Kidney International, Vol. 39 (1991), pp. 1184–1192

Blood pressure and tubuloglomerular feedback mechanism in chronically salt-loaded spontaneously hypertensive rats

YASUYUKI USHIOGI, TOSHIKAZU TAKABATAKE, and DIETER ALBERT HÄBERLE

Physiologisches Institut der Universität München, Munich, Germany, and First Department of Internal Medicine, School of Medicine, Kanazawa University, Kanazawa, Japan

Blood pressure and tubuloglomerular feedback mechanism in chronically salt-loaded spontaneously hypertensive rats. Experiments were performed to qualitatively characterize the effects of tubuloglomerular feedback (TGF) inhibition by chronic salt loading on salt sensitivity of blood pressure in spontaneously hypertensive rats (SHR). After two weeks of salt loading, systolic blood pressure (SBP) was significantly exacerbated and plasma volume (PV) was expanded in salt-loaded SHR compared with those in control SHR (SBP: 182 ± 1 vs. 159 ± 2 mm Hg; PV: 4.38 ± 0.06 vs. 4.04 ± 0.03 ml/100 g body wt, respectively). Plasma volume of WKY was also but only transiently expanded by salt loading, whereas plasma volume expansion in SHR had persisted over the entire dietary treatment period. TGF activity was assessed as the maximal reduction of single nephron GFR (SNGFR) on increasing loop of Henle perfusion rate from 0 to 40 nl/min using previously collected tubular fluid from salt-loaded rats (TFs) or control rats (TFc). Maximal TGF response in salt-loaded SHR with TFs was 14.9 ± 2.9% and 57.8 ± 2.6% with TFc. In control SHR the responses were 16.9 ± 2.5% with TFs and 52.7 ± 2.9% with TFc. In salt-loaded WKY the response with TFs were 3.1 ± 1.6% and 37.4 ± 2.8% with TFc. And in control WKY, the response with TFs were 8.2 ± 1.9% and 40.8 ± 2.8% with TFc, respectively. These results indicate the TGF resetting in chronically salt-loaded SHR and WKY is caused by the activation of humoral TGF inhibitory factor. The suppression of TGF in SHR was, however, far more variable and, on average, less than in WKY. In addition, the maximum TGF response elicited by TFs in SHR and systolic blood pressure in conscious donor SHR were significantly correlated (r = 0.8142). This indicates that in salt-loaded SHR less activation of the TGF inhibitory factor goes in hand with a greater blood pressure increase. It is thus reasonable to propose that the renal response to chronic salt-loading in SHR might be blunted by defective activation of the feedback inhibitory substance. This defect may contribute to the exacerbation and maintenance of hypertension in this strain.

A number of studies [1–5] have demonstrated that chronic dietary salt loading exacerbates the development and severity of hypertension in spontaneously hypertensive rats (SHR). Since previous studies have identified various functional abnormalities in the kidneys of these rats [6, 7] and since the renal function is involved in long-term blood pressure regulation [8], it is possible that additional, unknown abnormalities of the renal sodium balancing mechanisms participate in the exacerbation of hypertension during chronic volume expansion.

One mechanism of particular interest in this respect is the tubuloglomerular feedback (TGF) mechanism. This mechanism is generally believed to play a significant role in the regulation of water and electrolyte balance as well as in the long-term regulation of the blood pressure. Three observations have led to this idea. (i) The TGF mechanism influences the renal blood flow and glomerular filtration by changing the arteriolar flow resistance at the glomerular vascular pole in response to changes of the luminal sodium chloride concentration at the macula densa and, in consequence, stabilizes the salt and water delivery to both proximal and distal nephron [9]. (ii) Alterations of the activity of the TGF mechanism appear to influence the activity of the intrarenal renin-angiotensin system and, hence, the vascular tone of the arterioles of the parent glomerulus [9–11]. (iii) The magnitude of the feedback response is modulated by specific mechanisms under conditions of altered extracellular fluid volume [9] and sodium intake. In particular, when the dietary sodium load is increased, the feedback response is attenuated and contributes to the increase of sodium excretion [12–18] by several distinct mechanisms. In rats chronically fed a high salt diet, the resetting of feedback system is caused by the activation of an inhibitory factor in tubular fluid [19] rather than by a change in the intrinsic characteristics of the juxtaglomerular apparatus, as is the case in rats treated chronically with DOCA and isotonic saline for drinking [20]. In contrast, this inhibitory principle is not detectable in tubular fluid of rats acutely volume expanded by the infusion with Ringer's solution and appropriate amounts of fresh rat plasma [21].

Since the TGF function is reportedly disturbed in prehypertensive SHR [22], we investigated the activation and the effectiveness of the humoral inhibitory factor in SHR and WKY and its relationship to the increase of blood pressure in chronic dietary salt loading. An inverse correlation between the blood pressure increase and the activity of the inhibitory factor was found which may indicate an abnormal response of the SHR kidney and/or its hormonal regulating systems to dietary salt loading.

Methods

Dietary treatment

Experiments were performed on male 6- to 7-week-old spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar-Kyoto rats (WKY) (Savo-Ivanovas, Kisslegg, Germany). The rats were divided into two groups. One group was fed a high salt diet (Altromin C 1036, containing either 40 or 100

Received for publication August 27, 1990
and in revised form January 24, 1991
Accepted for publication January 25, 1991

g NaCl/kg food) for at least two weeks. The other group was fed a low salt control diet (Altromin C 1000, containing 0.35% Na, 0.48% Cl and 0.23% K). Food and water were available ad libitum throughout the study and the consumption of both was checked randomly. Systolic blood pressure and pulse rate in conscious, prewarmed rats were measured by the tail cuff method once weekly and twice in the three to five days prior to the micropuncture experiment. The average of four or five replicate measurements was used as an estimate of blood pressure. Body weight was determined on the same day as the blood pressure measurement.

Animal preparation

Micropuncture experiments were performed two to four weeks after beginning dietary treatment. Rats weighing between 190 and 270 g were anesthetized by intraperitoneal injection of Inactin, 100 mg/kg body weight (Byk-Gulden, Konstanz, Germany). The rat was placed on a thermostatically-controlled heated table to maintain rectal temperature at approximately 37.5°C. After tracheotomy, the right femoral artery and vein were catheterized. A solution of polyfructosan (125 g/liter in 150 mmol/liter NaCl) was infused at a rate of 4 ml/hr/kg body wt in control group and 5 ml/hr/kg body wt in the salt-loaded group. The arterial catheter was used to monitor blood pressure and to withdraw blood samples. The left kidney was then exposed through a left subcostal flank incision and supported in a plastic cup using oil-soaked cotton wool. The kidney was immobilized in agar (30 g/liter in 150 mmol/liter NaCl) and the renal surface bathed with warmed (38°C) mineral oil. The left ureter was catheterized for urine collection.

