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$(Na^+–K^+)$ATPase ACTIVITY IN ERYTHROCYTE MEMBRANES OF SPONTANEOUSLY, ONE KIDNEY-ONE WRAPPED, AND DEOXYCORTICOSTERONE ACETATE–NaCl HYPERTENSIVE RATS

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Abstract—$(Na^+–K^+)$ATPase activity in erythrocyte membranes of spontaneously (SHR), one kidney-one wrapped, and deoxycorticosterone acetate (DOCA)–NaCl hypertensive rats was studied. $(Na^+–K^+)$ATPase activity decreased in both prehypertensive (6 weeks old) and hypertensive (14 weeks old) stages of SHR, suggesting that the alteration of this enzymic activity may be due to a pre-existing defect in the membrane rather than being a consequence of hypertension. By contrast, $(Na^+–K^+)$ATPase activity remained unchanged in the one kidney-one wrapped hypertensive rats, whereas that of one kidney-one wrapped normotensive rats as well as that of DOCA–NaCl hypertensive rats was increased significantly ($P < 0.05$). These changes were specific for $(Na^+–K^+)$ATPase, since Mg^{2+}-ATPase activity was not altered. The susceptibility of $(Na^+–K^+)$ATPase to the inhibitory action of ouabain was not changed significantly. These findings indicate that $(Na^+–K^+)$ATPase activities of erythrocyte membranes isolated from the different types of hypertensive rats were subject to different changes. Whether this phenomenon applies to the clinical distinctions among the various types of hypertension remains a subject for further investigation.

Alterations in cellular properties, mainly of blood cells, have been described in humans and animal models in association with essential hypertension [1–4]. Abnormalities of erythrocyte membrane Na^+ transport in these models have been reported by several investigators [5–12]. These transport abnormalities were found to be similar in humans and rats with genetic hypertension [13]. This, therefore, validates the rat model for the study of these cell membrane abnormalities and their pathophysiological roles in genetic hypertension. $(Na^+–K^+)$ATPase is the biochemical manifestation of the Na^+ pump. It regulates the active transport of Na^+ and K^+ across the cell membrane [14]. Abnormalities in Na^+ handling by erythrocyte membranes, which partly depends on a change in ouabain-sensitive $(Na^+–K^+)$ATPase activity, have been reported in spontaneously hypertensive rats (SHR) [13, 15–18]. Therefore, it is worthwhile to investigate the changes of Na^+ transport and $(Na^+–K^+)$ATPase activity in the SHR. In a previous study, we found that the decreased $(Na^+–K^+)$ATPase activity of cardiac ventricle and kidney in SHR preceded the increase in blood pressure [19]. In the present study, we attempted to clarify the relationship between the alteration of erythrocyte $(Na^+–K^+)$ATPase activity and hypertension. SHR in the prehypertensive (6 weeks old), as well as in the hypertensive (14 weeks old), stages were used. Furthermore, Grollman and deoxycorticosterone acetate (DOCA)–NaCl hypertensive rats, which are two forms of volume-expanded, low renin type hypertension [20], were studied comparatively in order to characterize further the Na^+ and K^+ transport system in different types of hypertension.

MATERIALS AND METHODS

Rats. Male rats were used throughout the study. Spontaneously hypertensive and age-matched control Wistar–Kyoto rats (either 6 or 14 weeks old) were purchased from Charles River, Kanagawa, Japan.

Renal hypertension was produced in the Wistar rats (initial weight, 200–220 g). A figure-8 ligature was applied to compress the poles of one kidney of each rat as described by Grollman [21]. The kidney was exposed through a lumbar incision under ether anesthesia, the renal capsule was removed by gentle traction, and a figure-8 ligature was applied. The ligature was tight enough to deform the kidney but not tight enough to cut the tissue. Care was also taken to avoid damaging or constriction the blood vessels or ureters emerging from the hilus of the kidney. One week after the kidney was compressed, the contralateral kidney was removed. In the sham-wrapped group, the thread was applied loosely. Eight weeks after wrapping, the rats were used for experiments.

