion in both species tended to be increased after the high-salt diet, but this did not reach statistical significance ($P_{\text{diet}} = 0.06$; Table 2).

Urinary osmolality was significantly higher in spiny mice irrespective of the level of dietary salt ($P_{\text{species}} < 0.01$; Table 2). Both species showed a similar decrease in urinary osmolality after 7 days of the high-salt diet ($P_{\text{diet}} < 0.01$; Table 2). Total urinary osmolar excretions were similar between species, and both species showed a marked increase ($> 300\%$) following 7 days of the high-salt diet ($P_{\text{diet}} < 0.001$; Table 2).

**DISCUSSION**

This study describes for the first time the basal and activated (high salt load) renal function of the spiny mouse and compares it to that of the commonly studied laboratory C57BL/6 mouse. Under normal (basal) conditions, the spiny mouse has a lower
MAP and ERPF and a tendency for a lower GFR. On a high-salt diet, both species show a modest increase in MAP, but spiny mice showed a large increase in GFR and ERPF, a reduced RVR, and a reduced drinking response compared with the C57BL/6 mouse.

Low Basal MAP, ERPF, and GFR in the Spiny Mouse

The key differences in function of the anesthetized spiny mouse compared with the C57BL/6 mouse were a lower MAP, consistent with our previous findings of conscious MAP (10), and a lower ERPF. GFR tended to be lower in spiny mice under normal salt conditions, although this did not reach statistical significance. As reported in this study and previously (9), spiny mice have a significantly reduced kidney mass and volume, and fewer but larger glomeruli, compared with C57BL/6 mice (9). This is likely to affect the filtration surface area and capacity for glomerular ultrafiltration of the kidney. These structural and functional differences may account for the tendency toward a lower total GFR maintained by the spiny mouse at baseline. As a rough estimate of a single-nephron GFR (SNGFR), we divided the GFR in both species (present study) by their respective total number of nephrons (9). The calculated values obtained for SNGFR tended to be higher in spiny than in C57BL/6 mice, ~18 and 15 nl/min, respectively. Thus the apparently reduced total GFR in the spiny mouse likely reflects a lower number of nephrons rather than a reduced GFR per nephron. With the increased filtration surface area afforded by large glomeruli, one might have expected a larger SNGFR than that calculated for the spiny mouse; however, this might be offset by the lower systemic pressure and thus, presumably, a lower glomerular capillary pressure in the spiny mouse. Presently, there is no micropuncture data of the intraglomerular dynamics of the spiny mouse kidney.

Renal Blood Flow Response to High-Salt Diet

The key to maintaining Na⁺ and water homeostasis is the ability of the kidney to respond to changing Na⁺ loads. In response to the high-salt diet, both species demonstrated an increased filtration fraction, but this was apparently generated by different means. C57BL/6 mice on the high-salt diet demonstrated an increase in RVR associated with a small decrease in ERPF and little change in GFR, whereas the spiny mouse showed a significant decrease in RVR coupled with a large increase in both GFR and ERPF. The marked increase in GFR of the spiny mouse on the high-salt diet indicates larger falls in preglomerular resistance, increases in glomerular capillary pressure and ERPF, and decreases in total RVR. Changes to the glomerular capillary filtration coefficient $K_f$ cannot be ruled out in either species, and micropuncture experiments would be needed to precisely elucidate the mechanisms underlying the different responses in these species. If we calculate the SNGFR for these two species after exposure to the high-salt diet, we find little change for the C57BL/6 mouse at 16 nl/min, whereas the spiny mouse shows marked hyperfiltration of the glomeruli with a SNGFR of 34 nl/min.

Increased Urine Concentrating Ability or Altered Drinking Response in the Spiny Mouse?

We might have expected that the spiny mouse would respond to the salt load by increasing the urine osmolality quite significantly, perhaps to urinary osmolality values greater than 4,500 mosmol/kgH₂O as seen in some desert rodents (11), but this was not the case. The urinary osmolality of the spiny mouse after salt was not significantly different from that on normal salt. Furthermore, the spiny mouse maintained a higher plasma osmolality than the C57BL/6 mouse under both normal and high-salt conditions (although more markedly in the latter). This may suggest that the spiny mouse is able to maintain a higher plasma Na⁺ concentration than the C57BL/6 mouse, and so the thirst-provoking mechanism may be less sensitive than that of the C57BL/6 mouse. In response to the same high-salt load, the C57BL/6 mouse increased water consumption 3.5-fold, compared with 2.5-fold in the spiny mouse. This is likely a reflection of the environmental conditions experienced by the spiny mouse, since water is not readily available in the desert and high-salinity vegetation or invertebrates are often the only available source of nutrients and water (18). In support of an altered drinking or thirst response in this species is a study by Czech and Vander Zanden (8), who found that the spiny mouse is dipsogenically insensitive (relatively) to peripherally admin-
istered angiotensin II and that large doses were needed to elicit a drinking response from the spiny mice. Therefore, the major difference in the ability of the two species to adapt to a markedly increased salt intake resided predominantly in the ability of the spiny mouse to tolerate a larger increase in plasma osmolality, with a reduced (compared to C57BL/6) stimulation of water intake. We suggest that this study has therefore indirectly identified an altered drinking or thirst response in the spiny mouse.