Measurement of GFR and plasma volume

Rats were prepared as described above. After an equilibration period of approximately 60 minutes, a timed urine collection was begun for the determination of glomerular filtration rate and electrolyte excretion. Arterial blood samples were taken before and after urine collection. Serum and urinary polyfructosan concentrations were measured by perchloric acid hydrolysis to fructose and determination of the latter by the hexokinase/G-6-P dehydrogenase method, using a commercially available kit (Glucose/fructose Kit No. 139 106, Boehringer Mannheim, Mannheim, Germany). GFR (polyfructosan clearance) was determined using the standard formula. Serum and urinary sodium and potassium concentrations were determined by flame photometry. Chloride concentrations were determined by the Cotlove method (Chloridometer Buchler Instruments, Fort Lee, New Jersey, USA).

Plasma volume

In a separate series of experiments, plasma volume was measured by the Evans blue dilution technique at two, three and four weeks after beginning dietary treatment. After preparation as above and following an equilibration period of 30 minutes, a 500 μl blood sample was taken and subsequently used to prepare the standard concentrations of Evans Blue. After further 10 minutes, 100 μl of a 100 mg/dl solution of Evans Blue (E. Merck, Darmstadt, Germany) in Ringer's solution was injected i.v. as a bolus. A period of 10 minutes was allowed for distribution, then a second sample taken and the Evans Blue concentration measured. Plasma and standards were diluted similarly (1:10) and optical density determined in a dual beam spectrophotometer (model MPS 2000, Shimadzu) at wave lengths of 740, 660 and 610 nm. The difference in the optical densities at 660 and 610 nm was corrected by subtracting the optical density at 740 nm and plotted against Evan's Blue concentration.

Collection of tubular fluid

Late proximal tubular fluid (TF) was harvested under suction at a rate of 8 to 10 nl/min into an oil-filled siliconized micropipette (outer tip diameter 8 to 10 μm), the tip of which had been platinum glazed to make it visible, and which was mounted in a microperfusion/suction pump (Walter Klotz, Munich, Germany). Endogenous tubular fluid was collected from the experimental rat. Exogenous tubular fluid was collected from a rat from the other dietary group prepared in parallel.

Feedback response

The micropuncture protocol for estimating tubuloglomerular feedback response was as follows: The course of a nephron was identified by injecting small volumes of Ringer's solution containing FD & C green (2 g/liter) into a randomly-selected proximal tubular loop. The harvested tubular fluid or Ringer's solution was then perfused into the loop of Henle at a rate of 0, 10, 20 or 40 nl/min from the microperfusion pipette inserted into the last accessible proximal loop. Usually one Ringer's perfusion and two to three tubular fluid perfusions were performed per rat in random order. After an equilibration period of about two minutes, a micropipette (outer diameter 10 μm) filled with Sudan Black-stained paraffin oil was inserted into the earliest accessible proximal segment, an oil block injected, and a timed, quantitative collection of tubular fluid begun for the determination of single nephron glomerular filtration rate (SNGFR). After completion of the sample, the perfusion rate was changed and further collections made. Each subsequent collection was made slightly upstream to avoid the possibility of leakage.

The volumes of tubular fluid were measured by injection into a constant-bore microcapillary (Microcap 0.5 μl, Drummond) and measuring the length of the column by means of an ocular micrometer. Inulin in tubular fluid was determined by a microadaptation of the same method used for urine and plasma samples. SNGFRs were calculated using the standard clearance expressions and expressed in nl/min/g kidney weight.

NaCl loading

Another series of experiments were performed to examine the effect of more effective salt loading and possibly more remarkable volume expansion. Some SHR were fed 100 g/kg NaCl diet (Altromin C 1036, 100 g NaCl/kg dry food) during at least two weeks before the experiments. The systolic blood pressure and plasma volume were measured only at two weeks of salt loading. Micropuncture experiments were performed in the same manner as above, but the determinations of SNGFR were made only by loop perfusion with endogenous TF.

Statistics

Results are given as means ± SEM. Data were tested using analysis of variance and linear regression analysis. The significance of differences in means was established by Tukey's method for multiple comparisons [23] and by the Kruskal Wallis

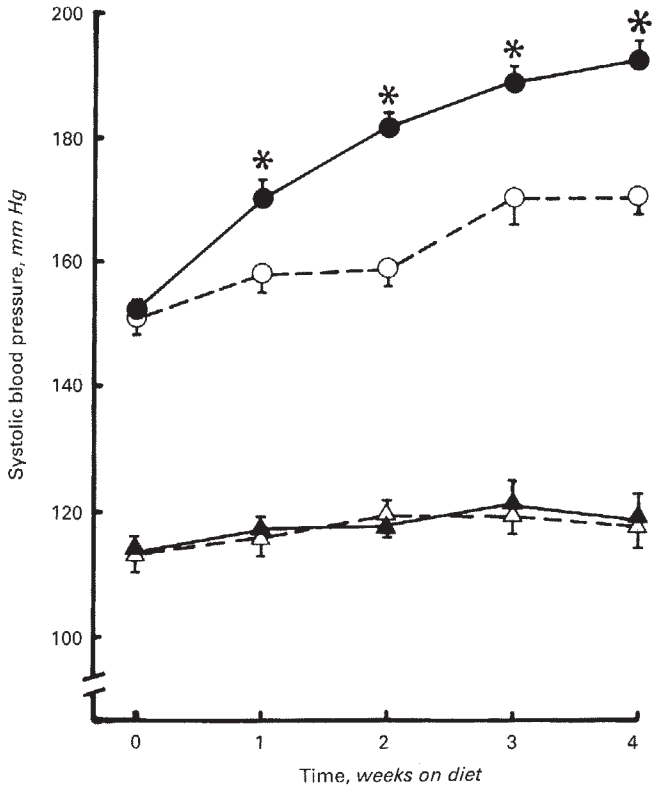

Fig. 1. *Effect of dietary salt loading on systolic blood pressure measured by tail-cuff method in salt-loaded and control SHR and WKY.* Values are given as means ± SEM. * Indicates significant differences from systolic pressure in control SHR. Symbols are: (–●–) salt-loaded SHR; (--○--) control SHR; (–▲–) salt-loaded WKY; (--△--) control WKY.

Test (H-test). A probability level of less than 0.05 was considered significant.

Results

Blood pressure, pulse rate and plasma volume

There was no difference in blood pressure between the two groups of SHR before the dietary pretreatment. After one week on the diets, systolic blood pressure in salt-loaded SHR was significantly higher than in control SHR. Systolic blood pressure in both groups of SHR continued to rise over four weeks of salt loading, but systolic blood pressure in salt-loaded SHR remained significantly higher than that in control SHR (Fig. 1). In salt-loaded conscious SHR, systolic blood pressure was higher than the mean blood pressure in anaesthetized rats. Both blood pressures were significantly correlated ($r = 0.5957$). The linear regression line is given by $BP_{mean,anesth.} = 25.5 + 0.696\ BP_{sys}$ ($N = 54$). Systolic blood pressure in WKY showed no significant response to salt loading. There was also no difference in pulse rate in the salt loaded and control groups of both strains. Food intake was comparable in all groups. Body weights of WKY were significantly greater than those of SHR, but high salt diet had no effect on body weight in either SHR or WKY (Table 1).

