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Body Fluid Alterations and Organ Hypertrophy in Age-Dependent Salt Hypertension of Dahl Rats

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Summary

The relationship between possible alterations in the volume or distribution of extracellular fluid and the development of salt hypertension was studied in inbred salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) Dahl rats. Blood pressure, cardiac and renal hypertrophy as well as body fluid volumes were determined in young and adult SS/Jr and SR/Jr rats of both sexes that were subjected to low, normal or high salt intake for various periods of time. Salt hypertension in young salt-sensitive rats fed a 4 % NaCl diet was not accompanied by any substantial intravascular or interstitial expansion as compared to salt-resistant rats that remained normotensive. There was no sex difference in the response of blood pressure or body fluids to high salt intake. Major expansion of plasma and blood volume, which was elicited by 8 % NaCl diet feeding from prepuberty, was not accompanied by a further blood pressure rise (compared to salt hypertensive SS/Jr rats fed 4 % NaCl diet). In conclusions, salt hypertension can occur in Dahl salt-sensitive rats without major salt and water retention. The degree of intravascular expansion is not directly related to blood pressure levels in salt-loaded Dahl rats. A high salt intake seems to exert its hypertensive effects in Dahl rats preferentially by influencing the balance of vasoconstrictor and vasodilator systems rather than by increasing the haemodynamically active intravascular volume.

Key words

Inbred Dahl rats – Salt intake – Age – Blood pressure – Plasma volume – Blood volume – Extracellular fluid volume – Interstitial fluid volume – Cardiac hypertrophy – Renal hypertrophy

Introduction

It is evident that the impairments of various vasoconstrictor and vasodilator systems might contribute to the development and/or maintenance of salt hypertension in Dahl salt-sensitive rats (Lüscher *et al.* 1987, Mark 1991). The important role of altered sympathetic nervous system in the pathogenesis of this form of experimental hypertension was well documented (Mark 1991, Friedman 1979, Bunag *et al.* 1983). Nevertheless, salt hypertension can be ameliorated or even prevented by thiazide diuretics (Iwai *et al.* 1977, Tobian *et al.* 1979, Sasaki and Bunag 1983) although these drugs do not modify sympathetic nerve activity or peripheral responsiveness to catecholamines in Dahl rats (Sasaki and Bunag 1983). Such findings seem to support a possible role of body fluid alterations in the pathogenesis of this low-renin form of hypertension (Iwai *et al.* 1973). Though balance studies (Roman and Osborn 1987) did not reveal any major water and sodium retention in Dahl rats subjected to increased salt intake, the prevention of fluid retention abolished the initial blood pressure elevation in salt-loaded Dahl animals (Greene *et al.* 1990). It was supposed that blood volume enlargement might trigger the increase of cardiac output which would be responsible for blood pressure changes seen in salt-sensitive rats within the first few days of salt loading. However, cardiac output was elevated to the same extent in both salt-sensitive and salt-resistant animals fed a high-salt diet for three days (Greene *et al.* 1990, Ganguli *et al.* 1979). It remains an open question whether the initial blood pressure rise in salt-loaded salt-sensitive Dahl rats is due to their

augmented vasoconstriction (Ganguli *et al.* 1979, Reddy *et al.* 1991) or attenuated vasodilation as compared to salt-resistant animals (Greene *et al.* 1990, Chen and Sanders 1991).

The experimental data concerning body fluid changes in Dahl rats are not quite consistent. Schackow and Dahl (1966) were the first who tried to estimate the degree of sodium retention in salt hypertensive Dahl rats. They found a shorter biological half-life of ^{22}Na in outbred salt-sensitive (DS) than in salt-resistant (DR) Dahl rats (on both low- and high-salt diets) but there was no significant increase of exchangeable sodium in salt hypertensive DS animals. Kotchen and colleagues (Whitescarver *et al.* 1984, 1986, Genain *et al.* 1988, Kotchen *et al.* 1991) did not disclose any significant expansion of plasma volume or extracellular fluid volume in DS rats exposed to high salt intake (4–7 % NaCl diets) for 1–11 weeks. On the other hand, in salt hypertensive animals fed 8 % NaCl diet for 4–10 weeks the enlargement of blood volume was reported not only in outbred (Brookhaven) DS rats (Simchon *et al.* 1989, 1991) but also in inbred (Toledo) salt-sensitive Dahl-Rapp rats (Overbeck *et al.* 1981, Zicha *et al.* 1987) in which a tendency to extracellular fluid volume expansion was observed. It should be mentioned that the intravascular expansion occurred in salt-sensitive animals exposed to high salt intake during youth but not in those subjected to the same dietary salt loading during adulthood only (Zicha *et al.* 1987).

The aim of our present study was to determine the volume and distribution of extracellular fluid between intravascular and interstitial compartments in inbred salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) Dahl-Rapp rats that were subjected to a low, normal or high salt intake since weaning. These experiments were scheduled to cover various phases of salt hypertension development in order to find the time relationship between body fluid changes and blood pressure elevation. We used the 4 % NaCl diet as a moderate but effective hypertensive stimulus in order to avoid the development of accelerated salt hypertension in young salt-sensitive rats with a high rate of premature death due to rapidly developing renal and/or cardiovascular lesions. This would be the case of 8 % NaCl diet feeding when the observed changes might be a mixture of those related to pathogenetic mechanisms and those resulting from cardiovascular and renal complications. The occurrence of organ lesions was one of the reasons for monitoring cardiac and renal hypertrophy in our experiments. We also performed some additional experiments to clarify the role of sex as well as the importance of salt intake level in prepuberty on hypertension development and body fluid alterations. Finally, we tried to investigate whether the changes of blood pressure and body fluids elicited by high-salt diet feeding in Dahl rats depend on the age at which salt intake was increased (Zicha *et al.* 1986).