Mineralocorticoid hypertension was induced in Wistar rats (120–140 g initial body weight). Unilateral nephrectomy was done under ether anesthesia. One week later, the rats were divided into four groups. The first group was fed with tap water (1K-control); the second one was injected subcutaneously with DOCA (20 mg/kg, Sigma) containing 0.25% Tween 80 and 0.125% carboxy-

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methylcellulose five times per week over 4 weeks (1K-DOCA). The third group was fed with 1% NaCl solution for 4 weeks (1K-NaCl), and the last group was injected with DOCA and concomitantly fed with 1% NaCl solution for 4 weeks (1K-DOCA/NaCl). Two weeks after finishing DOCA and/or NaCl treatment, the rats were used for experiments. In addition, rats with two intact kidneys were fed with tap water (2K-control) and used for another control group.

Systolic blood pressure was measured by the tail-cuff method using a Narco Sphygmomanometer.

Preparation of erythrocyte membrane. The rats were anesthetized with pentobarbital (50 mg/kg), and blood was drawn from the abdominal artery. Plasma and red blood cells were separated by centrifuging the blood at 3000 *g* for 10 min. Erythrocyte membrane was prepared as described by Mir *et al.* [22]. The red blood cell pellets were washed three times by 155 mM NaCl, 3 mM histidine, pH 7.5, and then hemolyzed by 3 vol. of lysing solution containing 87.7 mM sucrose, 50 mM imidazole, 0.15% deoxycholate and 2 mM EDTA, pH 7.5, for 1 hr. The suspension was centrifuged at 22,000 *g* for 20 min, and the pellet was washed with 40 vol. of washing solution containing 87.7 mM sucrose, 50 mM imidazole and 2 mM EDTA, pH 7.2, until the hemoglobin was totally eliminated.

Assay of ATPase activity. The erythrocyte membrane (0.2 to 0.5 mg/ml protein) was incubated for 20 min at 37° in the presence of a modified Krebs solution containing 118 mM NaCl, 4.7 mM KCl, 1.1 mM $MgCl_2$, 12.5 mM $NaHCO_3$, 0.5 mM ethyleneglycolbis(amino - ethylether)tetra - acetate (EGTA), 11.1 mM glucose, and 3 mM ATP as described previously [19]. $(Na^+–K^+–Mg^{2+})$ATPase activity was estimated from the amount of inorganic phosphate released from ATP and determined colorimetrically as described by Lanzetta *et al.* [23]. Mg^{2+}-ATPase activity, assayed in the presence of additional 1 mM ouabain in the modified Krebs solution, was subtracted from $(Na^+–K^+–Mg^{2+})$ATPase activity to calculate $(Na^+–K^+)$ATPase activity. Protein was determined by the method of Sedmak and Grossberg [24] using bovine serum albumin as the standard.

Drugs and statistical analysis. Ouabain octahydrate was purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Other chemicals were of reagent grade. Statistical analyses were performed with Student's *t*-test or analysis of variance (ANOVA).

RESULTS

Blood pressure, body weight, and plasma electrolytes in hypertensive rats. The systolic blood pressures of 6-week-old SHR and age-matched WKY rats were 140.8 ± 8.9 and 137.4 ± 6.6 mm Hg, respectively, which were not significantly different from each other, whereas that of 14-week-old SHR increased markedly to 203.5 ± 2.1 mm Hg (Table 1a). Body weight and plasma Na^+ of SHR were almost the same as those of WKY rats at either age. By contrast, plasma K^+ of all SHR, whether at the prehypertensive or the hypertensive stage, decreased as compared with that of age-matched WKY rats (Table 1a).

