Sexual dimorphism in renal function and hormonal status of New Zealand genetically hypertensive rats

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Abstract. Renal water and electrolyte handling and related plasma hormone levels were measured in male and female New Zealand genetically hypertensive and normotensive rats, in an attempt to establish any potentially important sex-related differences in these parameters. Male hypertensive rats had higher blood pressure than female hypertensive rats, but normotensive rats showed no such sex difference. Both groups of males had higher fluid turnover rates than respective females, and this was associated with raised plasma vasopressin in hypertensive males. Female hypertensive rats excreted more sodium, potassium and chloride in association with lower plasma aldosterone and higher corticosterone levels compared with the other groups. Plasma electrolytes did not differ between the four groups, but plasma osmolality was higher in hypertensive than normotensive rats of both sexes. A higher rate of electrolyte loss and lower fluid turnover in association with reduced plasma vasopressin may contribute to the lower blood pressure of female compared with male hypertensive rats.

It has been widely reported that in human essential hypertension males develop higher levels of blood pressure than females. Similarly in genetic models, hypertension is more severe or develops more rapidly in the male than in the female (1,2). However, there are few reports in the literature which have investigated potential sex differences in the hormones of body fluid homeostasis which may contribute to the maintenance of blood pressure. It is apparent that gonadal hormones play a role in determining the different levels of blood pressure achieved by males and females. Studies in the spontaneously hypertensive rat (SHR) have shown that castration of males at the pre-hypertensive stage resulted in lower blood pressure levels in the adult animal, similar to those shown by untreated females (2). Treatment of neonatal female SHR with androgens subsequently resulted in blood pressures similar to those of untreated males (2) and, conversely, administration of estrogen to males resulted in blood pressures similar to those of untreated females (3). Thus, the sexual dimorphism observed in the development of hypertension relies, in part, on the neonatal gonadal hormone complement. This may in turn contribute to the many potentially important sex-related differences in the secretion of the hormones which contribute more directly to body fluid homeostasis and thus may influence the severity of hypertension achieved.

Before specific study to assign a causal role it is essential that sex differences in these endocrine regulators of cardiovascular and renal function are fully described. Accordingly, in this study we have measured the circulating levels of important renally active hormones and have attempted to relate these measures to the patterns of renal handling of water and electrolytes and arterial blood pressure in males and females of a genetic model of hypertension, the New Zealand genetically hypertensive rat and its normotensive control.

A preliminary account of these data was presented to the Society for Endocrinology (4).
Material and Methods

Animals

New Zealand genetically hypertensive (NZGH) and normotensive (NZN) rats bred at Manchester University were maintained on a 12 h light: 12 h dark photoperiod and had free access to food (Labsure PMD diet, Biosure, Cambridgeshire, UK). Electrolyte content mmol/g Na⁺ 0.1±0.01, K⁺ 0.25±0.02, Cl⁻ 0.38±0.15) and water throughout the study.

Water and electrolyte management

Adult (20-25 weeks) male (N=5 per strain) and female (N=9 per strain) NZN and NZGH rats were housed individually in metabolism cages (Jencons, Herts, UK) and, following a 7-day equilibration period, 24-h water and electrolyte turnover were monitored for 5 consecutive days. Urinary sodium, potassium (Corning model 455 flamphotometer) and chloride concentration (Corning chloride analyzer 925), and osmolality (Roebling Automatic Osmometer, LH Roebling, Berlin FRG) were measured in 5-ml aliquots of the daily urine collection. Systolic blood pressure was measured by tail cuff plethysmography as described previously (5) (Narco Bio-systems Inc, Houston, TX).

Plasma electrolytes and hormones

Trunk blood samples were collected by decapitation into EDTA and heparin-treated tubes from groups of unanesthetised NZN (male N=25, female N=20) and NZGH rats (male N=25, female N=20). The separated plasma was stored at −20°C prior to the measurement of hormone levels by specific radioimmunoassays. Vaginal smears were taken from the female animals and stained with hematoxylin and eosin (BDH Ltd, Dorset, UK) to assess the phase of the estrus cycle. The adrenal glands of male (NZN N=6, NZGH N=9) and female (NZN N=9, NZGH N=10) animals were also removed, cleaned of connective tissue and the wet weight measured. Blood samples were also collected, by cardiac puncture, from Sagatal® (0.1 ml/100 g body weight sodium pentobarbitone 60 g/l, May and Baker Ltd, Dagenham, UK) anesthetised male (NZN N=6, NZGH N=9) and female (NZN N=9, NZGH N=10) normotensive and hypertensive rats for the determination of plasma sodium, potassium and chloride concentrations and plasma osmolality, without the potential artefactual changes in electrolyte composition resulting from tissue damage inevitable when collecting blood by decapitation (6).