The specific effects of dietary salt-loading on plasma volume are presented in Figure 2. After two weeks, plasma volumes were slightly but significantly expanded in both salt-loaded WKY and SHR (4.52 ± 0.05 and 4.38 ± 0.06 ml/100 g body wt, respectively, compared with 4.12 ± 0.04 and 4.04 ± 0.03 ml/100 g body wt in controls). Plasma volume expansion was still demonstrable at four weeks in salt-loaded SHR (4.37 ± 0.05 compared with 3.91 ± 0.03 ml/100 g body wt). In contrast, plasma volume in salt-loaded WKY decreased with increasing length of dietary treatment and was no longer different from that in controls at four weeks (4.08 ± 0.05 compared with 4.04 ± 0.07 ml/100 g body wt).

Clearance results

Mean blood pressure and renal clearances obtained during micropuncture experiments are presented in Table 2. Mean blood pressure was significantly higher in salt loaded SHR than in control SHR. There were no differences in urine volume and whole kidney GFR between salt-loaded and control groups within each strain. Urinary sodium excretion in salt-loaded groups was significantly greater than in controls. Urinary chloride excretion was increased after salt loading although the increases in WKY failed to reach significance. With the exception of mean blood pressure there was no difference between the two strains in any parameter measured.

Feedback analysis

Figure 3 and Table 3 demonstrate the response of SNGFR to loop of Henle perfusion in SHR. Three perfusates were used: endogenous TF, exogenous TF (from a rat of the other dietary group), or Ringer's solution. In control SHR, loop of Henle perfusion with endogenous TF elicited a significant decrease of SNGFR on increasing perfusion rate from 0 to 20 or 40 nl/min. In contrast, the feedback response was significantly attenuated when the loop of Henle was perfused with exogenous (high salt) TF. The response of SNGFR to loop perfusion with Ringer's solution was similar to that with endogenous TF.

The feedback response in salt-loaded SHR was significantly attenuated when the loop of Henle was perfused with endogenous TF, whereas much greater feedback responses were induced with exogenous TF or Ringer's solution. The feedback responses to any given perfusate were qualitatively similar in both control and salt-loaded SHR whereby the response to the high salt SHR TF was considerably less uniform than that to high salt WKY TF.

Figure 4 and Table 3 show the feedback response of SNGFR in WKY. Again, in both salt-loaded and control WKY, the feedback response was significantly attenuated when the loop of Henle was perfused with high salt TF, whereas perfusion with control TF elicited practically normal feedback responses. The characteristics of the tubuloglomerular feedback function in WKY were, however, different from those in SHR. Maximum stimulation of the TGF by loop perfusion at 40 nl/min with Ringer's solution in control SHR depressed SNGFR significantly more than in WKY (53.0 ± 3.2% vs. 35.6 ± 1.4%; Fig. 5). This may imply that TGF in normal SHR is hyperactive compared with WKY.

The feedback inhibitory activity was significantly different between the two strains on salt loading. Firstly, the maximal feedback response in salt loaded SHR was significantly higher than in WKY rats (Table 3, compare Fig. 7). Interestingly, the variance in salt loaded SHR was significantly larger than in salt

Table 1. Effects of two weeks dietary salt loading on systolic blood pressure (SBP), heart rate (HR) in SHR and WKY, and body weight (Weight)

	High salt diet			Control diet		
	SBP *mm Hg*	HR *beats/min*	Weight *g*	SBP *mm Hg*	HR *beats/min*	Weight *g*
SHR	182 ± 1 (29)[a]	354 ± 5 (29)	211 ± 3 (29)	159 ± 2 (28)	358 ± 4 (28)	207 ± 2 (28)
WKY	119 ± 2 (20)[b]	362 ± 4 (20)	237 ± 2 (20)[b]	119 ± 2 (20)[b]	354 ± 6 (20)	234 ± 2 (20)[b]

Data are shown as means ± SEM with number of experiments in parentheses.
[a] Significant difference from the corresponding control value
[b] Significant differences between SHR and WKY

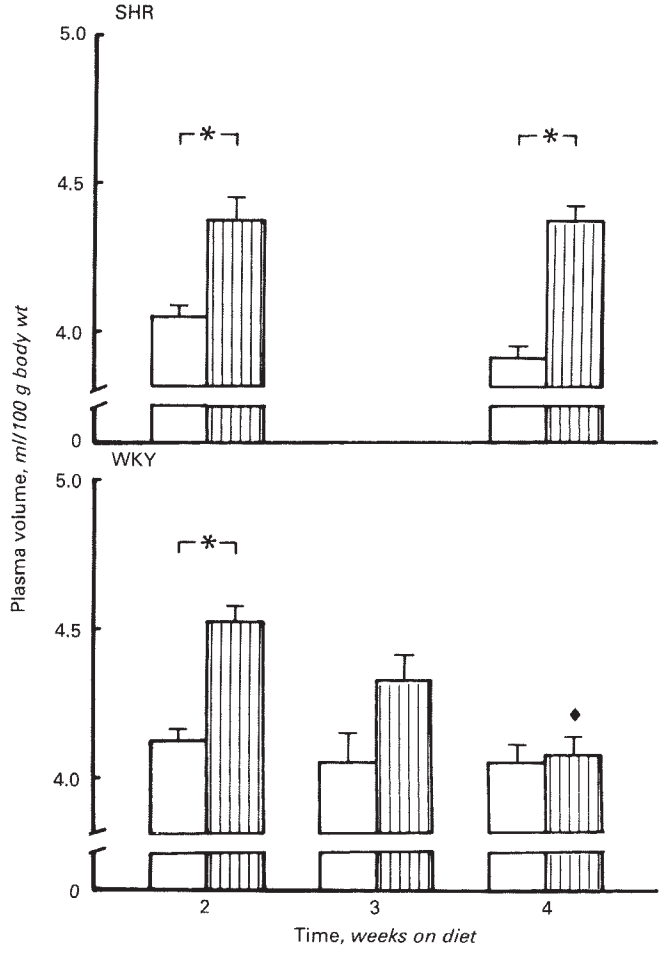

Fig. 2. *Effects of dietary salt loading on plasma volume in SHR and WKY.* Symbols are: (□) control rat; (▥) salt-loaded rat. Data are means ± SEM. The numbers of observations for the panels in the upper part of the figure were 8, 7, 9, and 9, respectively, and in the lower part, 7, 7, 5, 6, 8, and 7, respectively. * Indicates significant differences between salt-loaded and control rats. ◆ indicates a significant difference between salt-loaded SHR and WKY at four weeks.

loaded WKY. Secondly, the ratio of the maximum reduction of SNGFR on loop perfusion with endogenous tubular fluid to that with Ringer's solution in the same rat was in salt-loaded SHR, 0.33 ± 0.07 (18 pairs of tubules, 8 rats) and in salt-loaded WKY, 0.05 ± 0.04 (10 pairs of tubules, 6 rats). The difference between the ratios, which takes into account the difference of the intrinsic sensitivity of TGF in SHR and WKY, was also significant. The attenuation of TGF in salt-loaded SHR was, therefore, less pronounced than in salt-loaded WKY. There was no difference in the ratio between two control groups of rats, 1.03 ± 0.08 in control SHR, 1.16 ± 0.10 in control WKY. Figure 6 shows the relationship between maximum feedback response (% reduction in SNGFR) elicited by TF harvested in a salt-loaded SHR and the previously measured conscious systolic blood pressure in these donor rats. A comparable relationship exists between the TGF response and the mean arterial blood pressure of the same donor rat during anaesthesia. This relationship is described by the equation (% reduction of SNGFR) = 0.923 (mean arterial blood pressure) − 135 (r = 0.65, P = 0.0014). Thus, it may be concluded that there is an inverse, highly significant correlation between the maximum feedback response and the arterial blood pressure of the donor rat.

