An experimental rat model of salt-sensitive hypertension; biochemical and morphological parameters and sympathetic nervous system

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ABSTRACT

The objective of the study was to outline the characteristics of the development of hypertension and some neurohumoral, haematological and morphological factors contributing to development of high blood pressure in a genetic model of salt-sensitive rat. Characteristics of Dahl salt-sensitive (DS) rats, as compared to their Dahl salt-resistant (DR) controls were as follows: 1) DS rats display higher blood pressure and lower heart rate compared to DR rats as early as 1 month of age at weaning). They gradually develop hypertension at 2 months of age, irrespective of diet. Low-Na diet (0.5 % NaCl) does not prevent hypertension but delays its development and ameliorates it. High Na-diet (8 % NaCl) exacerbates hypertension. 2) DS rats have retardation in body weight gain. They develop mild hypochromic anaemia. 3) After 2 months of Na loading (3 months of age), DS rats express significantly increased Na and water retention and increased plasma volume by 15 % compared to 2.8 % increase in DR rats on high-Na diet. 4) DS rats showed renal parenchymal lesions, more pronounced after Na-loading, focal atrophy of cortical tubules, mesangial matrix expansion and glomerulosclerosis. Consistent with high blood pressure were changes in renal arterioles, fibromuscular proliferation, deposition of fibrinoid material in intima. 5) Sodium loading produced increased activity of the sympathetic nervous system (SNS), and sodium restriction reduced SNS responsiveness.

Key words: Dahl rat, pathogenesis of hypertension, salt-resistance, salt-sensitivity.

Somova L I, Channa M L, Khan M S **An experimental rat model of salt-sensitive hypertension; biochemical and morphological parameters and sympathetic nervous system**. *Journal of the South African Veterinary Association* 70(1): 14–17 (En.). Department of Human Physiology and Physiological Chemistry, University of Durban Westville, Private Bag X54001, Durban, 4000 South Africa.

INTRODUCTION

In the early 1960s Dahl *et al.*[3] selectively bred rats for susceptibility (DS rats) or resistance (DR rats) to the hypertensive effect of a high-salt diet (8 % NaCl). In the DR line the level of dietary salt intake had little effect on blood pressure, but in the DS line increased dietary salt caused markedly elevated blood pressure[15]. These 2 lines therefore provide an interesting model for the interaction of an environmental factor (salt) with genotype. The response to salt is inherited and polygenic. It has been estimated, using quantitative genetic techniques, that approximately 2–4 loci are involved in determining the variation in response to blood pressure difference between DS and DR rats[10]. Some of these loci have recently been defined[1,8,16].

Despite extensive studies of these genetically salt-sensitive rats[3,15], many controversial results have been reported; for example, there is a common misconception that DS rats develop high blood presure only when fed excess salt[26]. There is also controversy about the age at which DS rats become hypertensive[17] and the pathogenetic mechanism of developing high blood pressure, *e.g.* Na-water retention/ volume expansion[22]. However, there is conclusive evidence for the inability to excrete sodium by the kidney[3,26]. Some experiments showed[5,24] that an intact sympathetic nervous system (SNS) is required for DS rats to respond to salt with an increase in blood pressure[12].

The objective of the study was to study the contribution of some neurohumoral factors and SNS activity to development of high blood pressure in Dahl DS and DR rats. Since in many ways the DS rat is a good model for humans[11,21] who exhibit salt-sensitivity, we anticipate that our data will provide guidelines for future studies on salt sensitivity.

MATERIALS AND METHODS

Experimental animals and housing

The procedures followed were approved by the Ethics Committee of the University of Durban-Westville. Principles of laboratory animal care (WIH publication 85-23, revised in 1985) were followed.

Dahl DR and DS rats were imported from Sprague Dawley Inc, USA. Forty-eight, 1-month-old male weanling rats, from DR and DS lines respectively, were randomly divided into 4 experimental groups, 12 DR rats on low-Na diet (Group 1); 12 DR rats on high-Na diet (Group 2); 12 DS rats on low-Na diet (Group 3) and 12 DS rats on high-Na diet (Group 4). Twenty-four DR and DS (12 rats each) weanling, untreated rats were sacrificed at the beginning of the experiment as controls of the above experimental groups. All animals were exposed to a 12 h light/dark cycle and were accommodated in individual Nalgene metabolic cages, in order to monitor daily food and fluid intake, urine excretion and collection for electrolyte analysis. A week before starting the diets and during the last week of the experiment, on the basis of food and water consumption, urine excretion and sodium analysis, sodium- and water-balance was calculated. The averaged results of the 5 consecutive days' balance calculations are presented.

