PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 177, 240–246 (1984)

Renal Na^+–K^+-ATPase in Weanling and Adult Spontaneously Hypertensive Rats (41937)

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Abstract. The interrelationships among plasma renin activity (PRA, ng AI/ml plasma/hr), aldosterone concentration (ng%), and renal Na^+–K^+-ATPase activity (μmole PO_4/mg protein/hr) were studied in 9 weanling normotensive spontaneously hypertensive rats (SHR), 9 adult hypertensive SHR, and 9 weanling and 9 adult normotensive Wistar–Kyoto rats (WKY). All groups were placed on a normal (0.4% sodium) diet. PRA and plasma aldosterone, measured in samples drawn from the ether-anesthetized rat, were higher in weanling SHR (15.2 ± 2.0, 37 ± 4.2) than in WKY. PRA measured in samples collected from a separate group of unanesthetized weanling SHR was also greater than in age-matched WKY. In adult SHR, PRA (6.1 ± 0.9) and plasma aldosterone (20.0 ± 2.7) were decreased. During the weanling period Na^+–K^+-ATPase activity in SHR was not only greater than in age-matched WKY but was also increased compared to adult normotensive and hypertensive rats (137 ± 9 weanling SHR, 89 ± 7 weanling WKY, 73 ± 11 adult SHR, 84 ± 17 adult WKY). Thus, during the weanling period the renin–angiotensin–aldosterone (R–A–A) system and renal Na^+–K^+-ATPase activity are activated in SHR. The elevation of Na^+–K^+-ATPase activity may be due to increased aldosterone levels. It was noted, however, that plasma aldosterone was similar in adult WKY and weanling SHR, while Na^+–K^+-ATPase activity was higher in SHR. These findings involving R–A–A and renal Na^+–K^+-ATPase activity prior to the elevation of blood pressure suggest that the kidneys may play a role in the initiation of hypertension in SHR.

Longitudinal studies of the renin–angiotensin system in spontaneously hypertensive rats (SHR) have revealed elevated plasma renin activity (PRA) in the weanling rat (1). PRA decreases gradually with increasing age and is lower in mature SHR than in age-matched Wistar–Kyoto rats (WKY) (1–3). Activation of the renin–angiotensin system in weanling SHR may result in transient elevation of aldosterone and other mineralocorticoids. Although conflicting results have been reported (4–7), high plasma aldosterone concentration has been described in SHR from 5 to 6 weeks of age (4). In addition, others have found that the level of this steroid hormone appears to decline with increasing age (5) although in these studies plasma aldosterone levels were only slightly and not significantly increased in weanling SHR. Increased activity of adrenocortical steroids during the initial stage of the hypertensive process could lead to enhanced renal sodium and water reabsorption. The possible role of the renal–adrenal axis in the development of hypertension in SHR, however, has not been clearly defined. Renal clearance studies have demonstrated a decrease in fractional sodium excretion in 4- to 7-week-old SHR with subsequent normalization of sodium handling in the mature rat (8). It appears that SHR may achieve a positive sodium balance prior to the onset of sustained hypertension. Adrenocortical steroid hormones stimulate renal Na^+–K^+-ATPase activity, apparently by increasing the filtered load of sodium and/or enhancing luminal permeability of target sites along the nephron (9–11). If sodium retention results in part from high mineralocorticoid or glucocorticoid levels, renal Na^+–K^+-ATPase activity may mediate increased sodium reabsorption in weanling SHR. To evaluate this possibility, we examined the renin–angiotensin–aldosterone system (R–A–A) and renal Na^+–K^+-ATPase activity in both 5- (weanling) and 16-week-old (adult) SHR.