Methods

The experiments were carried out in inbred male and female salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) Dahl rats obtained from our breeding colony in Prague established due to the courtesy of Dr. John P. Rapp (Toledo, Ohio) who provided us with initial breeding pairs in 1985. All animals were weaned at the age of 28 days, randomly divided into particular experimental groups, offered tap water to drink and were fed natural ingredient diets with either low (LS, 0.3 % NaCl), normal (NS, 1 % NaCl), high (HS, 4 % NaCl) or very high salt content (8 % NaCl in Experiment C).

Volume and distribution of extracellular fluid during the development of salt hypertension (Experiment A)

Changes in blood pressure and body fluids were determined in 99 SS/Jr and 96 SR/Jr males at the age of 6, 9, 15 and 22 weeks, i.e. after 2, 5, 11 and 18 weeks of low, normal or high salt intake. Blood pressure was measured by direct puncture of the carotid artery under light ether anaesthesia.

Plasma volume (PV) was determined by the dilution of Evans blue (1 ml of 0.5 % solution per kg body weight, Fluka, Buchs, Switzerland) and extracellular fluid volume (ECFV) was measured as the distribution space of polyfructosane (0.2 ml of 25 % solution per kg body weight, Inutest, Laevosan, Linz, Austria). Briefly, 5 min after the intravenous injection of Evans blue, 0.2 ml of blood were withdrawn from tail vessels for the determination of haematocrit and dye concentration. Immediately thereafter, the animals were bilaterally nephrectomized and the corresponding amount of polyfructosane was injected into the jugular vein. The equilibration time for polyfructosane was 80 min after which blood was taken from the carotid artery. Polyfructosane concentrations were determined by means of a modified method for inulin measurement (Zicha *et al.* 1989). Interstitial fluid volume was calculated by subtracting PV from ECFV values whereas the blood volume was calculated from PV and haematocrit values. Kidney and heart weights as well as the weights of left and right ventricles were also determined.

The influence of sex, very high salt intake and age (Experiments B and C)

Weanling female (20 SS/Jr, 23 SR/Jr) and male (24 SS/Jr, 25 SR/Jr) Dahl rats were fed either LS (0.3 % NaCl) or HS (4 % NaCl) diets for 8 weeks, i.e. until the age of 12 weeks (Experiment B).

Thirty-one SS/Jr and 25 SR/Jr young females were exposed to the influence of 4 % and 8 % NaCl

diets for 6 weeks starting at the 4th week of age. Additional 22 SS/Jr and 16 SR/Jr adult females were subjected to the same dietary regimens from the age of 12 weeks (Experiment C).

Blood pressure as well as body fluid volume and distribution were measured at the end of Experiments B and C as described above.

Data were expressed as means ± S.E.M. and evaluated by one-way-analysis of variance with the calculation of least significant differences (LSD). Linear regression analysis was employed for the evaluation of the relationship between blood volume and blood pressure. $P<0.05$ was considered as significant.

