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Na, K-ATPase Activity in the Aged Erythrocytes of Hypertensive Rats

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ABSTRACT

To study the age dependent change of Na, K-ATPase in the erythrocyte of hypertensive rat, 1-kidey 1-clip hypertensive rat was made by the removal of right kidney and partial ligation of left renal artery. After 4 weeks, aged erythrocyte fraction was separated by density gradient centrifugation, and Na, K-ATPase activity and ^{3}H-ouabain binding with ghost cell membrane and ouabain sensitive Rb-uptake with whole cell were measured.

1) In the hypertensive rats, blood pressure was significantly increased to 165.5/119.0 mmHg (systolic/diastolic). Mean corpuscular volume and membrane protein(mg) per 10^9RBC were decreased and hemoglobin content was increased in the aged erythrocyte.

2) Na, K-ATPase activity in the solution containing 110 mM NaCl and 10 mM KCl, was decreased in hypertensive rat, and decreased in aged erythrocyte of both group.

3) Ouabain sensitive Rb-uptake by low RbCl concentration(4 mM) was slightly decreased in aged erythrocyte compared to that in young erythrocyte of each group, but slightly increased in young erythrocyte in hypertensive rat compared to that in normotensive rat.

4) Ouabain sensitive Rb-uptake by high RbCl concentration(16 mM) was decreased about 30 % to 50 % in aged erythrocyte in both group. And in hypertensive rat, especially in young erythrocyte it was significantly decreased compared to that in normotensive rats.

5) ^{3}H-ouabain binding at 0.13 or 1 $\times$ 10^{-6}M ouabain concentration was slightly decreased in aged erythrocyte of normotensive rat, and significantly decreased in aged erythrocyte of hypertensive rats.

6) ^{3}H-ouabain binding at 6 or 64 $\times$ 10^{-6}M ouabain concentration is slightly decreased in aged erythrocyte of both group, but significantly decreased in young and aged erythrocyte of hypertensive rats compared to that of normotensive rats.

The present results suggest that ① in the young erythrocyte of hypertensive rat, the alterations of Na-pump activity that slightly increased in weak stimulation and inhibited in strong stimulation, may be related to increased molecular activity and the decrease in the number of low affinity site without change in high affinity site, ② in the aged erythrocyte of normotensive rat, inhibited Na-pump may be related to the change in molecular activity of pump. ③ And in the aged erythrocyte of hypertensive rat, it may be related to the decrease in the number of high and low affinity site as well as the change in molecular activity

Key Words: Aged erythrocyte, Hypertensive rat, Na K-ATPase, Rb-uptake, ^{3}H-ouabain binding

INTRODUCTION

There are hundreds of researches that examined the relationship between the accumulation of body salt and the developmental process or physiological, biochemical change of hypertension, since McQuarrie(1936) have reported the blood pressure can be rised through ingested excessive salt. Some researches related to Na-

metabolism in the body were carried out in not only human being but also Dahl-salt hypertensive rat or spontaneously hypertensive rat (Canguli *et al.*, 1979; Takeshita *et al.*, 1978; Tobian *et al.*, 1979; De Mendonca *et al.*, 1980)

With the reduction of renal blood flow from one side of renal artery, it is possible that blood pressure is increased by renin release(2-kidney, 1-clip hypertension). But when the one side kidney is removed and remnant kidney is in ischemic state, at first, the blood pressure is increased because of releasing renin but soon, the renin activity in blood may be restored, extracellular or blood volume can be increased and hypertensive state is kept on(1-kidney, 1-clip hypertension). Futhermore extracellular volume may recover to normal, but there can be increased resistance of peripheral blood vessel and exitability of sympathetic nerve when 1-kidney, 1-clip hypertension lasts for a long time (Dargie *et al.*, 1976; Dargie *et al.*, 1977; Tanaka *et al.*, 1977; Guyton, 1986).

deWardener and MacGregor(1980), and Gruber *et al.*(1980) have reported that Na-transport inhibitor in blood is increased when the salt in the body(that is Na) is accumulated, and it binds with digoxin antibody. Poston *et al.*(1981) and Pamnani *et al.*(1980) have also reported that this substance inhibits the activity of Na, K-ATPase in hypertensive animal and hypertensive patient. By inhibition of Na, K-ATPase and increased intracellular Na-content, there can be caused the increase of blood vessel contractility and the increase of sympathetic nerve exitibility as it increases intracellular Ca content through Na-Ca exchange mechanism.