Materials and Methods. A total of 18 SHR

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0037-9727/84 $1.50

and 18 control WKY were studied. Our colony of SHR is derived from rats of the original strain provided by the National Institutes of Health for breeding purposes. Sibling mating has been strictly maintained to assure hypertensive offspring (12). Studies were carried out in a room with an ambient temperature of 26°C and a 12 hr light–12 hr dark cycle. All rats were housed in metabolism cages throughout the study and maintained on a standard chow diet (0.46% sodium) with tap water *ad libitum*. At the age of 28 days (weanling), 9 SHR and 9 WKY were studied while 9 SHR and 9 WKY were studied at 16 weeks of age (adult). A 5 day balance study was carried out in all rats, during which 24-hr urine samples were collected under oil for the measurement of urine flow, sodium and potassium concentrations. Food and water consumption was measured daily; systolic blood pressure was measured on Day 3 of the study. On the last day of the balance study, rats were bled beginning at 0800 hr by heart puncture under light ether anesthesia (45–60 sec exposure) for the determination of PRA and plasma aldosterone concentration. Although ether anesthesia leads to stimulation of the renin–angiotensin system, we previously showed the reproducibility of plasma renin activity measured in samples drawn from ether-anesthetized rats (13). The kidneys of each rat were then removed and decapsulated for the isolation of microsomal fractions obtained from whole kidney homogenates as previously described (14). These samples were subsequently assayed for Na^+-K^+-ATPase activity. A separate group of 6 weanling SHR and 6 age-matched WKY was also studied to determine plasma renin activity in the unanesthetized rat as an indication of basal renin activity. Those rats were housed individually and kept on a standard chow diet for 3 days. The following morning each rat was decapitated and blood was collected for the measurement of plasma renin activity. This determination might be of particular importance in weanling SHR in which increased activity of the sympathetic nervous system (15) could result in a greater renin response to ether stimulation even if basal plasma renin activity is normal.

Urinary sodium and potassium concentrations were measured by flame photometry. Systolic blood pressure was measured in conscious restrained rats by tail–cuff technique. PRA was determined by radioimmunoassay of angiotensin I generation (16) and plasma aldosterone concentration was determined by specific radioimmunoassay (17). The anti-aldosterone antibody used has been shown to have 100% cross-reactivity with aldosterone, but less than 0.01% cross-reactivity with corticosterone or deoxycorticosterone (17). Total and magnesium-dependent ATPase activities in microsomal fractions were determined by chemical assay of inorganic phosphate (Pi) generation (14). Na^+-K^+-ATPase activity was expressed as the difference between total and magnesium-dependent ATPase activities. Inorganic phosphate concentrations were measured by the method of Fiske and Subarrow (18). Protein concentrations were determined by the method of Lowry *et al.* (19). Statistical analysis of the data was done by Student's t test between groups. A P value less than 0.05 was considered significant. Values are expressed as the mean and standard error of the mean.

Results. The results of a 5-day balance study in weanling and adult SHR and age-matched WKY are summarized in Tables I and II. Body weight was similar in adult SHR and WKY (Table II), but was greater in weanling SHR than in age-matched WKY (Table I). No differences were observed in the food intake, urine flow rate (V), or dietary sodium and potassium intake between weanling or adult SHR and age-matched WKY. The urinary excretion of sodium and potassium was also similar in both groups of adult rats, and urinary potassium excretion was similar in weanling SHR and WKY. In contrast urinary sodium excretion was less in weanling SHR than in age-matched WKY. Consequently, the fraction of ingested sodium that was excreted in the urine was lower in weanling SHR than in WKY of the same age. Systolic blood pressure (not shown) was elevated in adult SHR (183 ± 6 mm Hg) compared to WKY (123 ± 4). A tendency for blood pressure to be higher in weanling SHR (126 ± 6) than in WKY of the same age (107 ± 11) was observed. This difference, however, was not significant.

Plasma renin activity in weanling SHR (Fig. 1) was higher than in weanling WKY.

TABLE I. BODY WEIGHT (BW) AND RESULTS OF A 3-DAY BALANCE STUDY EXPRESSED PER 100 g BW IN 5-WEEK-OLD (WEANLING) SHR AND AGE-MATCHED WKY

	BW (g)	Food intake (g)	V (ml)	Na^+ intake (meq/24 hr)	K^+ intake (meq/24 hr)	$U_{Na}V$ (meq/24 hr)	U_KV (meq/24 hr)
SHR	131 ± 8*	13.8 ± 0.5	7.5 ± 0.4	2.9 ± 0.1	3.8 ± 0.1	0.9 ± 0.1*	2.1 ± 0.1
WKY	106 ± 4	12.8 ± 0.5	7.9 ± 0.4	2.7 ± 0.1	3.5 ± 0.2	1.2 ± 0.1	2.2 ± 0.1

* Significantly different ($P < 0.05$) from values in WKY.

