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Pressure natriuresis in isolated kidneys from hypertension-prone and hypertension-resistant rats (Dahl rats)[1]

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Pressure natriuresis in isolated kidneys from hypertension-prone and hypertension-resistant rats (Dahl rats). Dahl described a strain of rats with genetically controlled propensities for hypertension. Chronic excess salt feeding increased blood pressure in sensitive (S) rats, whereas resistant rats (R) remain normotensive. We tested the pressure natriuretic function (urinary sodium excretion versus perfusion pressure) in isolated kidneys perfused with a cellular medium: in sodium-restricted normotensive sensitive (S_0) and resistant (R_0) animals; in sensitive rats receiving a high-salt diet for 3 weeks (S_3); and in both S and R animals exposed to excess sodium for 7 weeks (R_7 and S_7). The aim of these studies was to determine if a preset alteration of the pressure natriuretic function might be present in S animals prior to the development of hypertension. Systolic blood pressure in S_0, S_3, and S_7 animals were 123 ± 4, 136 ± 2, and 162 ± 4 mm Hg, respectively, whereas that of R_0 and R_7 were 121 ± 5 and 126 ± 5 mm Hg. An increase of the perfusion pressure of isolated kidneys from 105 to 185 mm Hg in stepwise fashion resulted in a pressure natriuresis whose slope was similar in R_0 and S_0 animals. Of interest was that the pressure natriuretic function slope of kidneys from R_0 (low sodium) and R_7 (high sodium) rats was as predicted by the Guyton system analysis of normal blood pressure control. Micropuncture of the proximal nephrons demonstrated that the origin of the natriuresis resulted from a site beyond the accessible proximal tubule. Results from S_7 kidneys contrasted with all others in that the natriuretic response was depressed ($P < 0.01$), which resulted from significantly lower filtration rates at higher perfusion pressures. We concluded (*1*) in normal R rats, the pressure natriuretic function is that predicted by the Guyton hypothesis, (*2*) Dahl S animals have no preset abnormality of this function until hypertension is present for some time, and (*3*) a depression of the pressure natriuretic function may aggravate hypertension in S rats once high blood pressure has persisted.

Natriurèse de pression dans les reins isolés provenant de rats de Dahl sensibles ou résistants à l'hypertension. Dahl a décrit une souche de rats ayant une propension génétique à l'hypertension. L'administration d'une alimentation riche en sel augmente la pression artérielle chez les rats sensibles (S) alors que les rats résistants (R) restent normotendus. Nous avons étudié la natriurèse de pression (excrétion urinaire de sodium en fonction de la pression de perfusion) dans des reins isolés perfusés avec un milieu cellulaire provenant de rats normotendus S_0 et R_0 soumis à une restriction de sodium, de rats sensibles ayant reçu une alimentation riche en sel pendant trois semaines (S_3) et de rats S et R exposés à un excès de sel pendant 7 semaines (R_7 et S_7). Le but de ces études est d'établir l'existence éventuelle d'une modification de la relation pression natriurèse avant le développement de l'hypertension. Les pressions systoliques des rats, S_0, S_3, et S_7 étaient de 123 ± 4, 136 ± 2, et 162 ± 4 mm Hg, respectivement, alors que celles de R_0 et R_7 étaient de 121 ± 5 et 126 ± 5 mm Hg. Une augmentation de la pression de perfusion de reins isolés de 105 à 185 mm Hg, par paliers, a eu pour résultat une natriurèse de pression dont la pente était la même chez R_0 et S_0. Il est intéressant de remarquer que la pente de cette relation pour les reins R_0 (sodium bas) et R_7 (sodium élevé) est celle qui est prévue par l'analyse de Guyton du contrôle normal de la pression artérielle. Des microponctions des néphrons proximaux ont montré que l'origine de la natriurèse est en aval du tuble proximal accessible. Les résultats des reins S_7 ont été différents de tous les autres en ce sens que la réponse natriurétique a été déprimée ($P < 0{,}001$) en raison des débits de filtration significativement plus faibles à des pressions de perfusion plus élevées. Nous concluons: (*1*) chez les rats R normaux la relation pression-natriurèse est celle prédite par l'hypothèse de Guyton; (*2*) les animaux Dahl S n'ont pas d'anomalie préexistante de cette fonction; (*3*) une dépression de la relation natirurèse-pression peut aggraver l'hypertension chez les rats S une fois que la pression artérielle élevée a persisté.

The kidney responds to an increase in perfusion pressure with increments in sodium and water excretion [1, 2]. Guyton et al suggest that this pressure natriuresis phenomenon plays an important physiologic role in the maintenance of normal blood pressure [3, 4]. A key element in their theory is that increments in blood pressure are dissipated by the volume loss caused by the pressure natriuresis and conversely that small degrees of hypotension result in sodium retention until normal blood pressure is

[1] Dr. Dahl, with whom we were collaborating, died just as this work was beginning. We wish to dedicate this paper to his memory.

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restored. In essence, the pressure natriuretic phenomenon is important in both blood pressure control and volume homeostasis.

Guyton et al [4] and Coleman and Guyton [5] further suggest that depression of the pressure natriuretic phenomenon may be the cause of several forms of hypertension. They postulate that in some disease states a higher blood pressure is required to excrete ingested sodium, and that this therefore sets the systemic blood pressure at a higher value. This hypothesis has led to the study of relationships between arterial pressure and salt excretion in several models of experimental hypertension [6, 7], and has led us to question whether aberrations of the pressure natriuresis function (PNF) might be the cause of hypertension in Dahl rats.