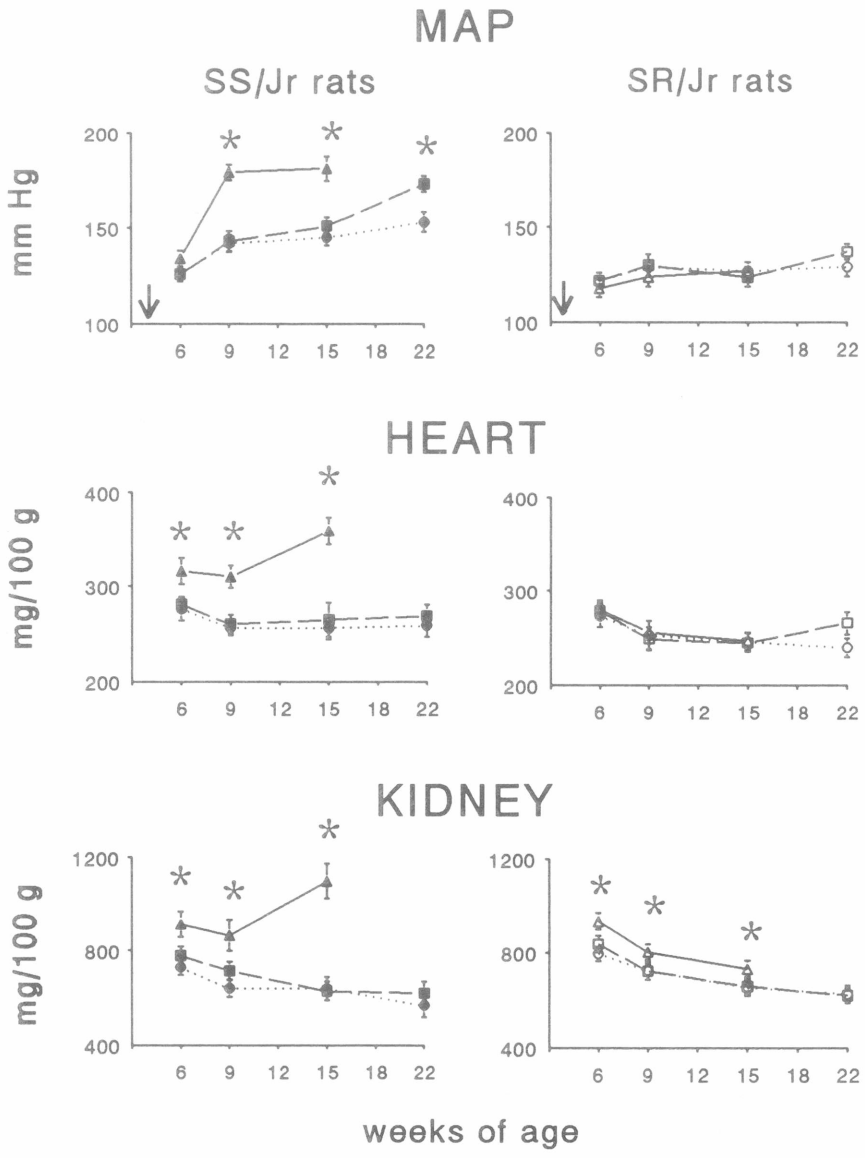

Fig. 1
Age-dependent changes of mean arterial pressure (MAP) and relative heart and kidney weights in male SS/Jr and SR/Jr Dahl rats fed either low-salt (0.3 % NaCl, circles, dotted line), normal (1 % NaCl, squares, broken line) or high-salt diet (4 % NaCl, triangles, full line) from the age of 4 weeks. Arrows indicate the onset of dietary regimens. Data are means ± S.E.M. Asterisks indicate differences ($p<0.05$) from animals fed a low-salt diet.

Results

Volume and distribution of extracellular fluid during the development of salt hypertension (Experiment A)

Blood pressure of salt-sensitive rats fed the 4 % NaCl diet was significantly elevated after 5 weeks but not after 2 weeks of dietary salt loading in comparison with all other experimental groups (Fig. 1). No animal of the group fed a high-salt diet survived until the age of 22 weeks when blood pressure rose even in SS/Jr rats fed a 1 % NaCl diet. In contrast, no blood pressure changes occurred in salt-resistant rats. In SS/Jr but not in SR/Jr animals fed a high-salt diet the relative heart weight was already increased after 2 weeks of salt loading, i.e. at the age of 6 weeks (Fig. 1). Early changes in relative heart weight were caused predominantly by enlargement of the left ventricle

which was followed by mild hypertrophy of the right ventricle (Table 1). Relative kidney weight was augmented after 2 weeks of high-salt diet feeding in rats of both genotypes, the increase being much greater in salt-loaded SS/Jr than in SR/Jr animals (Fig. 1). A major increase in relative heart and kidney weight occurred in salt hypertensive SS/Jr rats between the 9th and 15th week of age when mean arterial pressure was stabilized at the level of about 180 mm Hg (Fig. 1). There was a substantial rise of systolic but not of diastolic blood pressure during this period. The late augmentation of pulse pressure in salt hypertensive SS/Jr rats aged 15 weeks (LS: 48±2; NS: 52±4; HS: 66±3 mm Hg) might reflect not only the reduced compliance of great arteries but also vascular alterations in target organs.

Table 1
Relative weights of heart, right ventricle (RV), septum and left ventricle (LV) as well as the left/right ventricle ratio (LV/RV) in male Dahl SS/Jr rats aged 6, 9, 15 and 22 weeks that were fed either low-salt (LS, 0.3 % NaCl), normal (NS, 1 % NaCl) or high-salt diet (HS, 4 % NaCl) from weaning.

Diet	LS	NS	HS	F/LSD
6-week-old	n=5	n=5	n=5	
Heart	276±6	281±4	316±7*+	14.1/18
LV	135±5	140±6	154±12	n.s.
Septum	78±4	78±2	91±5	n.s.
RV	63±2	61±2	69±4	n.s.
LV/RV	2.16±0.09	2.30±0.14	2.29±0.27	n.s.
9-week-old	n=14	n=10	n=12	
Heart	256±4	260±5	310±4*+	49.9/12
LV	129±3	127±3	157±4*+	24.2/10
Septum	71±3	72±3	91±2*+	17.0/ 8
RV	55±1	57±2	61±2	n.s.
LV/RV	2.37±0.10	2.26±0.09	2.60±0.09	n.s.
15-week-old	n=14	n=9	n=10	
Heart	256±2	265±9	359±14*+	43.0/25
LV	135±2	148±8	204±11*+	27.2/20
Septum	63±2	62±1	93±5*+	31.7/ 9
RV	53±1	47±1	61±2*+	23.1/ 4
LV/RV	2.61±0.09	3.15±0.15*	3.40±0.22*	7.9/0.43
22-week-old	n=8	n=7		
Heart	259±6	269±6		
LV	142±3	151±4		
Septum	58±3	64±2		
RV	48±3	46±2		
LV/RV	3.07±0.20	3.36±0.19		

*Relative weights (means ± S.E.M.) are given in mg/100 g b.w. F/LSD – F ratio, least significant difference at 5 % level. Significantly different ($p<0.05$): * from animals fed low-salt diet, + from animals fed normal diet*