Diets

The low-Na diet contained 85 mmol Na/kg diet, and the high-Na diet 680 mmol Na/kg diet. The diets were prepared by Truka Inc., Johannesburg, and contained the correct mineral and vitamin mix, according to the prescribed formula by the American Institute of Nutrition and South African Research Programme for Nutritional Intervention. The rats were maintained after weaning on these 2 diets during the 2-month duration of the experiment.

Blood pressure measurement

Blood pressure and body weight were monitored twice a week. Before monitoring, rats were trained for 5 consecutive days for daily recording, so that stress reactions were minimised and reliable values obtained. A tail-cuff computerised blood pressure monitor (IITC Life Sciences 31,USA) was used. The method is accepted and used in all laboratories dealing with rodent hypertension[2]. It

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Received February 1998. Accepted January 1999.

works with IITC hardware blood presure system that determines both blood pressure and heart rate. The system employs an automatic scanner and pump, sensing cuff and amplifier to measure and count the pulse rate in the animal tail. The results were displayed as data plots and summary data of systolic, diastolic and mean blood pressure and heart rate on the computer screen. Small, medium and large restraining devices and 2 different tail cuffs (10 mm and 15mm) were used to compensate for the increase in body weight during the 8-week programme. Five readings were averaged for each rat per session. Owing to the sensitivity of the method, no prolonged preliminary warming of the animals was necessary, but a constant room temperature of 26 °C was maintained. According to Bunag[2], any tail-cuff method selected for chronic monitoring of blood pressure must first be tested for accuracy under the same experimental conditions that would exist when it is later used for actual measurements. Before being used routinely, the method should always be validated in the laboratory where it will be used.

We performed the validation of the method in a preliminary study by simultaneous (direct left common carotid artery) and indirect (tail-cuff method) measurements of anaesthetised (40 mg/kg body weight sodium thiopentane) animals. The simultaneous measurement on 6 male Wistar normotensive rats and on 6 Dahl hypertensive rats (body weight 260 g), showed a very good correlation between direct and indirect blood pressure measurement. For systolic blood pressure $r = 0.96$; for diastolic blood presure $r = 0.67$, and for heart rate $r = 0.87$. We consider the method very accurate for systolic blood presure measurement. The direct arterial pressure monitoring in the carotid artery was performed by using a low-volume pressure transducer (Statham, Model CP-01, Beckman, USA) and a Siemens Mingograph 62 recorder.

Electrolyte and catecholamine estimation

Urine samples were acidified to prevent mineral precipitation, and sodium, potassium and chloride were analysed by using a Beckman Synchron EL-ISE Electrolyte System. An ion-selective glass electrode is used in conjunction with a reference electrode to measure changes in potential due to ion (sodium, potassium) activity in diluted samples (indirect potentiometry). The rest of the urine samples were frozen and sodium and potassium were re-analysed by atomic absorption spectroscopy (Varian, Australia)[7] to compare and validate the above new method for routine analysis.

At the conclusion of the study, rats were fasted overnight and sacrificed after anaesthesia (40 mg/kg body weight sodium thiopentone i.p.) by exsanguination *via* cannulated left carotid artery for routine haematology and electrolyte analysis. Serum electrolytes were analysed using both methods described above, *e.g.* electrolyte analyser and atomic absorption spectroscopy. The correlation between the 2 methods was very good, for sodium $r = 0.81$ and for potassium $r = 0.87$. After validation of the electrolyte analyser precision by atomic absorption spectroscopy, all data were presented as results of Beckman electrolyte analysis.

The urine proteins were estimated by using 0.3 % tetrabromophenol blue AMES multiple reagents method (Ames-Miles, England).

On the basis of the haematocrit before and after the experiment, dV plasma volume was calculated by the following equation:

$$dV = (100/100–Hi) \times [100 \times (Hi–Hf)/Hf]$$

where dV is the percent change in plasma volume at the end of the experiment, and H*i* and H*f* are the initial and final haematocrit values, respectively.

The catecholamines were estimated in 1 mℓ pooled plasma of 2 rats (6 samples per group of animals) by using IBL (Germany) Amicyl-Test™ Kat Combi competitive radioimmunoassay (RIA) of derivatised noradrenaline (NA) and adrenaline (A). A cisdiole-specific baronat affinity gel was used for the extraction of NA and A from plasma before RIA. The extraction efficiency was 86 %.