Dahl rats are particularly suited to study the role of PNF in the development of hypertension. One strain of these animals have genetically controlled propensities for high blood pressure. Chronic excess salt feeding results in hypertension in the sensitive rats (S), but the resistant animals (R) remain normotensive [8]. Furthermore, data suggest that renal factors are important in producing and maintaining hypertension in these strains. When kidneys from S animals are grafted into R rats, hypertension develops, and conversely, when R kidneys are given to S animals, blood pressure is reduced [9, 10].

We therefore designed protocols to detect if the cause of high blood pressure in S rats was due to a genetically determined flattening of the PNF. The effect of pressure on renal sodium handling was examined in both Dahl strains subjected to a diet of either high- or low-sodium content, combining micropuncture and an isolated perfused kidney technique. By comparing the results of kidneys from S animals prior to and during the development of hypertension with those of R rats, we observed that Dahl rats display a normal PNF prior to the development of high blood pressure. A depression of the PNF was observed, however, after the development of frank hypertension. Alteration in the PNF therefore cannot be implicated in the salt-evoked hypertension in this strain. Once blood pressure is elevated, however, it may be maintained by an altered natriuresis function.

Methods

Materials. Dahl sensitive (S) and resistant (R) donor rats were used. From weaning, animals received a low- (0.02%) sodium diet. They were then divided into five groups. One R (R_0) and one S (S_0) group were maintained with the low-sodium diet till kidney isolation. One R (R_7) and one S (S_7) counterpart received for 7 weeks a high-sodium diet (8.02%). The fifth group (S_3) was exposed to the high-sodium diet only 3 weeks before kidney isolation (see Table 1). Normotensive Wistar rats fed standard laboratory chow were used for the experiments validating the isolated kidney perfusion technique. Food was withdrawn the evening before each study, but the animals had free access to water. At the time of kidney isolation, rats were 12 to 14 weeks old, and their weight was 290 to 350 g.

Isolated kidney perfusion technique: (1) Isolation of donor kidney. Isolated kideneys were perfused by a modification of the method of De Mello and Maack [11]. The major alteration introduced by us was the use of a perfusion medium containing 20 vol% of bovine red blood cells. Rats, anesthetized by i.p. injection of pentobarbital (10 mg/100 g body wt) (Nembutal®, Abott), were placed on a water-thermoregulated dissection table. A tracheotomy was performed, the femoral artery cannulated (with PE-50 polyethylene tubing), and arterial blood pressure monitored throughout the operation with a strain gauge (Endevco 8503-4), a Wheatstone bridge (Endevco 8630), and a graphical recorder (Hewlett Packard 7100 BM). Whenever pressure decreased more than 20 mm Hg, all surgical manipulations were temporarily suspended, and, if the decrement continued, 0.5 to 1.0 ml of isotonic saline was administered via the jugular vein. Unlike others [11], we did not canulate the vena cava, noting that this procedure occasionally leads to transient but very pronounced drops in arterial pressure when the final ligature of the vena cava is tightened. The remainder of the surgical preparation of donor rats, the procedure of kidney isolation, and arrangement of the isolated kidney in a micropuncture cup were

Table 1. Systolic blood pressure measured at time of kidney isolation in Dahl rats sensitive (S) or resistant (R) to high-sodium diet

Group	No. of animals	Duration of exposure to high-sodium diet *weeks*	Systolic blood pressure[a] *mm Hg*
R_0	7	0	121 ± 5
S_0	6	0	123 ± 4
R_7	7	7	126 ± 5
S_3	5	3	136 ± 2[b]
S_7	6	7	162 ± 4[c]

[a] Values are means ± SEM. These were obtained in vivo the day before the kidney isolation

[b] $P < 0.01$

[c] $P < 0.001$, compared with the mean blood pressure of the group S_0.

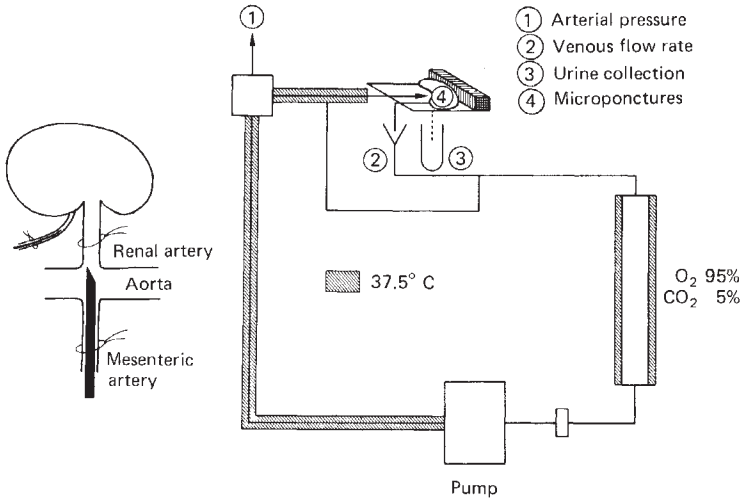

Fig. 1. *Arrangement used for cannulation of renal artery and perfusion of isolated kidney preparation.*

essentially that described by De Mello and Maack [11]. This also allowed stereomicroscopic examination of kidney surface during the perfusion. Only kidneys with an initial homogenous opening of the superficial tubules were studied. The perfusion circuit (Fig. 1) was also similar to that described by these authors except that all the perfusion tubes were included in tubes of larger diameter, within which water was circulated at 37.5° C. This thermoregulated water circuit minimized the temperature changes of the perfusate during the recirculation, as it is required by the presence of living erythrocytes in the perfusate.