Effect of 100 g/kg NaCl diet

After two weeks of salt loading, systolic blood pressure in SHR fed a 100 g/kg NaCl diet was significantly higher than in SHR fed a 40 g/kg NaCl diet (198 ± 4 mm Hg, N = 10, vs. 182 ± 1 mm Hg, N = 29). Plasma volume in the 100 g/kg SHR was 4.75 ± 0.14 ml/100 g body wt, not significantly different from that in the 40 g/kg NaCl SHR group. SNGFR during loop perfusion with endogenous TF at rates of 0, 10, 20 and 40 nl/min were 35.9 ± 1.9 (N = 16), 33.4 ± 1.4 (N = 4), 30.8 ± 3.4 (N = 8) and 23.0 ± 2.6 (N = 12) nl/min/g kidney wt, respectively. Maximal reduction of SNGFR on increasing the perfusion rate from 0 to 40 nl/min was 12.8 ± 1.1 nl/min/g kidney wt, which represents a 36.8 ± 3.1% reduction (Fig. 7). This is significantly higher than in the 40 g/kg NaCl SHR group. The response of SNGFR to loop perfusion was not as uniform as in salt-loaded or control WKY or control SHR.

Discussion

Different effects of salt loading in SHR and WKY

In the present study there were three major differences between WKY and SHR in the response to chronic dietary salt loading. Firstly, consistent with previous reports [1, 2, 5], systolic blood pressure increased more rapidly and to higher levels in salt-loaded SHR than in controls. WKY showed no response of blood pressure to salt loading. Secondly, after two weeks of salt loading plasma volume in both strains had increased to a comparable extent. However, plasma volume in WKY then declined progressively and returned to normal level

Table 2. Summary of systemic and renal function in the micropunctured kidney in experimental animals

	SHR		WKY	
	Control	Salt loaded	Control	Salt loaded
MBP *mm Hg*	152 ± 2 (19)	165 ± 21[a] (21)	104 ± 2[b] (14)	103 ± 1[b] (24)
GFR *ml/min/g kidney wt*	1.06 ± 0.03 (17)	1.10 ± 0.03 (17)	1.07 ± 0.01 (10)	1.10 ± 0.02 (15)
$\dot{U}V$ *µl/min/g kidney wt*	3.59 ± 0.17 (17)	4.00 ± 0.17 (20)	3.61 ± 0.31 (11)	3.97 ± 0.17 (14)
$\dot{V}U_{Na}$ *nmol/min/g kidney wt*	38.8 ± 6.9 (13)	572.6 ± 93.1[a] (14)	54.5 ± 7.7 (7)	465.4 ± 67.5[a] (10)
$\dot{V}U_K$ *nmol/min/g kidney wt*	864.5 ± 67.6 (13)	817.0 ± 39.3 (14)	845.9 ± 150.4 (7)	617.0 ± 61.4 (10)
$\dot{V}U_{Cl}$ *nmol/min/g kidney wt*	346.8 ± 60.2 (11)	805.6 ± 83.5[a] (13)	401.6 ± 56.6 (6)	703.0 ± 89.2 (10)
FE_{Na} %	0.022 ± 0.005 (9)	0.277 ± 0.055[a] (9)	0.034 ± 0.005 (6)	0.319 ± 0.040[a] (9)

Abbreviations are: MBP, mean blood pressure; GFR, glomerular filtration rate; $\dot{U}V$, urine volume; $\dot{V}U_{Na}$, $\dot{V}U_K$, $\dot{V}U_{Cl}$, urinary excretion of sodium, potassium and chloride, respectively; FE_{Na}, fractional excretion of sodium. Data are given as means ± SEM, with number of experiments in parentheses.

[a] Significant differences between salt-loaded and control rats

[b] Significant differences between the two strains

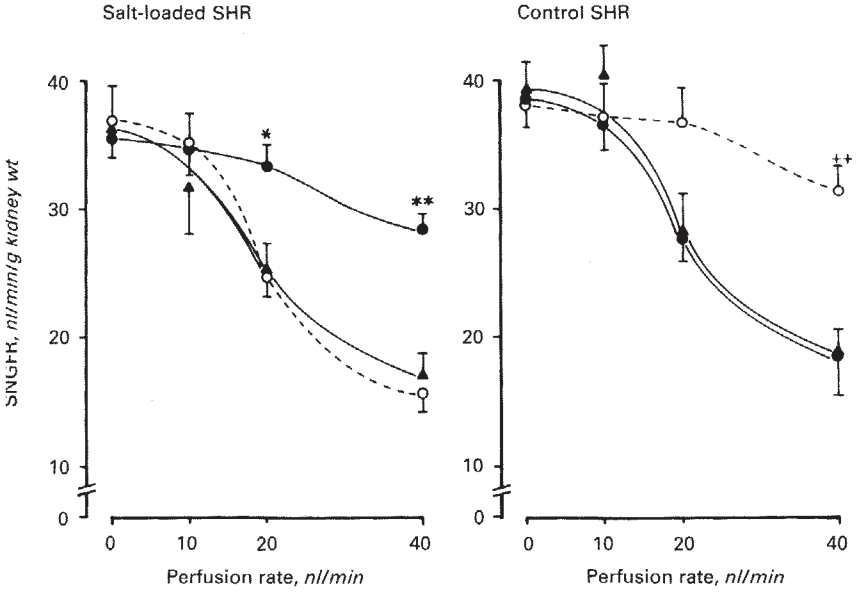

Fig. 3. *Relationship between SNGFR measured in proximal tubule and loop of Henle perfusion rate in salt-loaded and control SHR.* Symbols are: (-●-) endogenous tubular fluid; (--○--) exogenous tubular fluid; (-▲-) Ringer's solution. Data are given as means ± SEM. * Indicates a significant difference from the SNGFR at the same perfusion rate with exogenous tubular fluid. ** Indicates a significant different from SNGFR with exogenous tubular fluid or Ringer's solution. ++ indicates a significant difference from SNGFR with endogenous tubular fluid or Ringer's solution.