Hormone radioimmunoassays

Arginine-vasopressin. Plasma concentration was measured, following initial extraction with acetone and petroleum ether, using the method described by Ashton & Balment (5). Inter- and intra-assay coefficients of variation were 14.6 (N=12) and 17.1% (N=10), respectively.

Oxytocin. Plasma oxytocin was measured using the method described by Chard & Forsling (7). Inter- and intra-assay coefficients of variation were 8.3 (N=16) and 4.1% (N=6), respectively.

Aldosterone. Following separation from other steroids by LH2O chromatography, plasma aldosterone was measured using the method described by Milne et al. (8). Inter- and intra-assay coefficients of variation were 12.7 (N=42) and 14.4% (N=10), respectively.

Corticosterone. Plasma corticosterone was measured by radioimmunoassay of ethanol extracted samples as described by Kime (9). Inter- and intra-assay coefficients of variation were 10.1 (N=30) and 13.6% (N=10), respectively.

Angiotensin II. Following Sep-Pak extraction (C18 cartridges Waters Associates, MA), plasma angiotensin II levels were measured using the method described by Morton & Webb (10) and Düsterdieck & McElwee (11). (Antiserum kindly donated by Dr JJ Morton, MRC Blood Pressure Unit, Glasgow). Inter- and intra-assay coefficients of variation were 4.6 (N=6) and 4.4% (N=12), respectively.

Atrial natriuretic hormone. Following Sep-Pak extraction, plasma levels were measured using a commercial rANH radioimmunoassay kit (Amersham International plc, Bucks, UK). The intra-assay coefficient of variation was 4.0% (N=10).

Statistical Analysis

All values are presented as the mean ± SEM. Statistical comparisons between the strains and sexes were achieved by ANOVA and Student-Newman-Keuls (SNK) range test analysis.

Results

Blood pressure

As expected, systolic blood pressure was higher in hypertensive rats compared with normotensive animals (p<0.001). Hypertensive rats showed a sex-related difference in blood pressure, males having significantly higher blood pressure than females (NZGH male, N=5, 158.0±3.4 vs female, N=9, 147.8±2.5 mmHg, p<0.05), which was not observed in the normotensive group (NZN male, N=5, 94.0±3.7 vs female, N=9, 89.7±1.6 mmHg).

Water and electrolyte management

Since there were sex- and strain-related differences in the body weights of age-matched animals (NZGH male, N=5, 246.2±12.4 g vs female, N=9, 211.2±5.9 g, p<0.05, NZN male, N=5, 319.0±7.6
Table 1.
Daily water turnover, plasma osmolality and electrolyte balances (dietary intake – urinary excretion) in male and female New Zealand normotensive and genetically hypertensive rats: comparisons between the sexes in each strain are indicated by *p<0.05, **p<0.01.

<table>
<thead>
<tr>
<th></th>
<th>Normotensive</th>
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<th>Hypertensive</th>
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<tbody>
<tr>
<td></td>
<td>Male (5)</td>
<td>Female (9)</td>
<td>Male (5)</td>
<td>Female (9)</td>
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<tr>
<td>N</td>
<td></td>
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<td></td>
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<tr>
<td>Water turnover</td>
<td></td>
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<tr>
<td>Intake (ml · (100 g body weight)⁻¹ · (24 h)⁻¹)</td>
<td>14.1±0.5</td>
<td>**9.4±0.6</td>
<td>18.0±1.1</td>
<td>**9.6±0.9</td>
</tr>
<tr>
<td>Urine output (ml · (100 g body weight)⁻¹ · (24 h)⁻¹)</td>
<td>2.9±0.1</td>
<td>3.5±0.1</td>
<td>3.8±0.2</td>
<td>**5.0±0.4</td>
</tr>
<tr>
<td>Urine osmolality (mosm/kg)</td>
<td>2098.5±76.2</td>
<td>2059.8±84.3</td>
<td>1908.8±106.7</td>
<td>1883.4±136.3</td>
</tr>
<tr>
<td>Plasma osmolality (mosm/kg)</td>
<td>301.2±1.5</td>
<td>303.3±0.7</td>
<td>307.8±1.4</td>
<td>307.3±1.3</td>
</tr>
<tr>
<td>Electrolyte balance (μmol · (100 g body weight)⁻¹ · (24 h)⁻¹)</td>
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<tr>
<td>Sodium</td>
<td>412.4±20.4</td>
<td>**241.4±39.7</td>
<td>459.0±44.5</td>
<td>**121.0±22.9</td>
</tr>
<tr>
<td>Potassium</td>
<td>1053.5±33.5</td>
<td>**819.7±75.3</td>
<td>1291.6±48.2</td>
<td>**402.5±70.9</td>
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<tr>
<td>Chloride</td>
<td>1644.6±38.2</td>
<td>**484.2±51.4</td>
<td>2129.1±71.8</td>
<td>**331.3±51.8</td>
</tr>
</tbody>
</table>