This increased plasma renin activity was also observed in weanling SHR when blood samples were collected by decapitation (Table III). Stimulatory effects of ether anesthesia on the renin–angiotensin system were observed in both SHR and WKY. In adult SHR, however (Fig. 2), plasma renin activity was lower than in WKY. Differences in plasma aldosterone concentration between weanling and adult SHR (Figs. 1 and 2) paralleled those of the renin–angiotensin system. Plasma aldosterone concentration was greater in weanling SHR (Fig. 1) and lower in adult SHR (Fig. 2) than in age-matched WKY. Renal Na^+-K^+-ATPase activity was higher in 5-week-old SHR than in WKY (Fig. 3). Despite low plasma aldosterone levels in adult SHR, renal Na^+-K^+-ATPase activity was similar to that observed in adult WKY (Fig. 3).

Discussion. These studies demonstrate distinct differences in the R–A–A system and renal Na^+-K^+-ATPase activity in weanling 5-week-old and adult 16-week-old SHR. In weanling normotensive SHR, PRA, plasma aldosterone concentration, and renal Na^+-K^+-ATPase were persistently increased, as compared to WKY, while in adult SHR, PRA and plasma aldosterone levels were suppressed, but renal Na^+-K^+-ATPase values did not vary from normal controls. The increase in PRA was observed in both anesthetized and unanesthetized weanling SHR, such that both basal and stimulated renin levels are enhanced at this age. These findings support the concept that different mechanisms may come into play in the initiation and maintenance of hypertension in SHR.

Several hypotheses may be advanced to explain the exaggerated activity of the R–A–A system during the early period of development in SHR. Hypersensitivity of the sympathetic nervous system has been generally believed to participate in the pathogenesis of hypertension in SHR (20). Although it may be a plausible explanation for the increased activity of the R–A–A system, it is now recognized that it may not be the major mechanism in the elevation of blood pressure (21). Through our studies, however, this possibility cannot be discarded.

Another important observation we made in young SHR is that sodium handling was different from the control group. The weanling SHR excreted less sodium than WKY controls, while sodium intake was similar in both groups. Although extensive cumulative balance studies were not performed these data are consistent with an increased sodium retention in SHR. In fact, detailed studies of water and electrolyte balance by Beierwaltes *et al.* (8) have shown enhanced sodium reab-

TABLE II. BODY WEIGHT (BW) AND RESULTS FROM A 3-DAY BALANCE STUDY EXPRESSED PER 100 g BW IN ADULT SHR AND AGE-MATCHED WKY

	BW (g)	Food intake (g)	V (ml)	Na^+ intake (meq/24 hr)	K^+ intake (meq/24 hr)	$U_{Na}V$ (meq/24 hr)	U_KV (meq/24 hr)
SHR	284 ± 14	5.6 ± 0.3	7.7 ± 0.5	1.1 ± 0.1	1.4 ± 0.1	0.6 ± 0.1	0.9 ± 0.1
WKY	277 ± 16	5.1 ± 0.2	6.3 ± 0.6	1.0 ± 0.1	1.3 ± 0.1	0.6 ± 0.1	0.8 ± 0.1

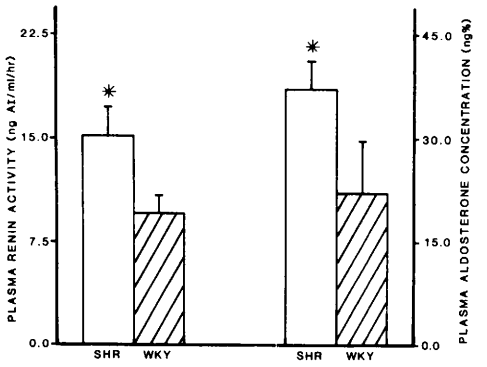

FIG. 1. Plasma renin activity and plasma aldosterone concentration in 5-week-old weanling spontaneously hypertensive rats (SHR) and age-matched Wistar–Kyoto rats (WKY). The asterisks denote values significantly different ($P < 0.01$) from corresponding values in WKY.