(2) Control of perfusion pressure. The perfusion pressure (PP) within the renal artery was maintained constant (± 2 mm Hg) at each selected level[2]. Perfusion flow rate (PFR) was measured by counting the drops of the renal effluent with two electrodes and an electronic integrator. Pressure above the renal canula was continuously recorded by a strain gauge (Endevco 8503-4) and a Wheatstone bridge (Endevco 8630). Both PFR and pressure were simultaneously recorded on a two-channel strip chart recorder (Hewlett Packlard 7100 BM), calibrated in milliliters per minute and millimeters of mercury.

(3) Perfusion medium. The medium preparation, except for the erythrocytes, is essentially that described by De Mello and Maack [11]. The composition of our recirculating perfusate is indicated in Table 2. The bovine albumin fraction V was supplied by Armour (Schweizerhalle Chemische Fabrik, Basel).

Ox blood, obtained by carotid section, immediately after slaughter in a packing house, was collected in a solution containing 12.5 mg/liter H_2O of glucose, 13.2 mg/liter citrate trisodium, 4.4 mg/liter citric acid, and 150 mg/liter tetracycline (200 ml of solution per liter of blood). The blood was filtered through gauze, and if clots or impurity were present, the material was discarded. The blood was stored 7 to 10 days at 4° C, and on the day prior to an experiment, the erythrocytes were washed four times in isotonic saline and two times in a modified Krebs solution. Care was taken to centrifuge at 4° C and 2500 rpm. The pellet of washed erythrocyte had an hematocrit of about 70%. A similar technique of erythrocytes preparation has been successfully used for isolated liver preparation [12]. We have used a 20% hematocrit (Table 2) in the perfusion solution. In this condition, the isolated kidney preparations display a much more stable GFR than do those perfused with a 40 vol% of ox red cells.

Experimental protocol. (1) Wistar control kidneys. Kidneys isolated from normotensive Wistar rats were perfused at a constant perfusion pressure of 125 mm Hg. We noted that, during the first 20 min of perfusion, the pump had to be accelerated slightly to maintain the desired perfusion pressure. Once a steady state was achieved, five clearance collections were made over a period of 100 min.

(2) Dahl experimental kidneys. In these experiments, perfusion pressure was increased from 105 mm Hg to 185 mm Hg in 20 mm Hg steps. (When

[2] The canula (a modified Luer Lock needle, 2R2 N° 12) offers an hydraulic resistance (R_h) so that the perfusate pressure drops from the beginning of the needle to its tip. This pressure drop (ΔP) is proportional to PFR because: $\Delta P = PFR \times R_h$. Therefore, calculated ΔP must be substracted from the pressure recorded above the renal canula to determine the actual perfusion pressure (PP).

Table 2. Composition of the recirculating perfusate[a]

Na	141.0 mEq/liter	Bovine erythrocytes	20 vol%
K	4.8 mEq/liter	Methionine	7.5 mg/dl
Ca	5.9 mEq/liter	Alanine	17.8 mg/dl
Mg	4.9 mEq/liter	Glycine	15.0 mg/dl
Cl	115.7 mEq/liter	Serine	21.0 mg/dl
H_2PO_4	0.15 mEq/liter	Arginine	21.0 mg/dl
SO_4	4.8 mEq/liter	Proline	23.0 mg/dl
HCO_3	25.0 mEq/liter	Isoleucine	13.0 mg/dl
HPO_4	1.2 mEq/liter	Aspartic acid	40.0 mg/dl
Acetate	10.0 mEq/liter	Antidiuretic hormone	5.5 mU/dl
Glucose	100.0 mg/dl	Aldosterone	28.0 ng/dl
Bovine albumin fract. V	6.0 g/dl	Inulin	100.0 mg/dl

[a] The initial perfusate used for washing the kidney had only the components indicated in the left part of this table. The total volume of perfusate used in the circulating circuitry was 120 ml.

the donor rats were hypertensive, the experiments began at a PP of 145 mm Hg, thus avoiding too large a pressure drop between in vivo values and initial PP.) Once the steady state was achieved, a clearance period lasting 10 to 15 min was obtained. Then pressure was increased in stepwise fashion. At each new pressure, 5 to 8 min of equilibrium time was allowed. (To verify that the steady-state conditions were maintained after increasing PP, once PP reached 185 mm Hg, the clearance procedures were performed twice at 20-min intervals. Urinary sodium excretion amounted to 9.76 and 9.90 μEq/min/g for the first test and 11.97 and 11.93 μEq/min/g for the second test.) Then, a 10- to 15-min clearance period was measured. An aliquot of perfusate was obtained in the middle of each period. All urine samples were collected under oil in preweighed vials, and the urinary flow rate (V) was determined gravimetrically. Concentrations of inulin, sodium (U_{Na}), and potassium (U_K) were determined on all samples by methods previously described [13, 14]. These measurements permitted calculations of the GFR, fractional tubular sodium reabsorption (% T_{Na}), urinary sodium excretion ($U_{Na}V$), urinary potassium excretion (U_KV), and filtration fraction (FF) by standard formulas.