after four weeks. In contrast, the plasma volume expansion in SHR persisted over the entire dietary treatment period. These results are consistent with previous reports on SHR [24] or Dahl-S rats [25], and with recent observations on Munich-Wistar rats in which plasma volume expansion on salt loading was also transient and in which the high salt intake was balanced *without* volume expansion (unpublished observations D.A. Häberle, V. Gross, C. Mast and C. Metz). Third, chronic salt loading markedly attenuated the feedback response in both SHR and WKY due to the appearance of an inhibitory factor in the tubular fluid. This finding is consistent with previous observations from this laboratory [13, 19] and elsewhere [18]. This attenuation is, however, less pronounced in SHR than in WKY. Three observations lead to this conclusion: (i) When TGF activity is assayed by loop of Henle perfusion with Ringer's solution, the dependency of SNGFR on the loop of Henle perfusion rate and, in particular, the response of SNGFR to a supramaximal challenge (loop of Henle perfusion at 40 nl/min) was similar in normal and salt-loaded WKY and in normal and salt-loaded SHR. However, in agreement with earlier reports [22, 26–29] both the amplitude of TGF curve, that is, the maximum response, and TGF sensitivity (the slope of the curve at the turning point) was greater in SHR than in WKY. Thus, in SHR TGF appears to be hyperactive and this hyperactivity appeared not to be affected by salt loading. (ii) Consistent with an earlier report [19], TGF resetting was caused by the activation of a humoral inhibitory principle in tubular fluid. This is concluded from the following: First, the feedback

Table 3. Single nephron GFR (SNGFR) values and maximal response of SNGFR (SNGFR max) in salt-loaded SHR (SHR salt), in control SHR (SHR cont), in salt-loaded WKY (WKY salt) or in control WKY (WKY cont) rats

	SNGFR *nl/min/g kidney wt*				ΔSNGFR max %
	0	10	20	40	
SHR salt					
TF end	35.5 ± 1.5 (30)	34.7 ± 2.3 (10)	33.4 ± 1.7[a] (21)	28.3 ± 1.3[b] (21)	14.9 ± 2.9[b,d] (21)
TF exo	36.9 ± 2.8 (10)	35.0 ± 2.5 (5)	24.9 ± 1.7 (9)	15.6 ± 1.3 (7)	57.8 ± 2.6 (7)
Ringer	36.3 ± 2.0 (9)	31.1 ± 3.0 (3)	25.2 ± 2.2 (6)	17.3 ± 1.5 (9)	52.9 ± 2.4 (9)
SHR cont					
TF end	38.6 ± 1.7 (14)	36.7 ± 2.0 (9)	27.8 ± 1.9 (9)	18.6 ± 1.3 (11)	52.7 ± 2.9 (11)
TF exo	38.1 ± 1.8 (21)	37.2 ± 2.7 (8)	36.8 ± 2.8 (12)	31.5 ± 2.0[c] (15)	16.9 ± 2.5[c] (15)
Ringer	39.2 ± 2.3 (10)	40.9 ± 3.0 (5)	28.3 ± 3.0 (5)	18.7 ± 1.9 (8)	53.0 ± 3.2[e] (8)
WKY salt					
TF end	34.5 ± 1.4 (22)	34.6 ± 1.8 (12)	33.5 ± 3.2 (8)	31.5 ± 0.8[b] (12)	3.1 ± 1.6[b] (12)
TF exo	36.1 ± 1.9 (12)	33.5 ± 2.3 (7)	30.2 ± 1.9 (7)	22.5 ± 1.8 (10)	37.4 ± 2.8 (10)
Ringer	35.2 ± 3.4 (10)	34.3 ± 3.5 (5)	26.2 ± 1.7 (7)	22.3 ± 2.1 (8)	39.3 ± 1.9 (8)
WKY cont					
TF end	41.2 ± 2.2 (18)	42.3 ± 2.6 (8)	31.8 ± 2.0 (10)	23.0 ± 1.7 (11)	40.8 ± 2.8 (11)
TF exo	41.3 ± 1.8 (15)	40.5 ± 1.8 (8)	36.7 ± 2.8 (8)	37.3 ± 1.4[c] (9)	8.2 ± 1.9[c] (9)
Ringer	41.7 ± 1.3 (9)	39.1 ± 0.4 (3)	32.0 ± 0.6 (4)	26.8 ± 1.1 (9)	35.6 ± 1.4 (9)

Perfusates are endogenous tubular fluid (TF end), exogenous tubular fluid (TF exo) or Ringer's solution (Ringer). Data are given as means ± SEM.
[a] Significant difference from SNGFR at the same perfusion rate with exogenous tubular fluid
[b] Significant difference from SNGFR and maximal response of SNGFR with exogenous tubular fluid or Ringer's solution
[c] Significant difference from SNGFR and maximal response of SNGFR with endogenous tubular fluid
[d] Significant difference from maximal response of SNGFR in salt-loaded WKY on loop perfusion with endogenous tubular fluid
[e] Significant difference from maximal response of SNGFR in control WKY on loop perfusion with Ringer's solution

response in both salt-loaded and control rats of each strain was markedly and similarly attenuated on loop perfusion with TF from high salt rats. Second, feedback responses in both salt loaded and control rats of each strain were similar and "normal" on loop perfusion with TF from control rats. Third, the TGF response in nephrons microperfused with TF from control rats was similar to that seen with Ringer's perfusion. Thus, since the TGF responses to the two types of tubular fluid differ in the same animal, the fluids must differ in their properties. Since the TGF response to Ringer's perfusion was similar to that with TF from control rats, the inhibition of TGF seen with TF from high salt rats must result from the appearance of an inhibitory principle in this tubular fluid. It is unlikely that the inhibitory principle simply causes a decrease in the intraluminal NaCl concentration at the macula densa segment. In comparable experiments on salt-loaded Munich-Wistar rats NaCl delivery to the early distal tubular segment was similar in salt-loaded and control rats [13]. Moreover, in both strains of rats dietary salt loading caused a significant increase of urinary sodium and chloride excretion compared with control. The nature and the mechanism responsible for activation of the feedback inhibitory factor are not clear and require further elucidation. (iii) As mentioned above, with TF from salt-loaded SHR the feedback activity in donor or normal SHR was less attenuated than with tubular fluid from salt-loaded WKY. This difference is even more evident when feedback response to the test challenge with Ringer's solution in salt loaded rats of both strains was taken into account. When the ratios between maximum reduction of SNGFR by loop perfusion with endogenous TF to that with Ringer's solution from each rat were compared between the strains, the ratio in salt-loaded SHR was significantly greater than that in salt-loaded WKY. There was no difference, however, between the ratio in control SHR and control WKY. This difference in the salt loaded rats appears to be due to a less homogeneous generation of the TGF inhibitory factor in SHR compared with WKY. Whereas salt loading in WKY resulted in similar TGF attenuation in all rats, the TGF in some SHR appeared not to be attenuated at all.