g vs female, N=9, 246.3±6.9 g, p<0.001), the standardisation of metabolism cage data with respect to body weight was appropriate for comparison between groups. However, it should be noted that while males of both strains were still growing fast (c. 10 g body weight gain) during the five-day study period, female NZN rats gained weight more slowly (c. 7 g body weight gain) and hypertensive females showed no significant change in body weight.

Water intake was greater in both groups of male animals, compared with their respective females. NZGH males, however, also drank (p<0.01) significantly more fluid than male NZN rats, though no such strain difference was evident in female animals. Although there was no sex difference in urine outputs of normotensive animals, NZGH females produced significantly more urine than NZGH males and the normotensive rats. Water balance (intake-urine output) was consistently greater for males than females (Table 1). The urinary osmolality was similar in all groups of animals. In accordance with their higher urine outputs, therefore, osmolar excretion (urine flow × urine osmolality) was higher in NZGH females than any other group. This raised total solute excretion was consistent with the higher excretion rates of sodium, potassium and chloride in NZGH females by comparison with the other three groups (Fig. 1). This did not reflect greater food intake by NZGH females, which showed similar food intakes to those of NZGH males and NZN females (NZGH female, N=9, 7.7±0.2 g · (100 g body weight)⁻¹ · (24 h)⁻¹, NZGH male, N=5, 7.2±0.1 g · (100 g body weight)⁻¹ · (24 h)⁻¹, NZN female, N=9, 9.7±0.2 g · (100 g body weight)⁻¹ · (24 h)⁻¹). NZN males ate significantly less than any other group (6.0±0.1 g · (100 g body weight)⁻¹ · (24 h)⁻¹, p<0.01). Thus for NZGH females electrolyte balances (intake – urine loss) were considerably lower than for NZGH males (Table 1). A similar sex difference in electrolyte balances was evident in normotensive rats, but it was notable that NZGH females showed lower balances for sodium, potassium and chloride than all other groups.

There were no significant differences between either the strains or sexes in plasma electrolyte concentrations and although plasma osmolality (Table 1) was higher in hypertensives than normotensives, there were no differences between sexes.

Plasma hormones

There were several important sex- and strain-related differences in the levels of plasma hormones.
Fig. 1.
24-h urinary electrolyte excretion rates (µmol · (100 g body weight)⁻¹ · (24 h)⁻¹) in male (open bars) and female (hatched bars) hypertensive (NZGH, male N=5, female N=9) and normotensive (NZN, male N=5, female N=9) rats. Statistical comparisons (SNK range test) between males and females (asterix above columns) and between strains (asterix linking columns) are indicated by **p<0.01.

measured (Table 2). Male NZGH rats had a higher plasma AVP concentration than NZGH females and NZN males (p<0.05). Interestingly, normotensive females showed levels comparable with those in NZGH males and higher than in NZN males. Plasma oxytocin did not differ between the groups

Table 2.
Plasma hormone concentrations in male and female New Zealand normotensive and genetically hypertensive rats. Comparisons between the sexes of each strain are indicated by *p<0.05, **p<0.01. Number of animals is given in parentheses.
(vaginal smears showed that the majority of female rats used for the measurement of oxytocin were in the estrus phase of the ovarian cycle). Aldosterone levels were comparable in male and female normotensive rats, but were much higher in NZGH males than NZGH females. Corticosterone presented a different pattern, normotensive males showing higher levels than females, while the converse was observed in the hypertensive animals. The higher plasma corticosterone concentration in female hypertensives was associated with a greater adrenal mass (adrenal wet weight mg/100 g body weight NZGH female, N=10, 55±5 cf NZGH male, N=9, 29±3, NZN male, N=6, 21±1, NZN female, N=9, 16±1, p<0.01). Plasma angiotensin II concentration was higher in male than female NZN rats, while levels were similar in both sexes of NZGH rats. Atrial natriuretic hormone was elevated to a similar extent in male and female hypertensive rats compared with normotensive animals (p<0.05). The sex difference in the NZN rats, females having higher (p<0.01) plasma ANH levels than males, was not evident in hypertensive animals.