Consistent with being a water-saving species, there is a significant difference in the estimated free water reabsorption between spiny and C57BL/6 mice after the high-salt diet. On the normal-salt diet, free water reabsorption is similar between species at ~7 ml/day. On the high-salt diet, estimated free water reabsorption in the spiny mouse is ~25 ml/day, whereas that in the C57BL/6 mouse is ~18 ml/day. This represents a 7 ml/day saving of free water in the spiny mouse. Another measure of water-saving ability is the urine-to-plasma osmolality ratio. In general, a higher urine-to-plasma osmolality ratio indicates a greater capacity to save water (22). The spiny mouse maintains an almost constant urine-to-plasma osmolality ratio on the normal salt (6.98) and high-salt diets (5.91), whereas the C57BL/6 mouse varied considerably between the normal (6.46) and high-salt diets (2.97). It must be noted that these calculations of urine-to-plasma osmolality and free water reabsorption are not within individual animals but are the means of animals after 7 days of high-salt from groups 1 (urine) and 3 (plasma). Therefore, under normal conditions, both species maintain a relatively similar water balance; however, following a high salt load, the spiny mouse maintains the concentration of its urine, whereas the C57BL/6 mouse does not.

There are likely several anatomical and hormonal differences between these two species that allow for such different responses to a high salt load, and these may include the architectural organization and transport properties of the different nephron segments, as well as the hormonal responses to thirst and drinking. Architecturally, the organization of the tubules and vascular bundles inside the renal medulla are recognized to play a key role in the concentration of urine and vary markedly between species (1). Furthermore, renal medullary thickness and loop length have been used as a general index of an organism’s ability to generate hypertonic urine (3).

To date, the evolutionary advantage of fewer but larger glomeruli as seen in the spiny mouse is unclear. One could speculate that a normal (nonhypertrophied), large glomerulus, as in the spiny mouse, may be more metabolically efficient. The spiny mouse is known to have a metabolic rate ~16% lower than predicted for its body mass (18). This is common among desert species, and it is thought that a low metabolic rate (i.e., a low rate of heat generation) also implies a saving of water that would otherwise be required to dissipate this heat (21) and is also thought to help maintain a low requirement for food, which, like water, is very scarce in the desert environment (17). It may be that by having fewer nephrons and a reduced cortex-to-medulla ratio, the spiny mouse effectively reduces the proportion of metabolically demanding proximal tubules and increases its proportion of medullary “water-saving” tubules.

This study has also highlighted the potential for significant hyperfiltration of the larger spiny mouse glomeruli in response to the high salt load, which was not present in the smaller C57BL/6/11011 glomeruli, suggesting that these larger glomeruli may play a key role in maintaining water balance for this species. Whether spiny mice also show an increased renal functional reserve is currently unknown.

**Future Direction**

Further investigations should include a comparison of the central circulating and renal concentrations of key renal and thirst provoking hormones, such as renin, vasopressin, AVP, and angiotensin II in these species, under different physiological conditions, as well as a description of the density and distribution of urea and water (aquaporin) transporters, which are known to be crucial in urinary concentrating ability (12, 23, 24). Furthermore, micropuncture studies should be utilized to precisely elucidate the intrarenal hemodynamics in this species. It would also be interesting to further test the capacity for increased filtration of the spiny mouse kidney by using a protein or amino acid load to examine the renal functional reserve and examine plasma urea levels and the fractional excretion of urea under these conditions.

**Summary**

The results of the present study highlight the efficiency of the spiny mouse kidney in filtering and excreting a remarkably high concentration of salt. The C57BL/6 mouse was also able to excrete the excess ingested salt but required a much larger volume of water to do so. Thus we have identified a different hemodynamic relationship, locally at the kidney and centrally in the drinking or thirst response, in these two species in response to a high salt load.

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