Defective TGF inhibition and blood pressure

Interestingly, the increase of blood pressure on salt loading in the latter rats was greater than in SHR in which the TGF was attenuated to a similar extent as in WKY. This conclusion is based on the following observations: Firstly, systolic blood pressure of the (conscious) salt-loaded donor SHR is significantly correlated to the maximum feedback response elicited by

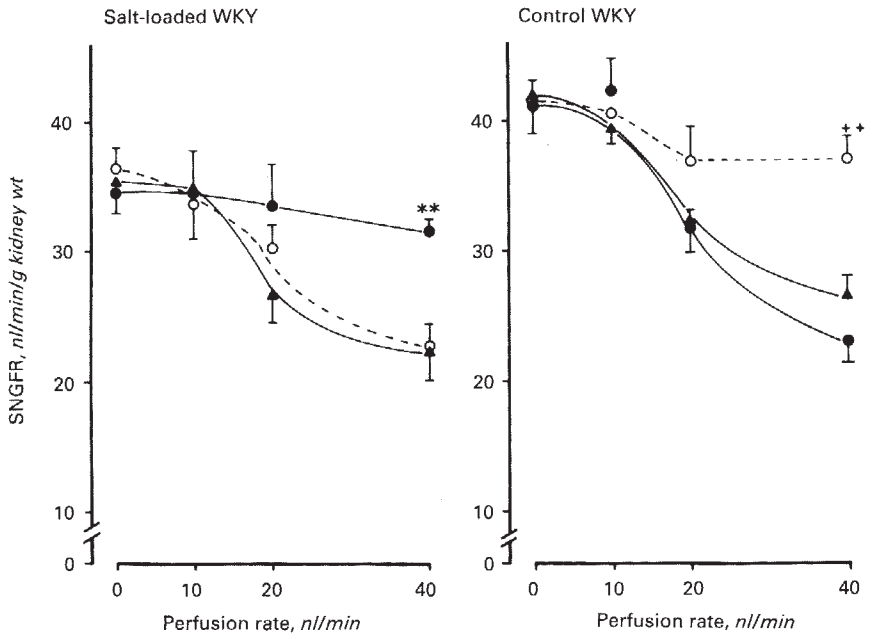

Fig. 4. *Relationship between SNGFR measured in proximal tubule and loop of Henle perfusion rate in salt-loaded and control WKY.* Data are given as means ± SEM. Symbols are: (–●–) endogenous tubular fluid; (--○--) exogenous tubular fluid; (–▲–) Ringer's solution. ** Indicates a significant difference from SNGFR at a same perfusion rate with exogenous tubular fluid or Ringer's solution. + indicates a significant difference from SNGFR with exogenous tubular fluid or Ringer's solution.

its TF in control or salt-loaded SHR. No such correlation was seen in control SHR. Two observations exclude the possibility that this correlation results from a different pressure natriuretic response within the group and hence a relative difference of sodium loading. First, there was no negative correlation between plasma volume and systolic blood pressure in salt-loaded SHR. Second, when salt loading of SHR was increased by feeding a 100 g/kg NaCl diet a similar relationship between systolic blood pressure and feedback activation with tubular fluid was observed. This suggests that the defective activation of the feedback inhibitory substance and the sensitivity of the blood pressure to salt loading in SHR might be in some way causally interrelated. The underlying mechanisms are not understood at present.

Defective TGF inhibition and volume expansion

It is not clear whether or not the persistence in plasma volume expansion is due to the defective feedback resetting in salt-loaded SHR rats. That plasma volume correlates neither with the level of systolic blood pressure (and nor, presumably, with the level of TGF activity) in salt-loaded SHR and that TGF resetting and plasma volume do not correlate in salt-loaded WKY might be regarded as evidence against such a relationship. However, in view of the other sodium retaining mechanisms activated in SHR and their coexistence with pressure diuresis, the above conclusion may be premature. Salt loading in SHR, for instance, has no effect on plasma atrial natriuretic factor (ANF) concentration [30]. In addition, the capacity for synthesis of vasodilatory prostaglandins on salt loading is impaired in SHR compared with WKY [31]. Moreover, sympathetic nerve activity, which has been shown to stimulate tubular salt and water reabsorption in both anaesthetized and conscious animals [32–34], is significantly increased in SHR and further enhanced by sodium loading. The pathophysiological relevance of this mechanism is apparent in the following observations: Stress in conscious SHR increases blood pressure and renal sympathetic nerve activity and decreases urinary sodium excretion [35]. This antinatriuretic effect of stress in SHR is enhanced by chronic sodium loading, whereas similar stress in conscious WKY has no effect on sodium excretion [36]. Finally, as discussed above, without the inhibitor in tubular fluid the tubuloglomerular feedback system is hyperactive in salt-loaded and normal SHR compared with WKY rats, also possibly a consequence of increased sympathetic nerve activity (see above). This follows from earlier observations which demonstrated a marked attenuation of this hyperactivity after renal denervation. Similar denervation in WKY did not influence the feedback activity [28]. It might thus be argued that firstly, in salt-loaded SHR, TGF inhibition, had it been present, would not have been adequate to overcome the increased sodium retention and to return the expanded plasma volume to normal. Secondly, since these SHR rats have a primary increase in blood pressure, the increased sodium retention will be partly balanced by enhanced pressure natriuresis resulting—as observed—in comparable urinary excretion of electrolytes during salt loading in both SHR and WKY. These mechanisms may compensate to some extent the defective TGF inhibition, resulting in virtual independency between plasma volume and TGF activity.

Unifying hypothesis

A unifying, but admittedly completely unproven, hypothesis might be advanced with respect to these considerations. If it were assumed that the TGF inhibitor in tubular fluid inhibits TGF by stimulating the production of a vasodilating and natriuretic substance in the JGA, this substance might not only prevent the vasomotor effect of a stimulation of the macula densa by a high luminal NaCl concentrations but also compensate the blood pressure raising effect of putative Na-K-ATPase inhibitor produced during chronic salt loading. In addition,

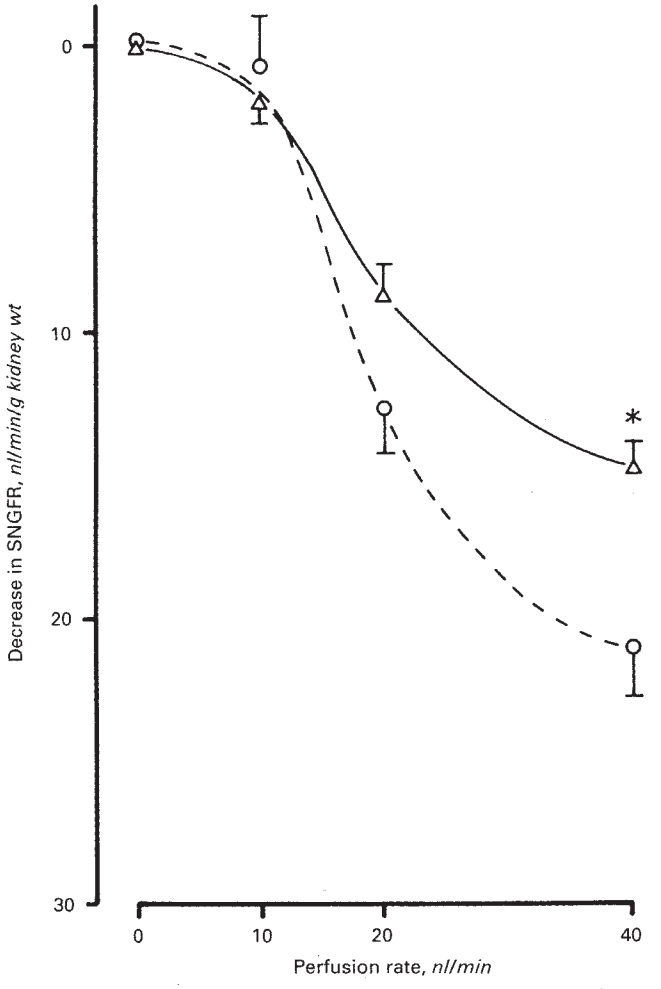

Fig. 5. *Relationship between the decrease of SNGFR and loop of Henle perfusion rate with Ringer's solution in control SHR (-○-) and control WKY (-△-).* Data are given as means ± SEM. * Indicates a significant difference from the corresponding value in control SHR.