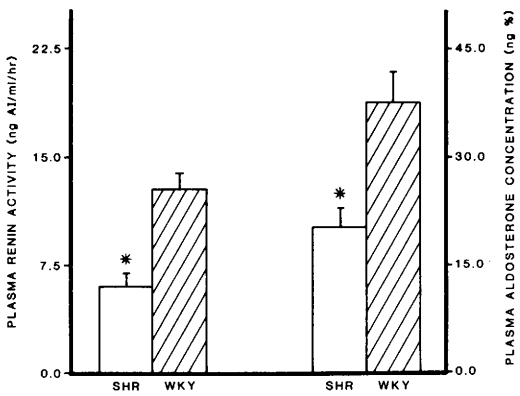

FIG. 2. Plasma renin activity and plasma aldosterone concentration in 16-week-old adult spontaneously hypertensive rats (SHR) and age-matched Wistar–Kyoto rats (WKY). The asterisks denote values significantly different ($P < 0.01$) from the corresponding values in WKY.

sorption during this early period. In their studies positive cumulative sodium balance was observed until the age of 7 weeks. In contrast, in adult SHR no changes in sodium handling were noted. The mechanism by which sodium retention might occur in young SHR is not known. An elevated renal vascular resistance with a reduced glomerular filtration rate and renal blood flow has been reported in 6-week-old SHR (22). A similar finding has been observed in another genetic strain of hypertensive rats, the Milan hypertensive strain (23). These renal hemodynamic changes may be the consequence of increased activity of the renin–angiotensin system, as reported herein. Alternatively, they may reflect an enhanced vascular reactivity to circulating vasoconstrictors as reported in the isolated perfused kidney from 4-week-old stroke prone SHR (24). Another possibility is that renal function changes may be mediated by the sympathetic nervous system. Increased efferent renal adrenergic tone (25) and increased renal α-adrenergic receptors (26) (two mechanisms which result in enhanced sodium reabsorption) have been reported in SHR.

Increased Na^+-K^+-ATPase activity will also lead to enhanced tubular sodium reabsorption. Weanling SHR showed increased Na^+-K^+-ATPase activity and high aldosterone, both of which, singly or combined, may

TABLE III. BASAL PLASMA RENIN ACTIVITY (PRA) MEASURED IN SAMPLES COLLECTED BY DECAPITATION OF 5-WEEK-OLD (WEANLING) SHR AND AGE-MATCHED WKY

	PRA (ng AI/ml/hr)
SHR	8.2 ± 0.1*
WKY	4.7 ± 0.1

* Significantly different ($P < 0.001$) from values in WKY.

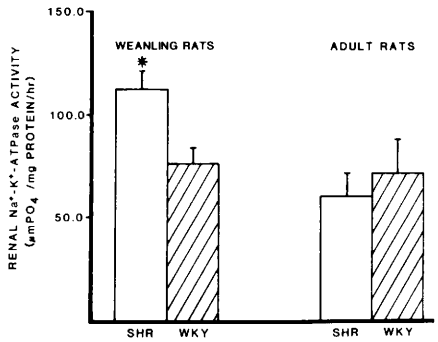

FIG. 3. Renal Na^+-K^+-ATPase activity in 5-week-old weanling and 16-week-old adult spontaneously hypertensive rats (SHR) and age-matched Wistar–Kyoto rats (WKY). The asterisk denotes a value significantly different ($P < 0.05$) from the values in the other three groups.

explain salt retention in this age group. It should be pointed out that steroid enhanced enzyme activity in proximal tubular cells of 20-day-old rats has been reported (27). Furthermore, in those studies dose-dependent increases in proximal tubular Na^+–K^+-ATPase were observed in 20-day-old rats (27). Although it has been suggested that steroid induction of proximal tubular Na^+–K^+-ATPase may be mediated through glucocorticoid receptors, a mineralocorticoid effect cannot be ruled out since physiologic doses of aldosterone also increased Na^+–K^+-ATPase activity.