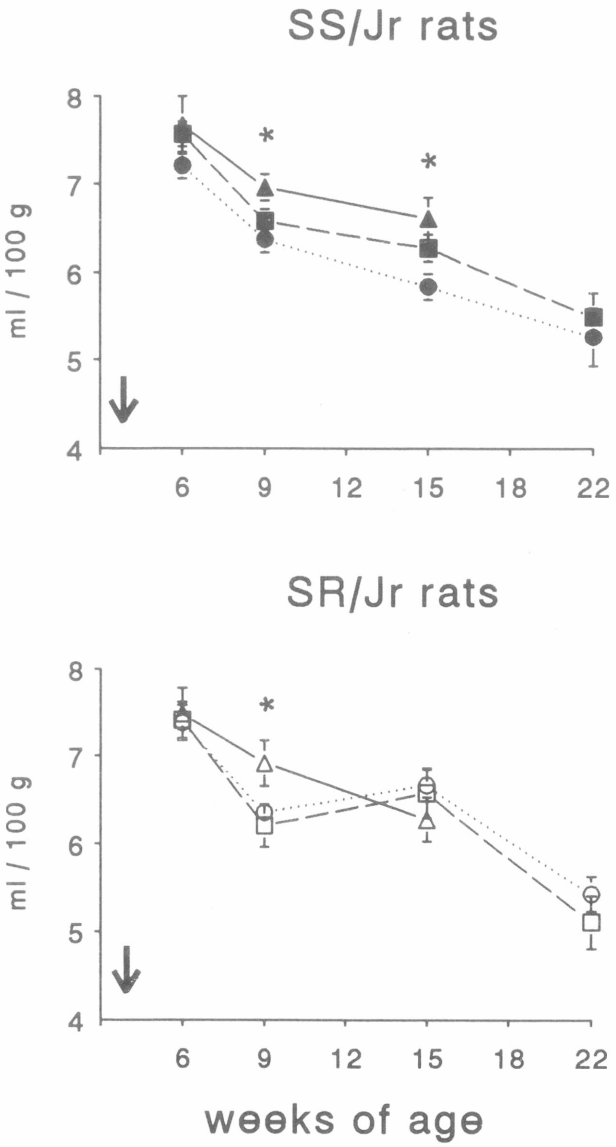

Fig. 2
Blood volume in male SS/Jr and SR/Jr Dahl rats aged 6, 9, 15 and 22 weeks that were fed either low-salt (0.3 % NaCl, circles, dotted line), normal (1 % NaCl, squares, broken line) or high-salt diet (4 % NaCl, triangles, full line) from the age of 4 weeks. For other legend see Fig. 1.

Though plasma and blood volumes tended to be expanded in all groups of SS/Jr rats fed a high-salt diet (compared to animals fed a low-salt diet), salt hypertensive SS/Jr animals did not differ significantly in plasma or blood volumes from salt-loaded SR/Jr rats which remained normotensive (Figs 2 and 3). There were no substantial differences in extracellular or interstitial fluid volumes among particular experimental groups except for a small increase seen in 15-week-old SS/Jr and SR/Jr rats subjected to dietary salt loading (Fig. 3). Plasma volume to interstitial fluid volume ratio (PV/IFV ratio) was similar in salt-loaded SS/Jr and SR/Jr rats indicating no redistribution of extracellular fluid in salt hypertensive SS/Jr rats (data not shown). No significant changes in volume or distribution of body fluids were revealed in moderately hypertensive 22-week-old SS/Jr rats fed the 1 % NaCl diet (data not shown).

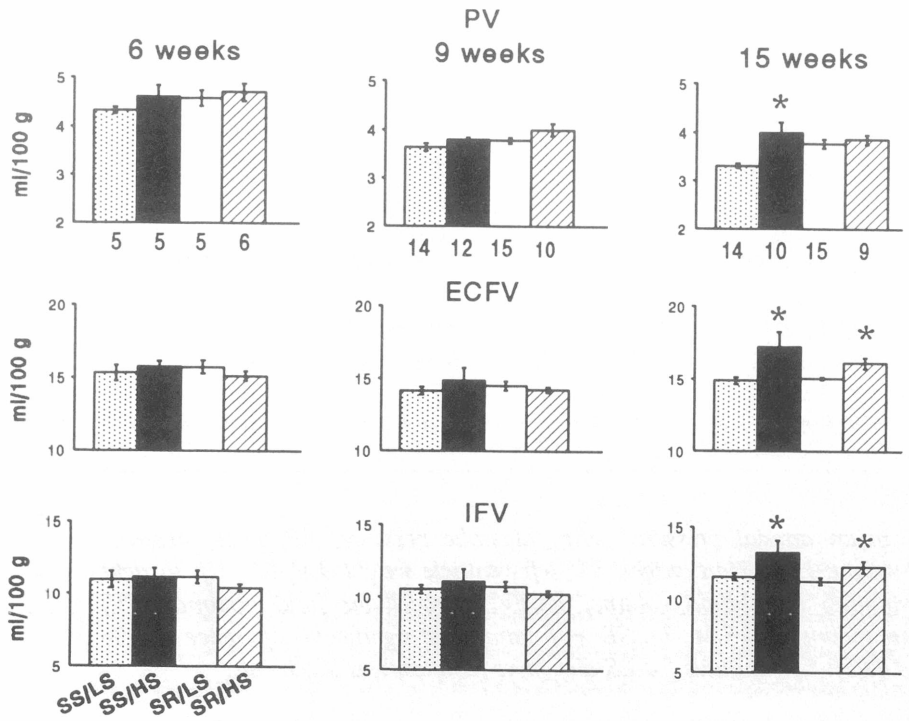

Fig. 3
Plasma volume (PV), extracellular fluid volume (ECFV) and interstitial fluid volume (IFV) in male SS/Jr and SR/Jr Dahl rats aged 6, 9 and 15 weeks that were fed either a low-salt (0.3 % NaCl, LS) or a high-salt diet (4 % NaCl, HS) for 2, 5 and 11 weeks starting from the age of 4 weeks. Data are means ± S.E.M. Number of animals is given at the bottom of the columns. Significant differences ($p<0.05$) from animals fed a low-salt diet are indicated by asterisks.