Histopathology

The renal tissue obtained was processed for light-microscopy. Half of each kidney was fixed in 10 % formalin solution. Sagittal slices were embedded in paraffin and 2 μm sections were excised for histological study. The sections were stained with haematoxylin and eosin, periodic acid silver methenamine and periodic acid Schiff. The samples were evaluated by an independent investigator without prior knowledge of the group to which the rat belonged. We defined glomerulosclerosis or hyalinisation as the disappearance of cellular elements from the tuft, collapse of capillary lumens, and folding of the glomerular basement membrane with entrapment of amorphous material. The extent of increase in mesangial matrix and the degree of glomerulosclerosis were evaluated and scored separately. Mesangial matrix expansion was defined by the presence of increased amounts of periodic acid Shiff positive material in the mesangial region. This change was occasionally accompanied by a variable increase in cellularity. However, increases in cellularity were not the predominant feature. In some glomeruli, the mesangial matrix expansion or the glomerulosclerosis affected only a small percentage of the glomerulus, while in others the process was diffuse. A semi-quantitative score according to the method described by Raij *et al.*[14] was used to evaluate the degree of damage. A minimum of 100 glomeruli in each specimen were examined and the severity of the lesions was graded from 0 to 4+ according to percentage glomerular involvement. Thus, a 1+ lesion represented an involvement of 25 % of the glomerulus, while a 4+ lesion indicated that 100 % of the glomerulus was involved. An injury score was then obtained by multiplying the degree of damage (0–4+) by the percentage of the glomeruli with the same degree of injury, that is, increase in mesangial matrix material or glomerulosclerosis. The extent of the injury for each individual tissue specimen was then obtained by the addition of these scores.

Similarly, scoring of the tubular damage was performed according to a modified by Uehara[25] method of Rosen *et al.*[20] Renal morphological alterations in the inner stripe and the medullary rays were semi-quantitatively evaluated and graded from 0 to 4+: 0 = no lesions; 1+ = very mild focal dilatation of tubules; 2+ = larger number of dilated tubules with widening of interstitium; 3 = fairly extensive dilatation of tubules with cystic formation and widening of interstitium; and 4+ = entire atrophy of tubules.

Statistical analysis

Values are expressed as mean ± standard error. For statistical analysis the Instat V2.04 programme was used, including 1-way analysis of variance and Tukey-Kramer multiple-comparison test. A P value of <0.05 was considered statistically significant.

RESULTS

Blood pressure, catecholamines, humoral changes and alterations in body weight in the 4 experimental groups after 2 months on either a low-Na or high-Na diet and in the 2 control weanling groups are presented in Tables 1, 2 and Figs 1, 2.

Morphological examination

The results have been presented and discussed in detail elsewhere[23]. They are summarised in Table 3. At weaning, both

Table 1: **Blood pressure, water-sodium balance and haematology of Dahl salt-resistant** (DR) **and Dahl salt-sensitive** (DS) **rats after 2 months on low-sodium and high-sodium diets.**

	DR control[a]	DS control	DR low-sodium diet	DS	DR high-sodium diet	DS
Systolic blood pressure (mm Hg)	102 ± 6.5[b]	138 ± 2.9*	122 ± 5.4	152 ± 6.2*	128±4.6	190 ± 6.5*
Diastolic blood pressure (mm Hg)	67 ± 8.5	93 ± 3.0*	91± 5.2	94± 6.4	98±5.9	130 ± 6.0*
Na-balance (mmol/100 g body weight/24 h)	1.010 ± 0.152	0.875 ± 0.094	0.480 ± 0.036	0.528 ± 0.053	3.606 ± 0.132	4.283 ± 0.137*
Water balance (mℓ/100 g body weight/24 h)	1.379 ± 0.460	1.282 ± 0.338	1.152 ± 0.460	1.428 ± 0.508	1.504 ± 0.152	2.097 ± 0.148*
Red blood cells (×1012/ℓ)	4.94 ± 0.29	4.78 ± 0.32	4.10 ± 0.38	3.60 ± 0.22*	4.06 ± 0.40	3.38 ± 0.42*
Haemoglobin (g/dℓ)	7.6 ± 0.43	8.86 ± 0.46	6.7 ± 0.54	6.3 ± 0.30	7.3 ± 0.40	6.2 ± 0.22*
Urinary protein (mg/100 g body wieght/24 h)	1.08 ± 0.27	1.03 ± 0.14	1.06 ± 0.14	1.45 ± 0.19	1.01 ± 0.13	4.35 ± 0.36*
dV (percentage change in plasma volume)			4.6	3.6	2.8	15.6

[a]Control groups are weanling (1 month old) DR and DS rats respectively.
[b]Mean ± standard error.
*Significantly different from respective salt-resistant group.