Mineralocorticoids other than aldosterone might also contribute to the production of a steroidal excess in weanling SHR. Ratios of either 18-OH-deoxycorticosterone or corticosterone to aldosterone are apparently decreased in 4-week-old SHR while circulating aldosterone and deoxycorticosterone levels are slightly increased, suggesting altered adrenal steroidogenesis in the prehypertensive rat (5). It is not yet clear, however, through what biochemical pathway aldosterone levels are maintained normal to high in the face of low corticosterone and 18-OH-deoxycorticosterone concentrations. Such alterations could, nevertheless, lead to increased levels of biologically active intermediates that might have significant mineralocorticoid activity. In addition, elevated urinary excretion of 19-nor-deoxycorticosterone has been reported in weanling SHR (28). The mineralocorticoid potency of this steroid appears to be similar to that of aldosterone when measured as the spironolactone-sensitive increase in short circuit current using urinary toad bladder (29). Increased levels of aldosterone, 19-nor-deoxycorticosterone and perhaps intermediates of steroid biosynthetic paths may give rise to enhanced mineralocorticoid activity in prehypertensive SHR.

Such mineralocorticoid excess in young SHR may be expected to enhance Na^+–K^+-ATPase in the proximal tubule as well as in other target sights along the nephron. With increasing age, the sensitivity to mineralocorticoids should fall, since their effect on proximal tubular cells is observed only during renal maturation (27). Consequently, it might be anticipated that for a given level of plasma aldosterone, renal Na^+–K^+-ATPase activity would be greater in weanling than in adult rats. In support of this concept, when aldosterone was plotted against Na^+–K^+-ATPase activity (Fig. 4) the slopes of the regression lines were significantly different in SHR and WKY, as determined by analysis of covariance. Consequently, it appears that weanling SHR have much higher Na^+–K^+-ATPase activity at aldosterone levels which are similar to those of adult WKY.

We propose that increased renal Na^+–K^+-ATPase activity in weanling SHR leads to sodium retention, which may in part trigger the onset of the hypertensive process. At some time between 5 and 16 weeks of age the increase in sodium reabsorption may lead to expansion of extracellular fluid volume. Interestingly, Mullins (30) has described an increase in both total body water and extracellular fluid volume in prehypertensive SHR. As the rat becomes older, volume regulatory

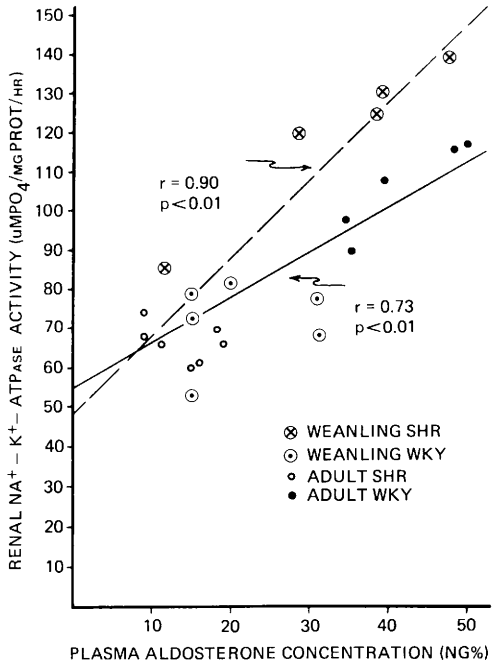

FIG. 4. Linear regression analysis of the correlation between renal Na^+–K^+-ATPase activity and plasma aldosterone concentration in spontaneously hypertensive rats (pooled data from weanling and adult SHR) and Wistar–Kyoto rats (pooled data from weanling and adult WKY).

mechanisms may come into play accounting for normalization of fluid volumes. During the adult period PRA and aldosterone levels are suppressed and mechanisms which normally stimulate their secretion are inoperative. Alternatively, mechanisms which inhibit these systems are operative.

In summary, activation of the renin–angiotensin–aldosterone system in weanling SHR was associated with increased renal Na^+-K^+-ATPase activity. Enhanced activity of this enzyme may be involved in the increased salt retention observed in SHR at this stage. Consequently, the kidneys through their capacity to expand extracellular fluid volume may play an important role in the genesis of hypertension in SHR.

We gratefully acknowledge the technical assistance of José Cruz, Eliseo Rivera, and Evelio Marrero, as well as the secretarial assistance of Maria Guerrero and Claribel Alvarez. This work was supported by a grant from the Veterans Administration.

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Received February 3, 1984. P.S.E.B.M. Vol. 177.
Accepted June 12, 1984.