Micropuncture studies were done in seven R_0 and six S_0 kidneys during the perfusion procedure. Tubular fluid collections at the end of proximal convolutions were made according to a conventional technique [14]. The oil blockage just distal to puncture site allowed complete collection of fluid. Therefore, single nephron glomerular filtration rate (SNGFR) could be calculated from the tubular fluid over plasma inulin concentration ($[TF/P]_{In}$) and the volume of fluid collected per unit of time. The fractional proximal fluid reabsorption (% T_{prox}) and the endproximal tubule fluid delivery (FD_{prox}) were calculated by the following formulas:

$$\% \, T_{prox} = (1-[1/(TF/P)_{In}]) \times 100$$
$$FD_{prox} = SNGFR \times (1/[TF/P]_{In})$$

Localizations were verified with injection of Microfil MV 122 (Canton Biomedical Product) and microdissection [15]. Micropressure measurements, with an adaptation of the technique of Wiederhielm et al [16], were determined in free-flow proximal tubules, stop-flow proximal tubules, free-flow distal tubules, and peritubular capillaries of about 5 μ in diameter [17]. Glomerular capillary pressure (P_{GC}) was estimated from the measurement of stop-flow proximal tubular pressure (P_{TSF}), and the calculated glomerular capillary colloid osmotic pressure (π_{GC}) by the Landis and Pappenheimer formula [18]:

$$\pi \text{ albumin} = 2.8 \, c + 0.18 \, c^2 + 0.012 \, c^3$$

where c is the afferent artery albumin concentration. Accordingly:

$$P_{GC} = P_{TSF} + \pi_{GC}$$

Measurement of arterial systolic blood pressure in vivo. The determinations were performed under light ether anesthesia by the technique of Friedman and Freed [19].

Statistics. The results are given as means ± SEM. The significance of the effect of increasing pressure among the different groups of kidneys was calculated by an analysis of variance or the least squares method. Differences in means were checked using Student's *t* test.

Results

Validation of the isolated kidney perfusion technique. In experiments designed to test the stability of our preparation, eight kidneys, obtained from Wistar rats, were perfused at 125 mm Hg for 100 min, a period exceeding that used when studying the Dahl rats. Results are summarized in Fig. 2, which illustrates the stability of the GFR, FF,

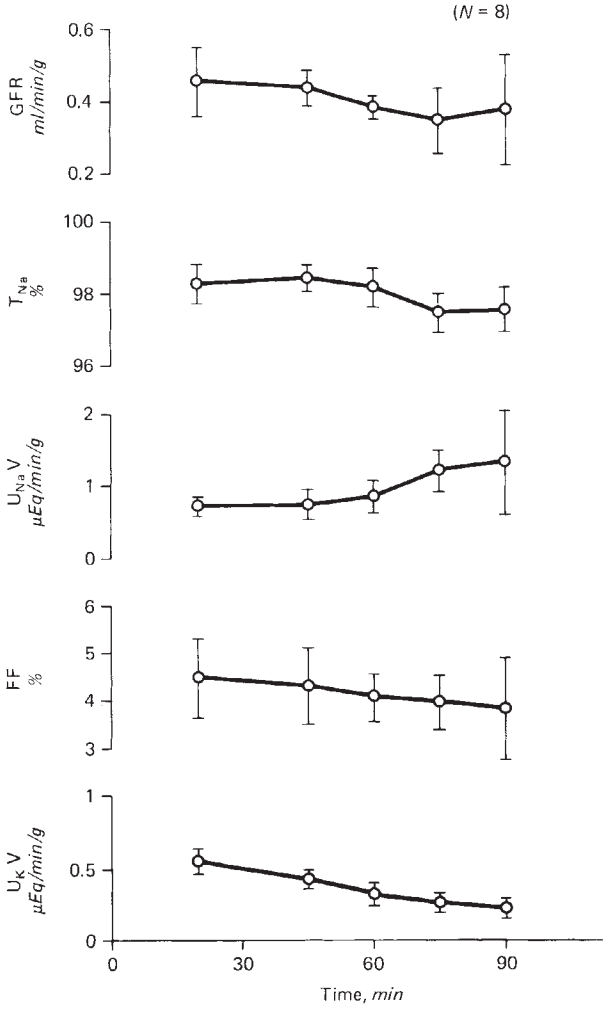

Fig. 2. *Renal function in isolated kidneys from Wistar control rats.* The data are plotted at the time corresponding to the middle of the clearance period. The last period was made between the 80th and the 100th min after the onset of the perfusion. Abbreviations are T_{Na}, fractional sodium reabsorption; and FF, filtration fraction.

$\%T_{Na}$, $U_{Na}V$, and U_KV during the 100 min of perfusion. GFR averaged 0.45 ml/min/g during the first two clearance periods and then decreased slightly to 0.38 ± 0.14 ml/min/g (P = NS) at the end of the experiment. The perfusate flow rate (PFR) did not significantly change from the first to the last clearance period. Indeed, PFR was 12.6 ± 0.8 (N = 8) and 11.9 ± 1.0 ml/min/g of kidney at the beginning and end of the perfusion, respectively. Filtration fraction (FF) was 4.5 ± 0.9% during the first two clearance periods and 4.0 ± 0.7% at the end of the experiment, values which contrast with the markedly lower FF seen in isolated kidney preparations perfused with acellular medium. This is a consequence of the lower blood flows required when the isolated kidney is perfused with a cellular medium. Fractional sodium reabsorption averaged 98.5% during the first and second clearance periods and then decreased only to 97 ± 0.6% (P = NS) in the last period. Thus, $U_{Na}V$, at constant perfusion pressure, was under 1 μEq/min/g during the first hour and rose only to 1.33 ± 0.7 μEq/min/g after 100 min of perfusion (P = NS). These values were achieved with physiologic albumin concentrations averaging 6 g/dl in the perfusate [20], and contrast with the 7.5 g/dl necessary to reach comparable values when using an acellular perfusion medium [21]. U_KV decreased slightly with the time from 0.55 ± 0.1 to 0.32 ± 0.08 μEq/min/g ($P < 0.05$). Table 3 demonstrates the similarities of glomerular and tubular functions in isolated kidneys as compared with anesthetized kidneys in vivo.