In the experiment with Grollman rats, the systolic blood pressures of the four groups of rats were analyzed by ANOVA: the F value (19.18) indicated that the difference among them was significant (Table 1b). It was noted that four out of fifteen 1K-1 wrapped rats had normal blood pressure as compared with that of 1K-1 sham-wrapped rats. The

Table 1. Blood pressure, body weight, and plasma ionic compositions of hypertensive rats

Rat group	Age (weeks)	N	Blood pressure (mmHg)	Body weight (g)	Plasma (mM)	
					Na^+	K^+
(a) Spontaneously hypertensive						
WKY	6	5	137.4 ± 6.6	126.0 ± 2.7	138.7 ± 0.6	4.54 ± 0.10
SHR	6	5	140.8 ± 8.9	108.6 ± 2.4	136.6 ± 0.9	4.07 ± 0.10*
WKY	14	8	138.1 ± 1.9	300.7 ± 2.8	136.0 ± 1.2	5.02 ± 0.23
SHR	14	8	203.5 ± 2.1*	299.4 ± 10.9	136.4 ± 1.1	4.13 ± 0.14*
(b) Grollman						
2K-control		11	116.4 ± 3.7	236.9 ± 11.0	137.2 ± 0.7	4.12 ± 0.17
1K-1 sham-wrapped		8	118.0 ± 3.6	252.1 ± 11.8	138.7 ± 0.7	4.94 ± 0.58
1K-1 wrapped (hypertensive)		11	179.8 ± 8.3†	241.7 ± 9.6	138.0 ± 1.0	4.50 ± 0.14
1K-1 wrapped (normotensive)		4	122.2 ± 3.0	251.9 ± 17.6	139.9 ± 1.1	4.78 ± 0.22
(c) DOCA/NaCl						
2K-control		13	116.0 ± 3.4	299.2 ± 12.8	139.5 ± 0.3	4.73 ± 0.36
1K-control		16	123.8 ± 1.6	286.2 ± 9.9	138.5 ± 0.7	4.84 ± 0.58
1K-DOCA		6	132.3 ± 7.5	310.3 ± 14.3	136.2 ± 0.9	4.45 ± 0.07
1K-NaCl		8	127.1 ± 5.8	276.2 ± 17.7	144.2 ± 2.4	4.11 ± 0.40
1K-DOCA/NaCl (hypertensive)		15	169.3 ± 2.7†	268.8 ± 15.2	139.5 ± 1.6	4.57 ± 0.18
1K-DOCA/NaCl (normotensive)		3	129.7 ± 5.0	258.6 ± 16.5	138.5 ± 3.1	4.96 ± 0.20

Values are presented as means ± SE; N represents the number of rats used in each group. 2K: two kidneys; 1K: one kidney.

* $P < 0.05$ as compared with age-matched WKY rats (Student's *t*-test).

† $P < 0.05$ as compared with 1K-1 sham wrapped or 1K-control rats (Student's *t*-test).

Table 2. ATPase activities, ouabain inhibition, hematocrit, and protein yields in the erythrocyte membrane of Wistar–Kyoto rats (WKY) and spontaneously hypertensive rats (SHR)

	Age (weeks)	ATPase (μmol P_i/mg protein/hr)		IC_{50}* (μM)	Hematocrit (%)	Protein yield† (mg/ml)
		$(Na^+–K^+)$ATPase	Mg^{2+}-ATPase			
WKY	6	1.90 ± 0.07	2.17 ± 0.27	48 ± 4	42.1 ± 0.6	0.654 ± 0.011
SHR	6	1.43 ± 0.08‡ (75.4 ± 4.2)	2.04 ± 0.13 (94.2 ± 6.0)	55 ± 5	41.9 ± 2.2	0.539 ± 0.020‡
WKY	14	1.61 ± 0.08	2.00 ± 0.14	50 ± 4	45.2 ± 1.6	0.687 ± 0.013
SHR	14	1.22 ± 0.10‡ (76.2 ± 6.1)	1.89 ± 0.11 (94.3 ± 5.6)	59 ± 5	45.2 ± 1.9	0.670 ± 0.011

Values are presented as mean ± SE (N = 4–5 for ATPase activity, N = 5 for protein yield, and N = 3 for IC_{50} values).