Table 2
Blood pressure (mm Hg), body weight (g), relative organ weights (mg/100 g b.w.) and relative volumes of body fluids (ml/100 g b.w.) in female and male Dahl SS/Jr and SR/Jr rats aged 12 weeks that were fed either low-salt (LS, 0.3 % NaCl) or high-salt (HS, 4 % NaCl) diet from weaning.

Diet	Females		Males		
Genotype	LS	HS	LS	HS	F/LSD
SS/Jr rats	n=9	n=11	n=13	n=11	
SBP	174±5	235±7*	167±3	208±6*#	35.7/15
MAP	148±5	198±6*	144±2	180±6*#	29.1/14
DBP	123±4	166±6*	120±2	154±5*	27.3/13
PP	51±2	69±1*	47±2	55±4*#	15.1/ 7
BW	202±5	184±7*	370±8#	323±12*#	114/25
KW	771±26	1356±57*	639±7#	971±61*#	54.2/124
HW	296±3	447±18*	256±2#	332±12*#	58.7/31
LV	170±3	253±11*	132±2#	178±11*#	42.7/22
LV/RV	3.06±0.08	3.72±0.13*	2.51±0.07#	2.96±0.20*#	15.7/0.37
PV	3.53±0.04	4.00±0.20*	3.45±0.05	3.88±0.15*	4.36/0.37
BV	6.31±0.08	6.85±0.17*	6.07±0.08	6.80±0.18*	8.24/0.39
ECFV	15.0±0.5	16.4±0.9	14.6±0.2	15.9±1.0	n.s.
IFV	11.5±0.5	12.4±0.8	11.1±0.2	12.1±0.9	n.s.
SR/Jr rats	n=8	n=15	n=10	n=15	
SBP	140±3$	143±3$	147±2$	145±3$	n.s.
MAP	121±3$	121±3$	128±2$	126±3$	n.s.
DBP	101±4$	101±3$	109±2$	107±3$	n.s.
PP	39±3$	42±2$	37±1$	38±2$	n.s.
BW	190±4	203±4	301±7#$	311±6#	129/16
KW	733±14	973±18*$	688±9#	756±12*#$	80.3/42
HW	266±2$	285±3*$	249±2#	250±2#$	52.3/ 7
LV	157±3	164±3$	126±2#	124±2#$	67.2/ 7
LV/RV	3.18±0.09	2.97±0.10$	2.30±0.07#	2.15±0.06#$	33.9/0.24
PV	3.75±0.06	3.97±0.05	3.78±0.05$	3.91±0.08	n.s.
BV	6.20±0.09	6.49±0.08	6.52±0.11#$	6.51±0.15	n.s.
ECFV	15.6±0.5	15.7±0.3	14.8±0.2	15.5±0.3	n.s.
IFV	11.9±0.5	11.8±0.3	11.0±0.2	11.6±0.4	n.s.

*SBP, systolic pressure; MAP, mean arterial pressure; DBP, diastolic pressure; PP, pulse pressure; BW, body weight; KW, kidney weight; HW, heart weight; LV, left ventricle weight; LV/RV, left to right ventricle ratio; PV, plasma volume; BV, blood volume; ECFV, extracellular fluid volume; IFV, interstitial fluid volume. Data are means ± S.E.M. F/LSD – F ratio, least significant difference at 5 % level. Significant differences ($p<0.05$): * vs animals fed LS diet, # vs females, $ vs SS/Jr rats.*

The role of sex (Experiment B)

The development of salt hypertension in young male and female Dahl rats was studied in animals subjected to 8 weeks of 4 % NaCl diet feeding (Table 2). A slightly higher blood pressure (especially systolic and pulse pressure) in salt hypertensive female SS/Jr rats was accompanied by a greater increase in relative kidney, heart and left ventricle weights as well as by higher left/right ventricle ratio. On the other hand, female and male hypertensive SS/Jr rats did not differ in the volume or distribution of body fluids. No sex differences in blood pressure or body fluid volumes were disclosed in salt-loaded SR/Jr rats. Organ hypertrophy induced by dietary salt loading was greater in female than in male SR/Jr rats (Table 2).