Table 2: **Plasma catecholamines, heart rate, body and organ weight of Dahl salt-resistant** (DR) **and Dahl salt-sensitive** (DS) **rats after 2 months on low-sodium and high-sodium diets.**

	DR control[a]	DS control	DR low-sodium diet	DS	DR high-sodium diet	DS
Body weight (g)	68 ± 7.6[b]	65 ± 5.3	321 ± 7.1	266 ± 14.8*	328±6.1	286 ± 10.2*
Heart ate (beats/min)	523 ± 10.2	476 ± 14.3*	502 ± 4.3	432 ± 2.3*	520 ±9.0	564 ± 11.2*
Epinephrine (pg/mℓ)	80 ± 10.2	120 ± 20.2*	75 ± 7.5	70 ± 4.8	325 ± 10.2	350 ± 2.2
Norepinephrine (pg/mℓ)	600 ± 12.2	700 ± 32.6*	500 ± 20.2	600 ± 21.2*	2160± 26.6	3320 ± 30.3*
Heart weight (g)	0.263 ± 0.02	0.280 ± 0.02	0.852 ± 0.08	0.862 ± 0.05	1.000 ±0.06	1.211±0.07*
Heart weight/body weight (mg/g)	3.87 ± 0.15	4.30 ± 0.05	2.65 ± 0.08	3.24 ± 0.10*	3.04 ±0.03	4.23 ± 0.14*
Paired kidney weight/body weight (mg/g)	14.58 ± 0.43	14.68 ± 0.39	7.44 ± 0.49	7.07± 0.10	7.25 ±0.13	6.18 ±0.45*
Paired adrenal weight/body weight (mg/g)	0.26 ± 0.02	0.25± 0.03	0.15 ± 0.02	0.22± 0.02*	0.10 ± 0.01	0.11 ± 0.02

[a]Control groups are weanling (1 month old) DR and DS rats respectively.
[b]Mean ± standard error.
*Significantly different from respective salt-resistant group.

DR and DS groups showed no arterial, glomerular or tubular changes. DR rats at the age of 3 months, on either 2 months' low-Na or high-Na diet also did not display any lesions. By 8 weeks on high Na diet (3 months), DS rats showed marked renal damage consisting of extensive fibromuscular proliferation around small muscular arteries, deposition of fibrinoid material in the intima of arteries, many proteinaceous tubular casts and focal atrophy of cortical tubules. Areas of interstitial fibrosis and cellular infiltrates were present primarily surrounding damaged tubules. Glomeruli revealed a diffuse increase in mesangial matrix (see methods) with evidence of cellular proliferation. Segmental glomerulosclerosis in scattered glomeruli was the predominant finding. It was displayed by collapse of capillary lumens and thickening and folding of the glomerular basement membrane with entrapment of amorphous PAS-positive material. An important characteristic of the renal pathology is the focal nature of damaged tubules and glomeruli. The DS rats fed low-Na diet for 2 months showed similar renal changes but parenchymal involvement was minimal and they exhibited almost normal tubules (Table 3).

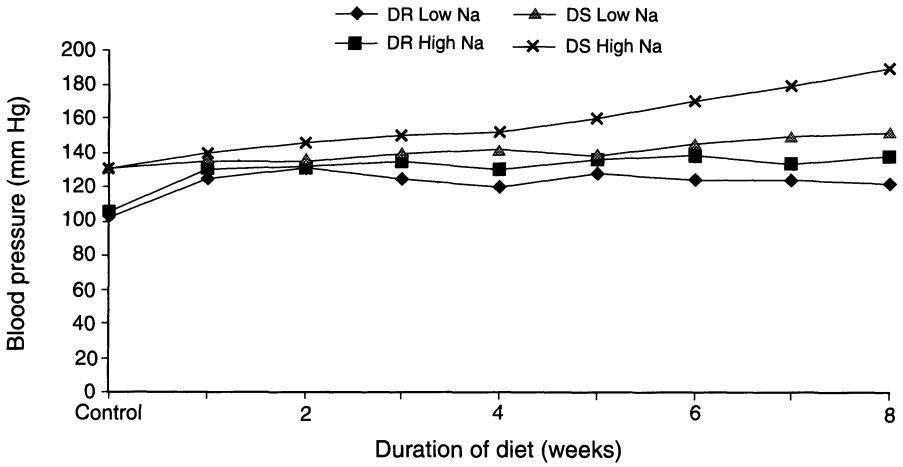

Fig. 1: **Systolic blood pressure responses of Dahl salt-resistant** (DR) **and Dahl salt-sensitive** (DS) **rats on low-salt** (0.5 % NaCl) **or high-salt** (8 %) **diets from weaning.** $n = 6$ rats per group; standard errors are presented in Table 1. All differences between groups were significant.