Studies on Dahl rats: (1) Effect of sodium intake on systolic blood pressure. As shown in Table 1, rats from both the R and S strains remained normotensive when fed a low-sodium diet. However, exposure of S animals to a high-sodium diet for 3 and 7 weeks provoked hypertension. R rats, exposed to the same high-sodium diet for 7 weeks, remained normotensive (Table 1).

(2) Experiments on isolated kidneys: (a) Animals fed low-sodium diet. These studies were designed to detect alterations in pressure natriuretic relationship in Dahl rats prior to their exposure to any hypertensionogenic stimulus. Figure 3 and Table 4

Table 3. Renal function in control isolated kidneys as compared to in vivo conditions

	Isolated Wistar kidneys perfused with cellular medium[a]	Values of antidiuretic rats in vivo taken from Refs. 20, 27, 28
GFR, *ml/min/g*	0.45	0.50[b]
Fractional sodium reabsorption, %	98.5	99[b]
Urinary sodium excretion, *μEq/min/g*	0.80	0.53[b]
Glomerular capillary pressure, *mm Hg*	47.8 ± 2.5	45.1 ± 0.8
Proximal tubule pressure, *mm Hg*	14.7 ± 1.0	13.4 ± 0.3
Distal tubule pressure, *mm Hg*	10.7 ± 0.9	7.4 ± 0.4
Peritubular capillary pressure, *mm Hg*	8.0 ± 2.0	8.6 ± 0.5

[a] Mean values during first hour of perfusion

[b] The values were calculated from data in Ref. 27 and using the kidney weight vs. body weight relationship found in our experimental rats, that is, 1 g of kidney for a body wt of 220 g.

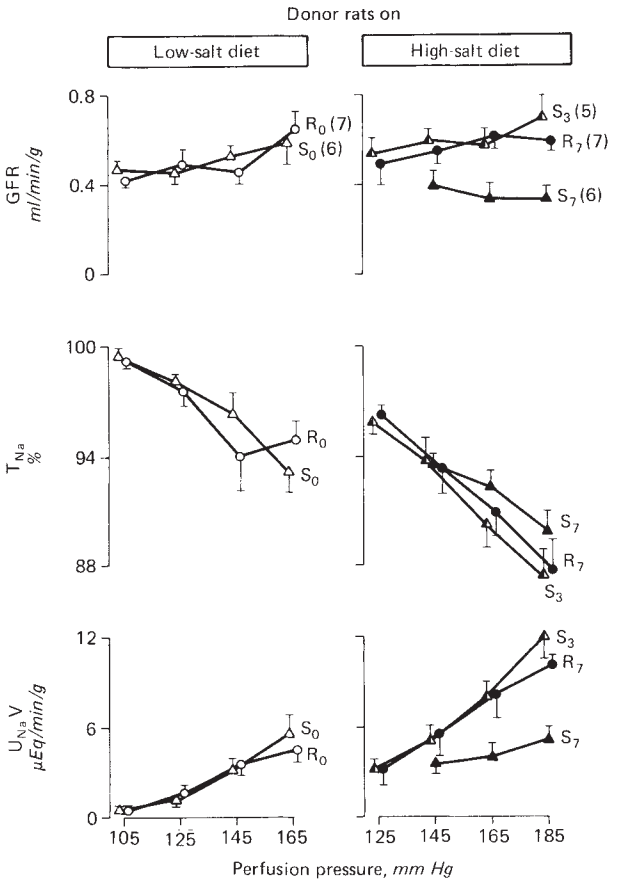

Fig. 3. *Effect of varying the renal perfusion pressure on GFR and sodium handling in sensitive (S) and resistant (R) Dahl rats.* Subscripts 0, 3, and 7 correspond to the number of weeks during which rats were exposed to the high-sodium diet. *Parentheses* enclose number of isolated kidneys. T_{Na} is fractional sodium reabsorption.

summarize effects on RPF, GFR, V, FF, $\%T_{Na}$, $U_{Na}V$, and U_KV in experiments where perfusion pressure was increased from 105 to 165 mm Hg after the low-salt diet. GFR increased slightly with increased PP in S and R animals, particularly after pressure had been increased above 145 mm Hg (P = NS), and the increments were similar in both strains of animals (Fig. 3). Fractional sodium reabsorption decreased with increased PP in S_0 kidneys from 99.7 ± 0.2% at 105 mm Hg to 93.2 ± 1.1% at 165 mm Hg and in R_0 kidneys from 99.2 ± 0.2% at 105 mm Hg to 95.2 ± 0.9% at 165 mm Hg. This decrease was similar in both strains (P = NS). Baseline $U_{Na}V$ was similar in R_0 and in S_0 kidneys, and excretion increased as PP was raised. The increment, too, was similar in both groups (P = NS): 0.5 ± 0.1 μEq/min/g at 105 mm Hg to 4.6 ± 0.8 μEq/min/g at 165 mm Hg in R_0 kidneys and 0.6 ± 0.2 μEq/min/g at 105 mm Hg to 5.7 ± 1.4 μEq/min/g at 165 mm Hg in S_0 kidney.