Discussion
The higher blood pressure of the NZGH male rat compared with the NZGH female and the reduced body weight of hypertensive rats compared with normotensive rats are both in accord with previous observations (1,12). There were apparent differences between both the strains and sexes in terms of renal water and electrolyte management. Both hypertensive and normotensive male animals drank more and appeared to excrete renally less of their water intake than their female counterparts. The NZGH males appeared to retain more fluid (high balance) despite having a higher urine output than the NZN male. This observation is, perhaps, surprising in view of previous reports which described reduced plasma volume (13,14), extracellular fluid volume (13), and total body water in NZGH animals (14). However, in these experiments animals were anesthetised with ether or in the case of total body water measures were killed by ether overdose, which may diminish comparability with our study on the conscious animal.

The apparent retention of water by NZGH males is clearly in accord with the greater plasma AVP levels observed in these rats compared with NZN males, although Crofton et al. (15) reported that 13-14 week male NZGH and NZN rats had similar plasma AVP levels. It appears that NZGH males may have difficulty in excreting an accrued water load. Simpson et al. (16) showed that conscious NZGH rats retain more water and sodium upon isotonic saline loading than NZN rats. We observed a similar retention of sodium with hypotonic saline loading (17).

The high urine flow rate of NZGH males has also been observed in hypertensive men, despite raised plasma AVP (18). This has been perported to reflect changes in renal function in the hypertensive state. An increase in glomerular filtration rate (GFR) is perhaps one of the more obvious mechanisms which could facilitate such a diuresis. Although the NZGH female does not exhibit altered GFR by comparison with NZN females (19), no data are available to our knowledge which describe GFR measures in the NZGH male rat. Other potential mechanisms include an insensitivity to AVP, although we found no supportive evidence for this in the anesthetised, hypotonic saline-infused NZGH rat preparation (17). The increased ANH levels observed, which are associated with high blood pressure in humans (20) and are commonly reported in models of hypertension (21), may contribute to the increased urine flow rate of the NZGH male. The NZGH females also had increased plasma ANH concentrations and higher urine outputs than NZN females, but these were associated with reduced plasma AVP in the hypertensive group. Clearly, a detailed study is required to establish the potential contributions of altered GFR, AVP and ANH to the abnormal renal water handling of hypertensive rats and the differing patterns in males and females.

The sex differences in water and electrolyte balances (intake – excretion), evident in both hypertensive and normotensive rats, appear to reflect their relative growth rates. The greater growth in males was associated with higher levels of water and electrolyte retention from the dietary intake. The hypertensive females were characterised by particularly low water and ion balances in association with their absence of growth. The high rates of urinary electrolyte excretion shown by the NZGH female, a factor shared with hypertensive women (18), was the most notable feature of the pattern of urinary electrolyte excretion observed in the four groups of animals. The relative natriuresis shown by these animals was associated with aldosterone levels reduced by 50% compared with the NZGH
male. The reason for this lower plasma aldosterone concentration in females is not immediately clear, since angiotensin II levels were comparable with those in males. The raised ANH levels observed in hypertensive rats would be expected to suppress aldosterone secretion directly and to block angiotensin II-induced secretion (22), but ANH concentrations were comparable in male and female NZGH animals. Our observations in the female NZGH rat of higher corticosterone and increased adrenal mass compared with males are in accord with the report by Iams et al. (23) in the spontaneously hypertensive rat. Undoubtedly, further information regarding the control of adrenal steroidogenesis in male and female NZGH rats is essential to clarify this intriguing picture.

The measures of renal water and electrolyte turnover and endocrine secretion described in this study give some indication of the altered physiology which may contribute to the differing blood pressures observed in hypertensive males and females. The greater renal sodium loss and reduced fluid turnover of the female compared with the male are perhaps important factors which could influence long-term maintenance of elevated blood pressure. Clearly the contribution, direct or indirect, of altered circulating hormone levels, particularly vasopressin and the adrenal steroids, to the divergence in blood pressures achieved in the two sexes should also be considered.

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