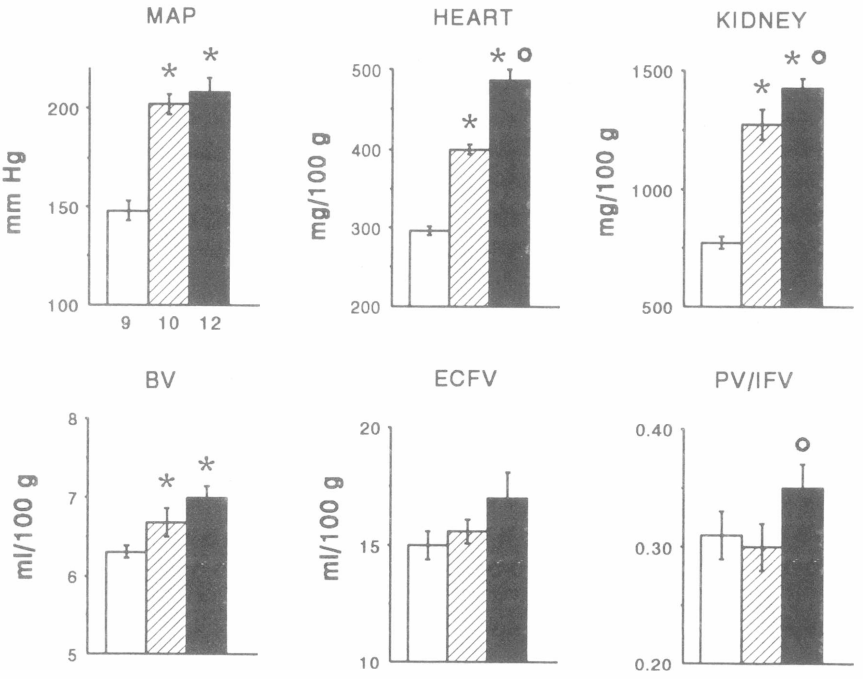

Fig. 4
The effects of 4 % and 8 % NaCl diet feeding for 6 weeks (hatched and full columns, respectively) on mean arterial pressure (MAP), relative heart and kidney weights, blood volume (BV), extracellular fluid volume (ECFV) and plasma volume to interstitial fluid volume ratio (PV/IFV) in 10-week-old female SS/Jr Dahl rats subjected to these dietary regimens from weaning (4th week of age). Number of animals is given at the bottom of the columns. Significant differences ($p<0.05$) from animals fed a low-salt diet (0.3 % NaCl, open columns) are indicated by asterisks whereas those between both high-salt diets by open circles.

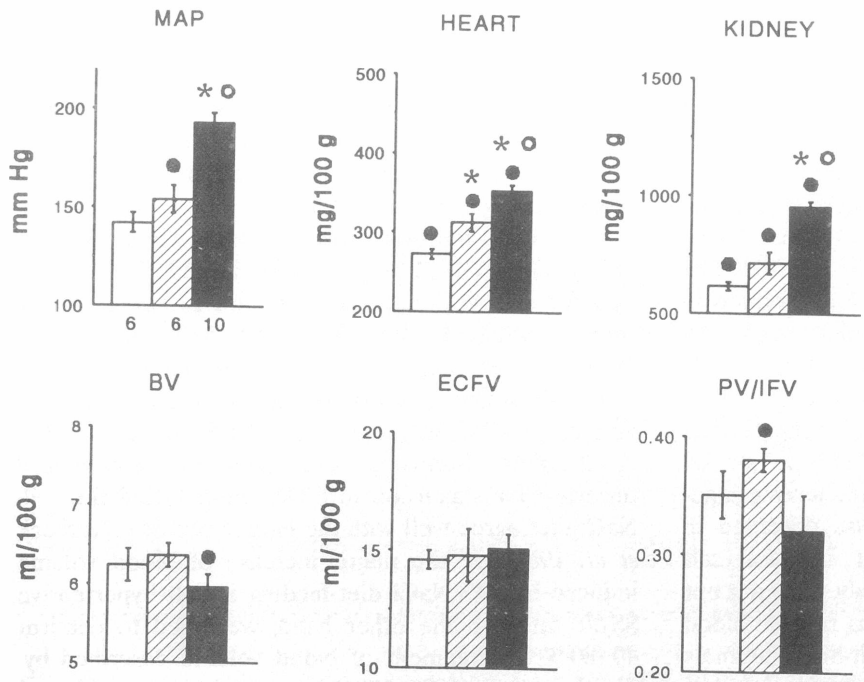

Fig. 5
The effects of 4 % and 8 % NaCl diet feeding for 6 weeks on MAP, HW, KW, BV, ECFV and PV/IFV ratio in 18-week-old female SS/Jr Dahl rats subjected to the respective dietary regimens in adulthood (from the 12th week of age). Significant differences ($p<0.05$) from young animals (Fig. 4) are indicated by full dots. For other legend see Fig. 4.

This experiment also confirmed a higher degree of intravascular (but not interstitial) expansion in SS/Jr than in SR/Jr rats. This was true for rats of both sexes. Though the magnitude of plasma and blood volume expansion in salt-loaded SS/Jr rats was twice as great as in SR/Jr animals fed a high-salt diet, plasma and blood volumes of salt hypertensive SS/Jr rats did not surpass the values found in salt-loaded SR/Jr animals that remained normotensive (Table 2).

Mean arterial pressure correlated positively with blood volume in SS/Jr ($r = 0.342$, $n=44$, $p<0.05$) but not in SR/Jr animals ($r = -0.146$, $n=48$, n.s.). This could be ascribed to the combination of groups with different means. It is therefore highly interesting to note that within the group of salt-loaded male and female SS/Jr rats there was a significant negative correlation between blood pressure and blood volume ($r = -0.442$, $n=22$, $p<0.05$).

The role of age and very high salt intake (Experiment C)

Feeding of the 8 % NaCl diet for 6 weeks did not cause a further significant blood pressure elevation in young female SS/Jr rats as compared to those consuming the 4 % NaCl diet. However, higher salt intake was associated not only with greater heart hypertrophy but also with a more pronounced intravascular expansion (Fig. 4). The increase in plasma volume (LS: 3.53±0.04; 4 % NaCl: 3.59±0.09; 8 % NaCl: 4.35±0.22 ml/100 g b.w.) was accompanied by a decreased haematocrit (53.4±0.3, 55.8±0.4 and 46.0±2.6 %) indicating not only blood dilution but also a reduced volume of erythrocyte mass in rats fed the 8 % NaCl diet (2.79±0.04, 3.09±0.09 and 2.62±0.05 ml/100 g b.w.).