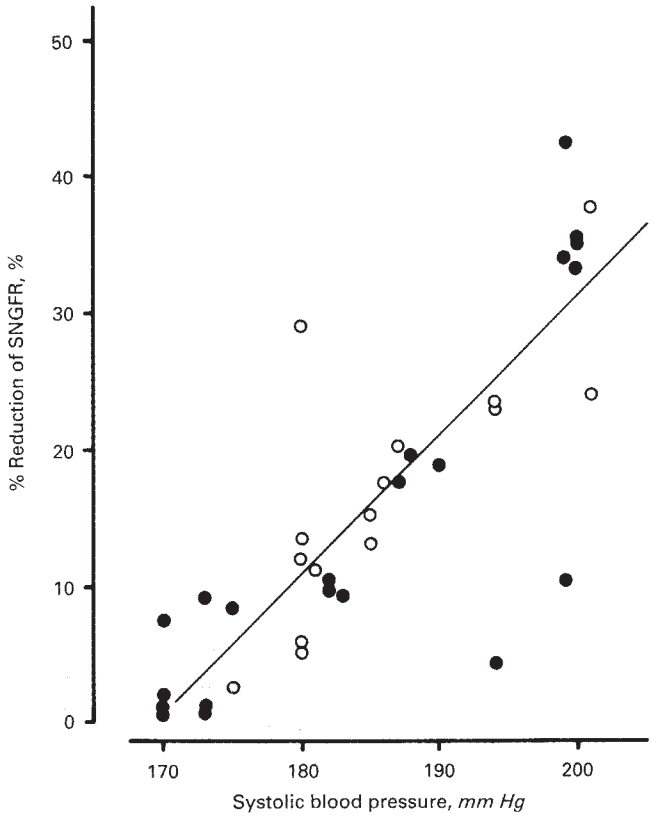

Fig. 6. *Relationship between the maximal response of SNGFR in salt-loaded (●) and control (○) SHR on loop perfusion with tubular fluid from salt-loaded SHR and the systolic blood pressure of the conscious donor SHR.* $r = 0.8142$; $P < 0.001$.

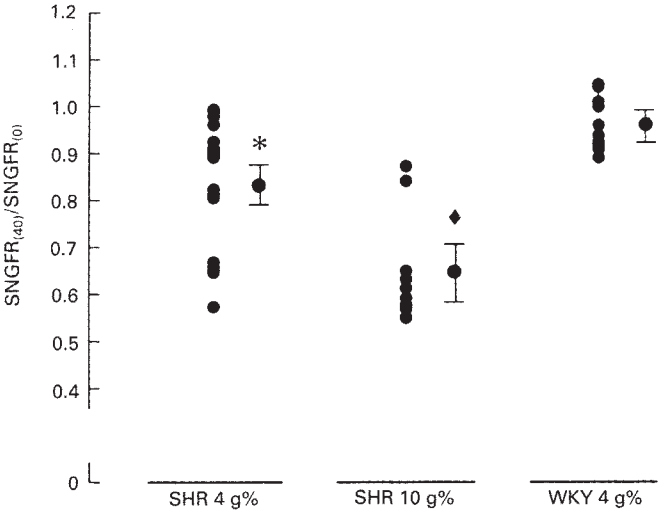

Fig. 7. *Maximal feedback responses by loop perfusion with endogenous tubular fluid in 40 g/kg NaCl diet-loaded SHR (SHR 4 g%) and WKY (WKY 4 g%), and 100 g/kg NaCl loaded SHR (SHR 10 g%).* * Indicates a significant difference from the value in 40 g/kg NaCl loaded WKY. ◆ Indicates a significant difference from the value in 40 g/kg NaCl loaded SHR.

because of its TGF inhibiting effects, this substance would contribute to increased sodium chloride excretion and hence, under chronic conditions and in concert with other mechanisms, enable the organism to excrete an increased sodium chloride intake without major expansion of the plasma and extracellular volume.

In summary, tubuloglomerular feedback resetting in chronically salt-loaded SHR and WKY rats is caused by the activation of humoral feedback inhibitory substance. However, the activity of this factor in tubular fluid from SHR was not uniform and, on average, less than in WKY. The maximum feedback response elicited by loop perfusion with tubular fluid from salt-loaded SHR and the systolic blood pressure in the conscious donor rat were significantly correlated. Thus, in salt-loaded SHR less activation of the feedback inhibitory substance goes in hand with a greater blood pressure increase. In addition, plasma volume expansion by salt loading persists in SHR but was only transient in WKY. It is thus reasonable to propose that the renal response to chronic salt loading in SHR might be blunted by defective activation of the feedback inhibitory substance. This defect may contribute to the exacerbation and maintenance of hypertension in this strain.

Acknowledgments

The authors gratefully acknowledge the support of Professor Dr. Klaus Thurau as well as financial support of the Alexander v. Humboldt Foundation. We also thank Dr. J. Davis for his help with the manuscript and Ms. M. Stachl for technical assistance.

Reprint requests to Yasuyuki Ushiogi, The First Department of Internal Medicine, School of Medicine, Kanazawa University, 13-1 Takaramachi, Kanazawa City, Ishikawa, 920 Japan.