Micropuncture of seven end-proximal convolutions in S_0 and eight in R_0 kidneys were performed to determine the single nephron GFR (SNGFR), tubular fluid over plasma inulin ratio ($[TF/P]_{In}$), proximal tubular fluid reabsorption ($\%T_{prox}$), and fluid delivery at the end of the proximal tubules (FD_{prox}). None of these values correlated with the change in renal perfusion pressure from 105 to 145 mm Hg. There was no significant difference of these values in the two groups of rats. SNGFR was 58.3 ± 8.4 and 50.5 ± 9.3 nl/min; $(TF/P)_{In}$, 2.7 ± 0.2 and 2.6 ± 0.1; $\%T_{prox}$, 0.61 ± 0.04 and 0.62 ± 0.02; and FD_{prox}, 23.0 ± 3.6 and 20.2 ± 4.7 nl/min in S_0 and R_0 kidneys, respectively. Constancy in $\%T_{prox}$ and FD_{prox} indicates that pressure natriuresis might be due to a decreased sodium reabsorption located beyond the proximal tubule. These results are in accordance with the observation made by Kunau and Lameire [22] in special conditions and by Di Bona, Kaloyanides, and Bastron in isolated dog kidney [23].

(b) Animals fed high-sodium diet. The response to increased PP in R_7, as well as S_3 and S_7, were designed to investigate the PNF in S strain animals after exposure to hypertensionogenic stimuli. Figure 3 and Table 4 summarize effects on RPF, GFR, V, FF, $\%T_{Na}$, $U_{Na}V$, and U_KV in experiments where perfusion pressure was increased from 125 to 185 mm Hg. Variations in perfusion pressure between 105 and 145 mm Hg resulted in minor (4 to 6%) changes in GFR in all groups studied (Table 4). This indicates that the isolated kidney preparation used in this study is capable of maintaining GFR constant in response to change in perfusion pressure within the range where autoregulation can be observed in vivo. At perfusion pressure higher than 145 mm Hg, a significant rise in GFR in all groups with the exception of the S_7 animals occurred, as occurs in vivo. In this latter group, no increase in GFR was observed even at perfusion pressure of 185 mm Hg. The decrements in $\%T_{Na}$ during increments of PP were present in all groups and were similar in S_3 and R_7 groups. At 145 mm Hg, the $\%T_{Na}$ of S_7 was comparable to others; thereafter, the response of S_7 kidneys seemed to diminish, but by 185 mm Hg, the difference was not significant (Fig. 3). Because GFR failed to rise, however, in S_7 kidneys and $\%T_{Na}$ tended to be higher, $U_{Na}V$ rose significantly less in S_7 kidneys than it did in the other two groups. The $U_{Na}V$ of S_7 was 5.2 ± 1.0 μEq/min/g at 185 mm Hg, and the $U_{Na}V$ of R_7 and S_3 was, respectively, 10.3 ± 0.4 and 12.3 ± 1.6 μEq/min/g.

Table 4. Effect of varying the renal perfusion pressure on renal hemodynamics and on sodium and potassium transport of the five groups of rats studied

Groups	Perfusion pressure *mm Hg*	Perfusion flow rate *ml/min/g*	GFR *ml/min/g*	Urinary flow rate *ml/min/g*	Filtration fraction %	Fractional sodium reabsorption %	Urinary sodium excretion *μEq/min/g*	Urinary potassium excretion *μEq/min/g*
R_0 (N = 7)	105	13.0 ± 0.4	0.43 ± 0.06	0.0065 ± 0.0025	4.2 ± 0.6	99.2 ± 0.2	0.45 ± 0.1	0,51 ± 0.1
	125	12.9 ± 0.8	0.50 ± 0.09	0.0160 ± 0.0040	4.8 ± 0.8	97.5 ± 0.8	1.70 ± 0.5	0.65 ± 0.1
	145	12.6 ± 0.6	0.47 ± 0.06	0.0275 ± 0.0045	4.5 ± 0.5	94.2 ± 1.8	3.56 ± 0.7	0.76 ± 0.1
	165	12.7 ± 0.4	0.67 ± 0.10	0.0335 ± 0.0055	6.3 ± 0.9	95.2 ± 0.8	4.59 ± 0.8	0.83 ± 0.2
S_0 (N = 6)	105	13.6 ± 1.8	0.49 ± 0.02	0.0090 ± 0.0020	4.4 ± 0.6	99.7 ± 0.2	0.57 ± 0.2	0.63 ± 0.1
	125	13.7 ± 1.5	0.46 ± 0.05	0.0120 ± 0.0040	4.3 ± 0.7	98.4 ± 0.4	1.14 ± 0.3	0.72 ± 0.1
	145	13.9 ± 1.6	0.55 ± 0.04	0.0260 ± 0.0050	5.0 ± 0.6	96.7 ± 1.0	3.24 ± 0.7	1.05 ± 0.1
	165	14.5 ± 2.1	0.60 ± 0.10	0.0430 ± 0.0080	5.1 ± 0.7	93.2 ± 1.1	5.73 ± 1.4	1.31 ± 0.2
S_3 (N = 5)	125	15.6 ± 1.2	0.56 ± 0.06	0.0210 ± 0.0045	4.6 ± 0.5	96.2 ± 0.5	3.01 ± 0.5	1.04 ± 0.2
	145	16.6 ± 1.3	0.61 ± 0.06	0.0350 ± 0.0060	4.6 ± 0.2	93.9 ± 1.2	5.09 ± 0.9	1.33 ± 0.2
	165	17.5 ± 1.4	0.61 ± 0.05	0.0540 ± 0.0060	4.3 ± 0.1	90.3 ± 1.1	8.11 ± 0.9	1.51 ± 0.3
	185	18.3 ± 1.4	0.73 ± 0.10	0.0790 ± 0.0090	5.0 ± 0.5	87.5 ± 1.6	12.28 ± 1.6	1.56 ± 0.3
R_7 (N = 7)	125	15.3 ± 0.9	0.51 ± 0.01	0.0215 ± 0.0055	4.4 ± 0.7	96.4 ± 0.6	3.01 ± 1.1	0.46 ± 0.1
	145	15.0 ± 1.0	0.55 ± 0.06	0.0365 ± 0.0065	4.6 ± 0.5	93.5 ± 1.3	5.56 ± 1.7	0.57 ± 0.1
	165	14.9 ± 1.2	0.64 ± 0.07	0.0510 ± 0.0050	5.1 ± 0.6	91.1 ± 1.3	8.11 ± 1.2	0.63 ± 0.1
	185	16.2 ± 1.7	0.60 ± 0.06	0.0640 ± 0.0030	4.1 ± 0.6	87.8 ± 1.8	10.26 ± 0.4	0.71 ± 0.1
S_7 (N = 6)	145	14.4 ± 1.3	0.42 ± 0.07	0.0240 ± 0.0040	3.5 ± 0.3	93.8 ± 0.5	3.65 ± 0.7	0.71 ± 0.1
	165	14.1 ± 1.4	0.37 ± 0.08	0.0260 ± 0.0060	3.1 ± 0.5	92.5 ± 0.7	4.04 ± 1.0	0.60 ± 0.1
	185	13.4 ± 1.5	0.35 ± 0.05	0.0325 ± 0.0055	3.3 ± 0.3	89.9 ± 1.0	5.24 ± 1.0	0.61 ± 0.1
P								
R_0 *vs.* S_0		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*< 0.05*
R_7 *vs.* S_7		*NS*	*< 0.01*	*< 0.01*	*< 0.01*	*NS*	*< 0.01*	*NS*
S_3 *vs.* S_7		*< 0.05*	*< 0.01*	*< 0.01*	*< 0.01*	*NS*	*< 0.01*	*< 0.01*
R_0 *vs.* R_7		*< 0.01*	*NS*	*< 0.01*	*NS*	*< 0.05*	*< 0.01*	*< 0.05*
S_0 *vs.* S_3		*NS*	*NS*	*< 0.01*	*NS*	*< 0.01*	*< 0.05*	*NS*