Neither level of salt intake affected mean arterial pressure of young SR/Jr rats significantly (LS: 121.0±3.4 mm Hg, n=10; 4 % NaCl: 121.4±3.9 mm Hg, n=7; 8 % NaCl: 120.9±3.0 mm Hg, n=8). Salt-induced organ hypertrophy was greater in SR/Jr females fed the 8 % NaCl diet than in those consuming 4 % NaCl diet (heart: 266±2, 278±4 and 291±4 mg/100 g b.w.; kidney: 733±14, 933±20 and 1009±23 mg/100 g b.w.). On the other hand, blood volume in SR/Jr females was expanded to a similar degree by both high-salt diets (LS: 6.20±0.09; 4 % NaCl: 6.47±0.07; 8 % NaCl: 6.48±0.12 ml/100 g b.w.).

As far as adult SS/Jr females are concerned, a significant blood pressure rise occurred only in rats fed the 8 % NaCl diet (Fig. 5). A characteristic age-dependent blood pressure response was observed in SS/Jr rats fed the 4 % NaCl diet because salt hypertension appeared after 6 weeks only in young but not in adult animals. In contrast to young rats, no blood volume expansion was observed in adult SS/Jr animals kept on the 8 % NaCl diet. Organ hypertrophy in adult salt hypertensive rats was substantially smaller than in the corresponding young group.

Blood pressure of adult SR/Jr rats was not influenced by either level of salt intake (LS: 123±4 mm Hg, n=6; 4 % NaCl: 124±5 mm Hg, n=5; 8 % NaCl: 130±5 mm Hg, n=5). There were no significant salt-induced changes in relative heart weight (266±3, 279±6 and 274±4 mg/100 g b.w.), whereas the high salt intake moderately augmented relative kidney weight (644±20, 781±22 and 823±28 mg/100 g b.w.). Blood volume, which tended to generally higher values in adult than in young SR/Jr rats, was significantly increased only in rats fed the 4 % NaCl diet (6.84±0.16, 7.31±0.11 and 6.78±0.27 ml/100 g b.w.). It should be noted in adult females fed the 8 % NaCl diet that blood volume was larger in normotensive SR/Jr rats than in salt hypertensive SS/Jr ones.

Discussion

The present study indicated that pronounced salt hypertension in inbred salt-sensitive Dahl rats need not be associated with a major expansion of intravascular or interstitial compartments. In our experiments the enlarged blood volume of young salt hypertensive SS/Jr rats did not differ significantly from that of age- and sex-matched normotensive salt-loaded SR/Jr rats even if we examined different phases of salt hypertension development. Blood volume expansion in young SS/Jr rats was augmented by very high salt intake (8 % NaCl diet) although this intervention was not associated with a further blood pressure rise compared to animals fed the 4 % NaCl diet. Moreover, no increase of blood volume was observed in adult salt hypertensive SS/Jr rats fed the 8 % NaCl diet. Finally, in young salt hypertensive SS/Jr rats there was negative correlation between blood volume and blood pressure as was reported earlier in salt hypertensive monkeys (Kuneš *et al.* 1978). Thus, the intravascular expansion need not always participate in the maintenance of high blood pressure (Tarazi 1976).

Our findings on moderate blood volume enlargement (8–12 %) in young inbred SS/Jr Dahl rats fed the 4 % NaCl diet are not in variance with those obtained by Kotchen and co-workers (Whitescarver *et al.* 1984, 1986, Kotchen *et al.* 1991) who only found a non-significant increase of plasma volume (5–8 %) in young outbred DS rats fed 4–7 % NaCl diets. Similarly, our data on pronounced intravascular expansion in SS/Jr animals fed the 8 % NaCl diet agree well with the earlier report (Overbeck *et al.* 1981) on the major increase of blood volume induced by 8 % NaCl diet feeding in salt hypertensive SS/Jr rats. On the other hand, we failed to confirm 40–80 % enlargement of blood volume described by Simchon *et al.* (1989, 1991) in young DS rats subjected to 8 % NaCl diet for 4–8 weeks. It should be noted

that the "extreme intravascular expansion" in salt hypertensive Dahl rats fed 8 % NaCl diet is usually associated with a substantial haematocrit reduction (Overbeck *et al.* 1981, Simchon *et al.* 1989). These low haematocrit values in salt-sensitive Dahl rats exposed to a very high salt intake (Kazda *et al.* 1982, Zicha and Duhm 1990) might be ascribed rather to the microangiopathic haemolytic anemia than to simple plasma volume expansion (Luckhaus *et al.* 1982). The absence of intravascular expansion in our adult salt hypertensive SS/Jr rats are in accordance with a similar observation in 46-week-old hypertensive DS rats fed the 1 % NaCl diet (Simchon *et al.* 1989).