References

1. WYSS JM, LIUMSIRICHAROEN M, SRIPAIROJTHIKOON W, BROWN D, GIST R, OPARIL S: Exacerbation of hypertension by high chloride, moderate sodium diet in the salt-sensitive spontaneously hypertensive rat. *Hypertension* 9(Suppl. III):III-171–III-175, 1987
2. GRADIN K, DAHLÖF C, PERSSON B: A low dietary sodium intake reduces neuronal noradrenaline release and the blood pressure in spontaneously hypertensive rats. *Naunyn-Schmiedeberg's Arch Pharmacol* 332:364–369, 1986
3. LOUIS WJ, TABEI S, SPECTOR S, SJOERDSMA A: Studies on the spontaneously hypertensive rat: Geneology effects of varying salt intake and kinetics of catecholamine metabolism. *Circ Res* 24 and 25(Suppl. I):I-93–I-102, 1969
4. DIETZ R, SCHÖMIG A, RASCHER W, STRASSER R, KÜBLER W: Enhanced sympathetic activity caused by salt loading in spontaneously hypertensive rats. *Clin Sci* 59:171–173, 1980
5. YAMASHITA Y, TAKATA Y, TAKISHITA S, KIMURA Y, FUJISHIMA M: Enhancement of brain renin-angiotensin system induced by long-term salt loading in spontaneously hypertensive rats. *J Hyperten* 4(Suppl 3):S361–S363, 1986
6. ROMAN RJ, COWLEY AW: Abnormal pressure diuresis natriuresis response in spontaneously hypertensive rats. *Am J Physiol* 248 (Renal Fluid Electrol Physiol 17):F199–F205, 1985
7. ARENDSHORST WJ: Autoregulation of renal blood flow in spontaneously hypertensive rats. *Circ Res* 44:344–349, 1979
8. KAWABE K, WATANABE T, SHIONO K, SOGABE H: Influence on blood pressure of renal isografts between spontaneously hypertensive and normotensive rats, using F1 hybrids. *Jpn Heart J* 19:886–894, 1978
9. SCHNERMANN J, BRIGGS JP: Function of the juxtaglomerular apparatus: Local control of glomerular hemodynamics, in *The Kidney: Physiology and Pathophysiology*, edited by DW SELDIN, G GIEBISCH, New York, Raven Press, 1985, pp. 669–697
10. NAVAR LG, ROSIVALL L, CARMINES PK, OPARIL S: Effects of locally formed angiotensin II on renal hemodynamics. *Fed Proc* 45:1448–1453, 1986
11. SCHNERMANN J, BRIGGS JP: Role of the renin-angiotensin system in tubuloglomerular feedback. *Fed Proc* 45:1426–1430, 1986
12. DEV B, DRESCHER C, SCHNERMANN J: Resetting of tubuloglomerular feedback sensitivity by dietary salt intake. *Pflügers Arch* 234:263–277, 1974
13. DAVIS JM, HÄBERLE DA, KAWATA T: The control of glomerular filtration and renal blood flow in chronically volume-expanded rats. *J Physiol* 402:473–495, 1988
14. KAUFMANN JS, HAMBURGER RJ, FLAMENBAUM W: Tubuloglomerular feedback: Effect of dietary NaCl intake. *Am J Physiol* 231: 1744–1749, 1976
15. MOORE LC, YARIMIZU SH, SCHUBERT G, WEBER PC, SCHNERMANN J: Dynamics of tubuloglomerular feedback adaption to acute and chronic changes in body fluid volume. *Pflügers Arch* 387:39–45, 1980
16. PERSSON AEG, SCHNERMANN J, WRIGHT FS: Modification of feedback influence on glomerular filtration rate by acute isotonic volume expansion. *Pflügers Arch* 381:99–105, 1979
17. PLOTH DW, RUDOLPH J, THOMAS C, NAVAR LG: Renal and tubuloglomerular feedback responses to plasma expansion in the rat. *Am J Physiol* 235:F156–F162, 1978
18. SCHNERMANN J, SCHUBERT G, BRIGGS JP: Tubuloglomerular feedback responses with native and artificial tubular fluid. *Am J Physiol* 250:F16–F22, 1986
19. HÄBERLE DA, DAVIS JM: Resetting of tubuloglomerular feedback: Evidence for a humoral factor in tubular fluid. *Am J Physiol* 246 (Renal Fluid Electrol Physiol 15):F495–F500, 1984
20. HÄBERLE DA, DAVIS JM, KAWATA T, DAHLHEIM H, SCHMITT E: The effect of acute adrenalectomy on the resetting of tubuloglomerular feedback and renal renin release in chromic volume expansion, in *The Juxtaglomerular Apparatus*, edited by AEG PERSSON, U BOBERG, Amsterdam, Elsevier, 1988, pp. 177–188
21. DAVIS JM, TAKABATAKE T, KAWATA T, HÄBERLE DA: Resetting of tubuloglomerular feedback in acute volume expansion in rats. *Pflügers Arch* 411:322–327, 1988
22. ARENDSHORST WJ, POLLOCK DM: Altered reactivity of tubuloglomerular feedback in the spontaneously hypertensive rat, in *The Juxtaglomerular Apparatus*, edited by AEG PERSSON, U BOBERG, Amsterdam, Elsevier, 1988, pp. 297–312
23. WALLENSTEIN S, ZUCKER CL, FLEISS JL: Some statistical methods useful in circulation research. *Circ Res* 47:1–9, 1980
24. HARRAP SJ, DINICOLANTONIO R, DOYLE AE: Sodium intake and exchangeable sodium in hypertensive rats. *Clin Exp Hypertens* A6:427–440, 1984
25. GENAIN CP, REDDY SR, OTT CE, VANLOON GR, KOTCHEN TA: Failure of salt loading to inhibit tissue norepinephrine turnover in prehypertensive Dahl salt-sensitive rats. *Hypertension* 12:568–573, 1988
26. LEYSSAC PP, HOLSTEIN-RATHLOU NH: Tubuloglomerular feedback response: Enhancement in adult spontaneously hypertensive rats and effects of anaesthetics. *Pflügers Arch* 413:267–272, 1989
27. PLOTH DW, DAHLHEIM H, SCHMIDMEIER E, HERMLE M, SCHNERMANN J: Tubuloglomerular feedback and autoregulation of glomerular filtration rate in Wistar-Kyoto spontaneously hypertensive rats. *Pflügers Arch* 375:261–267, 1978
28. TAKABATAKE T, USHIOGI Y, OHTA K, HATTORI N: Attenuation of enhanced tubuloglomerular feedback activity in SHR by renal denervation. *Am J Physiol* 258:(Renal Fluid Electrol Physiol 27): F980–F985, 1990
29. TAKABATAKE T, USHIOGI Y, OHTA H, YAMAMOTO Y, ISHIDA Y, HARA H, KAWABATA M, HATTORI N: Enhanced tubuloglomerular feedback activity is partially mediated by blood volume reduction in spontaneously hypertensive rats. *J Hypertens* 4(Suppl. 3):383–385, 1986
30. JIN H, CHEN Y-F, YANG RH, MENG QC, OPARIL S: Impaired release of atrial natriuretic factor in NaCl-loaded spontaneously hypertensive rats. *Hypertension* 11:739–744, 1988
31. MARTINEAU A, ROBILLARD M, FALARDEAU P: Defective synthesis of vasodilator prostaglandins in the spontaneously hypertensive rats in vivo. *Hypertension* 6(Suppl. I):I-161–I-165, 1984
32. BENCSATH P, SZENASI G, TAKACS L: Water and electrolyte transport in Henle's loop and distal tubule after renal sympathectomy in the rat. *Am J Physiol* 249(Renal Fluid Electrol Physiol 18):F308–F314, 1985
33. NOMURA G, TAKABATAKE T, ARAI S, UNO D, SHIMAO M, HATTORI N: Effects of acute unilateral renal denervation on tubular sodium reabsorption in the dog. *Am J Physiol* 232:(Renal Fluid Electrol Physiol 1):F16–F19, 1977
34. TAKABATAKE T: Feedback regulation of glomerular filtration rate in the denervated rat kidney. *Kidney Int* 22(Suppl. 12):129–135, 1982
35. KOEPKE JP: Renal responses to stressful environmental stimuli. *Fed Proc* 44:2823–2827, 1985
36. KOEPKE JP, DIBONA GF: High sodium intake enhances renal nerve and antinatriuretic responses to stress in spontaneously hypertensive rats. *Hypertension* 7:357–363, 1985