DISCUSSION

At weaning (1 month old), both salt-resistant (DR) and salt-sensitive (DS) lines had similar body weights, but at the end of the experiment (3 months old) the DS line showed a significant reduction in body-weight gain. At weaning all animals had a similar sodium-water balance. At low-Na regimen both DR and DS had a similar sodium-water balance and plasma volume. The main and significant difference between DR and DS rats was measured after 2 months of sodium loading. DS rats expressed significantly increased sodium and water retention, estimated as a difference between baseline and final sodium- and water-balance. The plasma volume had increased by 15 % compared to 2.8 % increase in DR rats.

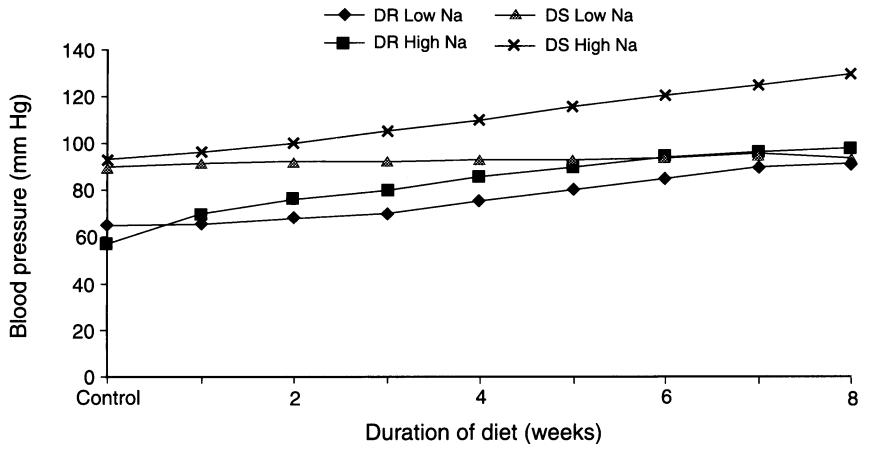

Fig. 2: **Diastolic blood pressure responses of Dahl salt-resistant** (DR) **and Dahl salt-sensitive** (DS) **rats on low-salt** (0.5 % NaCl) **or high-salt** (8 %) **diets from weaning.** $n = 6$ rats per group; standard errors are presented in Table 1. All differences between groups were significant.

Table 3: **Morphological changes in Dahl salt-resistant** (DR) **and Dahl salt-sensitive** (DS) **rats after two months on low-sodium** (0.5 % NaCl) **and high sodium** (8 % NaCl) **diets.**

	DR low-sodium diet	DR high-sodium diet	DS low-sodium diet	DS high-sodium diet
Mesangial matrix injury score	0	8.0± 2.2[a]	56 ± 2.0	122 ± 8.2
Glomerular sclerosis injury score	0	0	6 ± 2.0	18 ± 6.0
Renal tubular injury score[b]	0	0	1.0 ± 0.1	3.4 ± 1.2

[a]Mean ± standard error.
[b]Combined from the medullary ray and inner stripe.

The results of blood-pressure measurement showed that at weaning, without Na-loading or any treatment, DS rats displayed higher systolic and diastolic blood pressure, which indicates the genetic component of the hypertension. Irrespective of the diet, DS rats start gradually to develop hypertension (BP above 150/95 according to WHO criteria) at the age of 2 months. Sodium loading shortened the period for development of hypertension and exacerbated it. DS rats at the age of 3 months were anaemic, with more pronounced hypochromic anaemia in salt-loaded DS rats.

The mechanism for the rise of blood presure in the Dahl rat genetic model of hypertension has been debated since the strain was first introduced by Dahl in 1962. Renal sodium-water handling problems[6,19,26], central nervous system mechanisms[5,24] and various hormonal systems[2,4,9,13,18] have been suggested to be responsible for the chronic rise in blood pressure. In the present study we confirmed the genetic predisposition for development of hypertension, renal sodium-water handling problems and noradrenergic involvement.

Additional studies at molecular level will clarify the mechanisms of the described neurohumoral changes.

ACKNOWLEDGEMENTS

We thank P. Gathiram for assisting with direct blood pressure measurement and Ms C. Govender for typing the manuscript.

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