(c) Comparison of results obtained between high- and low-sodium diet. The effect of sodium intake itself on kidneys isolated from normotensive donors may be seen by comparing results in R_0 and R_7 groups (Fig. 3 and Table 4). GFR changes were similar in both R_0 and R_7 kidneys, but $\%T_{Na}$ was clearly greater ($P < 0.05$) in the kidneys from salt-restricted animals, and the difference was maintained at all PP levels.

(d) Potassium excretion. The results are summarized in Fig. 4 and in Table 4. U_KV was similar in R and S kidneys from normotensive animals at PP of 105 and 125 mm Hg. With further increments of pressure, however, the increase in U_KV was always greater in the S_0 strain ($P < 0.01$). After high-salt feeding, the difference became more striking in the S_3 animals. The exaggerated kaliuresis, however, is no longer present in the S_7 kidneys perhaps due to the relatively low GFR at high PP.

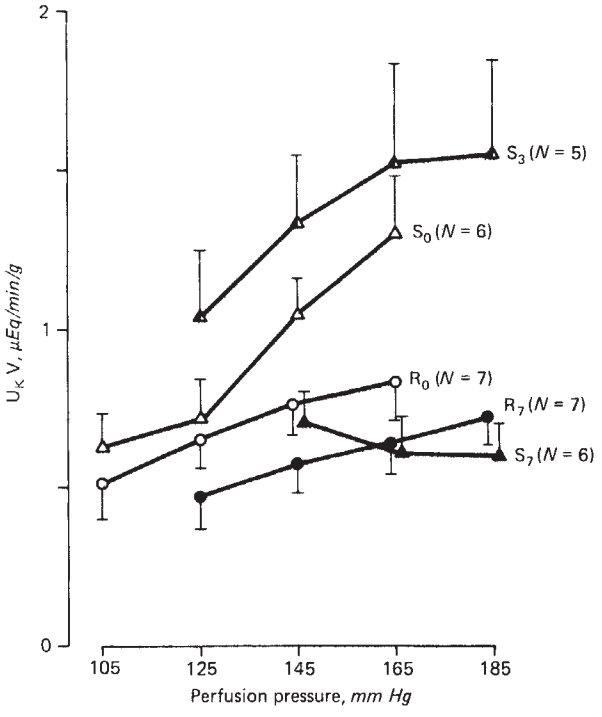

Fig. 4. *Effect of varying the renal perfusion pressure on urine potassium excretion in sensitive (S) and resistant (R) Dahl rats.* Subscripts 0, 3, and 7 correspond to the number of weeks during which rats were exposed to high-sodium diet. *Parentheses* enclose number of isolated kidneys.

Discussion

This study was designed to determine if an alteration of the intrinsic renal pressure natriuretic function might be a cause of salt-evoked hypertension in Dahl sensitive (S) rats. The data demonstrate that there is no preset abnormality of this function when S rats are normotensive or at early stage of their