Values in parentheses represent the percentage of those of the age-matched WKY rats.

* IC_{50} is the concentration of ouabain required for 50% inhibition of $(Na^+–K^+)$ATPase activity.

† Protein yield is presented as milligram protein per milliliter of blood.

‡ $P < 0.05$ as compared with the age-matched WKY rats by Student's *t*-test.

body weights and plasma Na^+ and K^+ concentrations among all these groups were not significantly different from one another (Table 1b).

As with Gollman rats, the systolic blood pressures of six groups of rats, including control and DOCA and/or NaCl treated rats, were significantly different from one another as analyzed by ANOVA (F value, 13.44, Table 1c). The other variables, e.g. body weight and plasma electrolytes, however, were not changed in the 1K-DOCA and/or NaCl rats as compared with that of 1K-control rats. Three out of eighteen 1K-DOCA/NaCl rats had normal blood pressure as compared with that of 1K-control rats (Table 1c).

ATPase activities in the erythrocytes of spontaneously hypertensive rats. As shown in Table 2, $(Na^+–K^+)$ATPase activity of SHR at both the prehypertensive (6 weeks old) and the hypertensive (14 weeks old) stage decreased as compared with that of WKY rats, whereas Mg^{2+}-ATPase activity remained unchanged. The susceptibility of $(Na^+–K^+)$ATPase activity of all rats to the inhibitory action of ouabain was almost the same, as indicated by IC_{50} values (Fig. 1a and Table 2). The hematocrit was also unaltered in SHR, whereas protein yield of the erythrocyte membrane of 6-week-old SHR was lowered significantly compared with that of the age-matched WKY rats (Table 2).

ATPase activities in the erythrocytes of Grollman hypertensive rats. $(Na^+–K^+)$ATPase activity was significantly different among all four groups of rats by ANOVA (F value, 8.99). This enzymic activity of 1K-1 wrapped hypertensive rats was not changed significantly, compared with 1K-1 sham-wrapped rats, whereas that of 1K-1 wrapped normotensive rats increased by 33.1% (Table 3). Mg^{2+}-ATPase activity of these rat groups was not altered. The susceptibility of $(Na^+–K^+)$ATPase activity of all rats to the inhibitory action of ouabain was almost the same, as indicated by IC_{50} values (Fig. 1b and Table 3). The hematocrit and protein yield were also unaltered in these rat groups (Table 3).

Table 3. ATPase activities, ouabain inhibition, hematocrit, and protein yields in the erythrocyte membrane of normotensive and Grollman hypertensive rats

Rat group	ATPase (μmol P_i/mg protein/hr)		IC_{50}* (μM)	Hematocrit (%)	Protein yield† (mg/ml)
	$(Na^+–K^+)$ATPase	Mg^{2+}-ATPase			
2K-control	1.97 ± 0.08	2.71 ± 0.29	54 ± 5	45.0 ± 1.4	0.633 ± 0.020
1K-1 sham-wrapped	1.81 ± 0.14 (92.1 ± 7.0)‡	3.00 ± 0.25 (110.7 ± 9.1)	59 ± 3	45.6 ± 1.2	0.658 ± 0.021
1K-1 wrapped (hypertensive)	1.73 ± 0.11 (95.6 ± 6.1)§	2.59 ± 0.12 (86.3 ± 4.0)	56 ± 3	45.0 ± 1.8	0.646 ± 0.024
1K-1 wrapped (normotensive)	2.41 ± 0.10‖ (133.1 ± 5.5)§	2.70 ± 0.16 (90.0 ± 5.3)	56 ± 4	44.5 ± 1.5	0.627 ± 0.032

Values are presented as mean ± SE (N = 4–6 for ATPase activity, N = 6 for protein yield, and N = 4 for IC_{50} values).