The sophisticated experiments of Greene *et al.* (1990) represent the best attempt to demonstrate the important role of fluid retention in the pathogenesis of salt hypertension in Dahl rats. Using a servo-controlled system for the maintenance of stable body weight they succeeded in preventing the early salt-induced blood pressure rise in adult DS females in which blood volume was kept constant at the baseline level. Nevertheless, under such conditions, the increased plasma sodium levels exhibited a severe water deficit (at least 15 % of body weight) which had to be associated with considerable water redistribution from the intracellular to the interstitial compartment. This might modify the mechanisms responsible for blood pressure elevation in salt-dependent forms of hypertension. Our previous experiments (Zicha *et al.* 1989) on DOCA-salt hypertension in Brattleboro rats demonstrated that a pronounced water deficit can almost abolish hypertension development although sodium retention was normal and blood volume was even greatly expanded. The chronic administration of an antidiuretic vasopressin analogue (dDAVP) reduced blood volume expansion but fully restored the hypertensive response of vasopressin-deficient Brattleboro rats to DOCA-salt treatment. Similar observations on the role of dehydration were also made by Hofbauer *et al.* (1984) who prevented DOCA-salt hypertension in Sprague-Dawley rats by chronic blockade of antidiuretic vasopressin action. These findings suggest that salt hypertension development in the experiments of Greene *et al.* (1990 might be blocked by other mechanisms than by the absence of intravascular expansion.

It is well known that the efficiency of both arterial and cardiac baroreceptors is reduced in DS rats compared to DR ones and this difference is further augmented by a high salt intake (Mark *et al.* 1987, Nedvídek and Zicha 1993). At present, we cannot exclude the possibility that blood volume expansion seen in salt-sensitive Dahl rats fed the 8 % NaCl diet would be a part of the protective mechanisms lowering abnormally high sympathetic discharges that would result from the resetting of cardiac sensory endings to higher pressure thresholds. Salt-loaded DS rats indeed responded to acute volume expansion by a greater fall in systemic resistance than DR animals (Reddy and Kotchen 1992), although cardiopulmonary baroreflex efficiency was reported to be impaired just in the former group (Mark *et al.* 1987), this might suggest that blood volume expansion exerts a more efficient reduction of augmented efferent sympathetic outflow *via* stimulated cardiopulmonary baroreceptors in salt-loaded DS rats (Reddy *et al.* 1991, Reddy and Kotchen 1992).

The absence of important interstitial expansion in salt hypertensive Dahl rats is not surprising because no major increase in exchangeable sodium was observed in these animals (Schackow and Dahl 1966). Minimal changes of extracellular fluid volume that were described by Genain *et al.* (1991) represent the effects of very short-term salt feeding (lasting one week only). The data on extracellular fluid volume expansion in salt-loaded SS/Jr and SR/Jr Dahl rats obtained by Overbeck *et al.* (1981) should be considered with great care because the radiosulfate method yielded unusually high values of "extracellular" distribution space.

Increased relative heart and kidney weights in salt hypertensive Dahl rats are usually ascribed to the deleterious effects of high blood pressure (Luckhaus *et al.* 1982, Jaffe *et al.* 1970, Fernandez *et al.* 1984, Pfeffer *et al.* 1984, Sterzel *et al.* 1988). High salt intake seems to increase organ weights in SS/Jr rats by two different mechanisms. The early cardiac and renal enlargement that was rather independent of blood pressure increase, already began within two weeks of salt loading. A moderate augmentation of the kidney weight was also seen in SR/Jr rats.

The trophic influence of high-salt diet feeding on the left ventricle mass, which was independent of cardiac volume or pressure overload, was recently reported in normotensive Wistar and Wistar-Kyoto rats (Yuan and Leenen 1991, Frohlich *et al.* 1993). Yuan and Leenen (1991) have also observed that the effect of high salt intake on heart weight was greater in young than in mature animals. Nevertheless, they did not find any significant change of cardiac mass in young salt-resistant Dahl rats kept on 8 % NaCl diet for one month. Our experiments indicated that the elevation of salt intake for 6 weeks slightly increased the relative heart weight in young female but not male SR/Jr rats (see Table 2). This effect of 8 % NaCl diet feeding was significant only in young but not in adult SR/Jr females.

The late pronounced heart and kidney hypertrophy in SS/Jr rats fed 4 % NaCl diet was evidently caused by high blood pressure. This phase of organ growth appeared in the course of the second month of high salt intake when salt hypertension development was associated with a reduced arterial compliance (evidenced by augmented pulse pressure) and a moderate hypertrophy of the right ventricle. The increments of heart and kidney weights were greater in

young than in adult salt hypertensive SS/Jr rats. The relative cardiac mass was increased by 64 % in young and by 29 % in adult SS/Jr females fed 8 % NaCl diet for 6 weeks. The same was true for the relative renal mass (+85 % vs +55 %) although both age groups achieved similar elevation of blood pressure. The age-dependent involvement of vascular damage and/or local circulatory disturbances in these two organs remains to be determined.

In conclusion, our findings support the earlier view of Dahl and Schackow (1966) that salt hypertension can develop in Dahl rats without gross sodium retention. Our data obtained in young salt-sensitive rats fed the 8 % NaCl diet are not in variance with the findings of other investigators (Overbeck *et al.* 1981, Simchon *et al.* 1989, 1991) who reported intravascular expansion in salt hypertensive Dahl rats. However, we are not convinced about the pathogenetic importance of increased blood volume in this form of experimental hypertension because a similar degree of salt hypertension can occur without accompanying blood volume enlargement. The alterations in the balance of vasoconstrictor and vasodilator systems induced by high salt intake seem to play a more important role than the direct or indirect haemodynamic effects of salt and water retention. This is in concert with some of our earlier observations. No signs of volume-dependent sodium pump inhibition can be demonstrated in erythrocytes of young salt hypertensive SS/Jr Dahl rats (Zicha and Duhm 1990) whereas the catecholamine content is altered in the blood vessel wall of these animals (Kuneš *et al.* 1991).

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