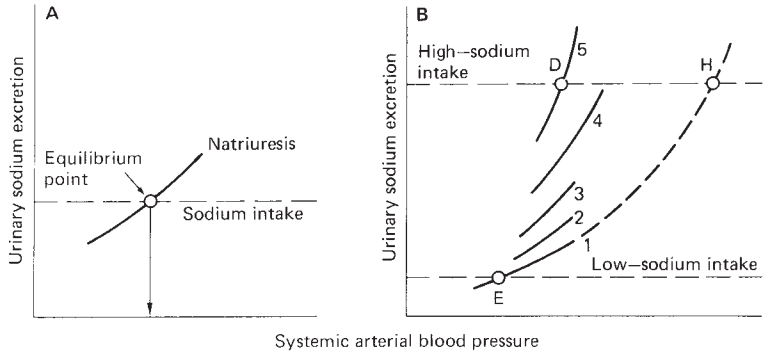

Fig. 5. *Some aspects of Guyton's theory on control mechanisms of arterial blood pressure (adapted from Ref. 4).* According to this theory, the slope characteristics of renal pressure natriuresis function (PNF) in vivo defines an intercept at which an equilibrium between sodium intake and excretion is achieved. As a consequence, arterial blood pressure is stabilized to a pressure level determined by the location of the intercept. Guyton et al clearly stated [4] that renal PNF, although describing a natriuretic effect of pressure acting through renal hydraulic mechanism alone, is modified in vivo when, for example, sodium intake increases. In the latter situation, PNF is progressively changed, and as schematically presented in B, curve number 1 becomes number 5 and equilibrium point D is achieved with very little change in arterial pressure (compare point D and E). In the absence of this adaptation, blood pressure would have risen to a hypertensive level defined by equilibrium point H. Some hypertensive states might be due to a depression of PNF and a shift to the right of the equilibrium point.

hypertensive disease. Once hypertension has been present from some time, however, there seems to be a resetting of the pressure natriuretic function (PFN), and this alteration may be a factor that sustains or aggravates high blood pressure in these rats.

Because we have chosen to analyze our results in relation to the Guyton hypothesis [4], this theory is briefly recalled in Fig. 5 and its legend.

The data from the resistant-strain rats (R) are in accordance with Guyton's views, and furthermore demonstrate that in vitro PNF is not merely a renal hydraulic mechanism, but is dependent on the sodium intake of the animal whose kidney was isolated. This is most evident when comparing results from R_0 and R_7 rats. In vivo blood pressure was similar regardless of the diet, but in the isolated kidney $\%T_{Na}$ was lower, and $U_{Na}V$ was greater in the R_7 animals, a difference that persisted at every perfusion level. As predicted by Guyton, the PNF curve of R_7 is shifted to the left when compared with R_0 animals. If such in vitro data are applicable to the intact animal, PNF in the rat may be an important factor in maintaining blood pressure with increased salt intake.

Guyton further suggested that certain forms of hypertension result from depression of the PNF [4, 5]. In the present work, we used an isolated kidney preparation to ascertain whether the renal determinent of the slope of PNF might be responsible for the different location of the equilibrium points (Fig. 5) in the five groups of rats studied. In such situations, high blood pressure could be produced by displacement to the right of PNF. It seemed plausible to us that this could well be the case in Dahl S rats because they remain normotensive on sodium-restricted diets and become hypertensive with chronic excess salt feeding. We looked at the perfusion characteristics of the S_0 kidneys, for it is already here where a preset abnormality should be detectable if our hypothesis were correct. The effect of pressure changes were identical, however, in R_0 and S_0 animals. Furthermore, S_3 animals, which are already at an early stage of hypertensive disease, still had an unaltered PNF in vitro because we observed a shift to the left of the function similar to that of the resistant rats. Therefore, our experiments do not suggest that a preset abnormality in PNF may be the primary causal factor in the development of hypertension in S rats. Nevertheless, an alteration in PNF appearing after the sodium-induced rise in blood pressure may aggravate the hypertension state in S rats. At 7 weeks, the systolic blood pressure had increased to 162 mm Hg, and then for the first time an alteration in the pressure natriuretic relationship was present (Fig. 3). The slope of this relation is less steep. This could be due in part to an absence of rise in GFR, and also to a possible trend for an increased $\%T_{Na}$ at high perfusion pressure.

The reason why S_7 kidneys behave differently than all others is not apparent from our data. With regard to the trend for a slightly higher $\%T_{Na}$ at high PP, it is possible that the maintenance of a high level of 18-OH-DOC observed in S rats fed a high-salt diet [24] may contribute to this phenomenon. Dis-

crete histopathologic changes in the kidneys of normotensive sensitive rats have been observed [25]. These alterations become more pronounced with sustained hypertension in S_7 rats (Chatelanat and Grandchamp, unpublished data). Such histologic modifications would support the concept that the depression of PNF is more likely a pathologic consequence of the sustained hypertension rather than an adaptive response allowing the maintenance of sodium balance. Such a secondary change in intrinsic PNF may contribute to fix hypertension in S_7 rats.

Experiments performed simultaneously to ours [26] have presented evidence that isolated Dahl S kidneys display a subnormal rate of natriuresis before the development of hypertension. The apparent discrepancy between this observation [26] and the foregoing results may be due to a difference in the perfusion medium. With an artificial medium, our experiments allowed an assessment of the intrinsic capacity of the renal tubule to excrete sodium in response to variations in perfusion pressure. In contrast, in the study by Tobian et al, in which the kidneys were perfused with whole blood of donor rats, the hydraulic effects of varying pressure are tested, in the presence of plasma factors that might interact with substances present in the renal tissue, and thereby generate products that might alter the pressure natriuresis relationship. If the latter would be the case, this discrepancy would be of interest, because it would mean that the intrarenal endocrine system plays a critical role in depressing the pressure natriuresis relationship in S rats.

It is known that transplantation of R kidneys to S animals ameliorates or prevents high blood pressure [9, 10]. This suggests the existence of an intrarenal mechanism responsible for the development of hypertension. From the experiments presented above, one can, however, eliminate that an intrinsic alteration in hydraulic PNF represents the mechanism that initiates hypertension in Dahl sensitive rats.

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