* IC_{50} is the concentration of ouabain required for 50% inhibition of $(Na^+–K^+)$ATPase activity.

† Protein yield is presented as milligram protein per milliliter of blood.

‡ Values represent percentage of those of 2K-control rats.

§ Values represent percentage of those of 1K-1 sham-wrapped rats.

‖ $P < 0.05$ as compared with 1K-1 sham-wrapped rats (Student's *t*-test).

ATPase activities in the erythrocytes of DOCA–NaCl hypertensive rats. (Na^+–K^+)ATPase activity was significantly different among all six groups of rats by ANOVA (F value, 3.38). This enzymic activity of 1K-control rats was not changed significantly, compared with that of 2K-control rats (Table 4). That of 1K-DOCA and 1K-NaCl was also unchanged, compared with that of 1K-control rats. As for DOCA–NaCl hypertensive rats, the (Na^+–K^+)ATPase activity increased by 49.3%, whereas that of DOCA–NaCl normotensive rats was not altered (Table 4). Mg^{2+}-ATPase activity of these rat groups remained unaltered. The susceptibility of (Na^+–K^+)ATPase activity of all rats to the inhibitory action of ouabain was almost the same, as indicated by IC_{50} values (Fig. 1c and Table 4). The hematocrit and protein yield were also unchanged in these rat groups (Table 4).

DISCUSSION

The present investigation indicates that the (Na^+–K^+)ATPase activity of erythrocyte membrane decreased in both the prehypertensive and hypertensive stages of SHR; that of Grollman hypertensive rats remained unchanged, while that of Grollman normotensive and that of DOCA–NaCl hypertensive rats increased. These changes were specific for (Na^+–K^+)ATPase, because Mg^{2+}-ATPase activity was almost the same, compared with that of their respective controls. The protein yield was not changed except for that of the 6-week-old SHR which was reduced compared with the age-matched WKY rats. The susceptibility of (Na^+–K^+)ATPase of erythrocyte membrane to the inhibitory action of ouabain did not differ significantly from one type of hypertensive rat to another.

The decrease in (Na^+–K^+)ATPase activity appeared already in the 6-week-old prehypertensive SHR, suggesting that the alteration of this enzymic activity may be a genetic defect rather than the consequence of hypertension. This finding was in accord with the result obtained in essential hypertensive patients and their normotensive offspring that the deficiency of Na^+–K^+ cotransport in red cells is due to an inherited defect [25]. There are reports that erythrocyte membranes from SHR exhibit various structural abnormalities such as decreases in calcium binding and transport [1, 2], probably due to a decrease in integral membrane calcium-binding protein [26]; a decrease in calmodulin-dependent Ca^{2+} transport [27]; modified phosphoinositide metabolism [28]; and changes in physicochemical properties of inserted probes [3]. Some of these changes were shown to be of genetic origin since they could be found in prehypertensive SHR [2–4, 26]. In this paper, we provide data showing that erythrocyte (Na^+–K^+)ATPase was diminished already in prehypertensive SHR. Sowers *et al.* [29] also reported a decrease of this enzymic activity in 9- and 12-week-old hypertensive SHR. The decrease in (Na^+–K^+)ATPase activity may have been due to an abnormality that was intrinsic to the cell membrane between SHR and WKY rats as suggested by Van deVen and Bohr [30] and Feig *et al.* [13]. The decreased (Na^+–K^+)ATPase activity in SHR may lead to an increased intracellular Na^+ concentration as Feig *et al.* [13] found in SHR. Lasker *et al.* [31] also found a significant inverse correlation between (Na^+–K^+)ATPase activity and erythrocyte Na^+ concentrations. Since higher erythrocyte Na^+ concentrations have also been observed in patients with essential hypertension as compared with normotensive subjects [5, 6], Lasker *et al.* [31] suggested

Table 4. ATPase activities, ouabain inhibition, hematocrit, and protein yields in the erythrocyte membrane of normotensive and DOCA–NaCl hypertensive rats

Rat group	ATPase (μmol P_i/mg protein/hr)		IC_{50}* (μM)	Hematocrit (%)	Protein yield† (mg/ml)
	(Na^+–K^+)ATPase	Mg^{2+}–ATPase			
2K-control	1.62 ± 0.07	2.31 ± 0.30	50 ± 3	45.0 ± 1.4	0.632 ± 0.040
1K-control	1.50 ± 0.11 (92.1 ± 7.0)‡	2.56 ± 0.21 (110.7 ± 9.1)	53 ± 3	45.6 ± 1.2	0.631 ± 0.019
1K-DOCA	1.58 ± 0.06 (105.1 ± 4.1)§	2.62 ± 0.06 (102.3 ± 2.5)	52 ± 3	43.8 ± 1.7	0.614 ± 0.020
1K-NaCl	1.54 ± 0.10 (102.7 ± 6.7)§	2.57 ± 0.05 (100.4 ± 2.0)	55 ± 4	43.0 ± 1.7	0.630 ± 0.029
1K-DOCA/NaCl (hypertensive)	2.24 ± 0.20‖ (149.3 ± 13.3)§	2.24 ± 0.14 (87.5 ± 5.5)	60 ± 6	43.4 ± 1.3	0.622 ± 0.017
1K-DOCA/NaCl (normotensive)	1.72 ± 0.11 (114.7 ± 7.3)§	2.40 ± 0.09 (93.8 ± 3.5)	51 ± 4	44.1 ± 1.5	0.644 ± 0.059

Values are presented as mean ± SE (N = 4–6 for ATPase activity, N = 5 for protein yield, and N = 3 for IC_{50} values).

* IC_{50} is the concentration of ouabain required for 50% inhibition of (Na^+–K^+)ATPase activity.

† Protein yield is presented as milligram protein per milliliter of blood.

‡ Values represent percentage of those of 2K-control rats.

§ Values represent percentage of those of 1K-control rats.

‖ $P < 0.05$ as compared with 1K-control rats (Student's *t*-test).

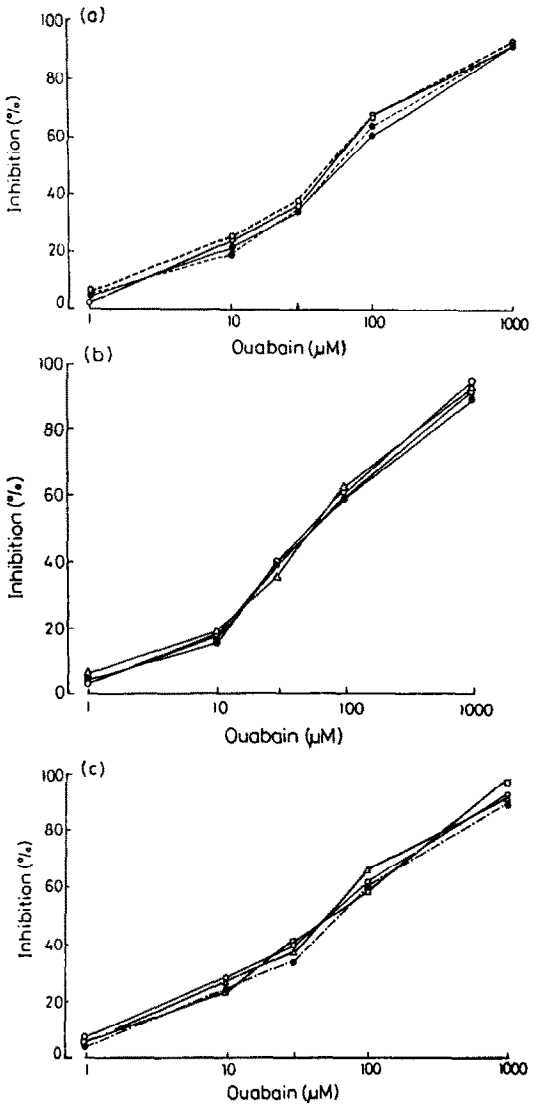

Fig. 1. Concentration–inhibition curve of ouabain on erythrocyte membrane (Na⁺–K⁺)ATPase activity of (a) SHR: (○---○) 6-week-old WKY rats; (●---●) 6-week-old SHR; (○——○) 14-week-old WKY rats; (●——●) 14-week-old SHR, (b) Grollman: (○——○) 2K-control rats; (□——□) 1K-1 sham-wrapped rats; (●——●) 1K-1 wrapped hypertensive rats; (△——△) 1K-1 wrapped normotensive rats, and (c) DOCA–NaCl hypertensive rats: (○——○) 2K-control rats; (□——□) 1K-control rats; (●---●) 1K-DOCA/NaCl hypertensive rats; (△——△) 1K-DOCA/NaCl normotensive rats.

that higher intracellular Na^+ concentrations in blacks and men may contribute to the greater predisposition of these groups to essential hypertension. Therefore, the decrease (Na⁺–K⁺)ATPase activity in SHR may have a greater predisposition to develop hypertension as reported by Lasker *et al.* [31]. We found previously that the decreased (Na⁺–K⁺)ATPase activity of cardiac ventricle and kidney in SHR also appears in the prehypertensive stage [19]. These results and the intrinsic abnormality in erythrocyte (Na⁺–K⁺)ATPase activity may represent a more generalized membrane defect resulting in an increase in arteriolar tone as suggested by Sowers *et al.* [29], Lasker *et al.* [31] and Van deVen and Bohr [30]. This factor may contribute to the pathogenesis of elevated arterial blood pressure in SHR and essential hypertension. From the inhibitory curve and IC_{50} values of ouabain on (Na⁺–K⁺)ATPase activity of SHR and WKY rats, there was no change in the specificity of this enzyme in SHR. The decreased enzymic activity may be due to reduced Na^+ pump sites in SHR erythrocyte membrane.

The (Na⁺–K⁺)ATPase activity was unchanged in Grollman hypertensive rats but was increased in Grollman normotensive rats. These results contrasted with those for SHR erythrocyte membrane but were similar to that of kidney in this type of renal hypertensive rats (unpublished data). Whether the increased (Na⁺–K⁺)ATPase activity of erythrocyte membrane in Grollman normotensive rats is related to renal adaptation [32] was unclear. It has been reported that there are Na^+ pump inhibitors in Grollman hypertensive rats [20]. Therefore, the unaltered (Na⁺–K⁺)ATPase activity in the Grollman hypertensive rats suggests that (Na⁺–K⁺)ATPase inhibitors reversibly blocked the enzymatic activity and were removed by the washout of erythrocyte membrane. In DOCA–NaCl hypertensive rats, the (Na⁺–K⁺)ATPase activity was increased. DOCA is a mineralocorticoid and it increases this enzymic activity of renal cortex [33]. *In vitro,* aldosterone could increase (Na⁺–K⁺)ATPase activity of human erythrocytes [34]. In this paper, however, we demonstrated that neither DOCA nor NaCl treatment increased the enzymic activity. Therefore, in DOCA–NaCl hypertensive rats, the increased (Na⁺–K⁺)ATPase activity was not due to DOCA or NaCl treatment but rather was related to hypertension. Duhm *et al.* [35] have reported that Na^+ leakage is increased in this type of hypertensive rats. The increased Na^+ leakage resulted in an increase of intracellular Na^+, thus stimulating the Na^+ pump. Therefore, we obtained the increased (Na⁺–K⁺)ATPase activity in this type of hypertensive rats.

In conclusion, the alteration of (Na⁺–K⁺)ATPase activity in these three types of hypertensive rats was different. It was decreased, unchanged and increased in SHR, Grollman and DOCA–NaCl hypertensive rats respectively. Whether this phenomenon could apply to clinical distinctions of various types of hypertension requires further investigation.

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