Katano *et al.*(1985) have reported that numbers of high affinity site and Na, K-ATPase activity in cardiac muscle of aged rat is decreased, but Na leak influx is increased in this tissue and rubidium-uptake can be increased through Na-pump. Katano *et al.*(1984) and Kennedy and Seifen(1988) have reported that Na-pump in aged rat cardiac muscle is decreased in reserve capacity or maximum stimulation capacity by electrical stimulation.

Also it has been reported that cell membrane properties or enzyme function in erythrocyte can be changed by the aging(Blostein *et al.*, 1990; Turner *et al.*, 1974).

Although there are lots of reports on the change of Na K-ATPase activity in the erythrocyte membrane of hypertensive animal or hypertensive patient, but nothing on the change of membrane properties or Na, K-ATPase activity by aging of erythrocyte. So the authors made artificially 1-kidney, 1-clip hypertensive rat and made an experiment to examine any change of Na-pump in erythrocyte by aging and hypertension outburst.

MATERIALS AND METHODS

Experimental animals

Male 6-week-old Sprague-Dawley rats weighing 120 to 150g were used in this study. Hypertension was produced in the experimental group by partial ligation(e.d. 0.38 mm) of left renal artery and removal of the right kidney(1-kidney, 1-clip hypertensive rat). Only the right kidney was removed in the control group. All rats were maintained for 4 weeks on normal rat chow. Blood pressure was recorded in physiograph(Narco-Biosystem, MK III) via a polyethylene cannular which was inserted into carotid artery under light ether anesthesia.

Age related fractionation of erythrocyte

Heparinized fresh blood was obtained from carotid artery, and the leukocyte and platelet were removed as described by Beutler *et al.* (1976). Whole blood was passed through an α-cellulose: microgranular cellulose(1:1, w/w) column in normal salline solution. Column was made in 5 ml plastic syringes with an inner diameter of 1.26 cm. Packed erythrocytes were subjected to a density gradient centrifugation separation employing percoll/hypaque: 1 ml of leukocyte-free packed red cells were mixed with 10 ml of total solution containg 35% percoll, 22.8% hypaque meglumine, and then centrifuged at 32,000×g for 40 min at 4°C (Vettore *et al.*, 1980). The cells(young in the top layer, older in the bottom layer) were separated into three fractions according to density(Fig.1). The cell count, hemoglobin content and mean corpuscular volume were determined by the Coulter Counter(Coulter. STKR).

Preparation of erythrocyte ghost membrane

Ghost cell membrane was prepared from

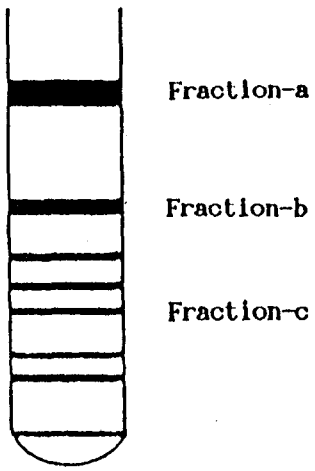

Fig. 1. Fractionations of erythrocyte separated by percoll density gradient centrifugation with 10 ml of the cell suspension. Details are in methods.

packed red cell of each cell layer as previously described by the method of Rosenberg and Guidotti(1968). Each of the washed cell fraction was suspended in a 30-fold volume of destilled water and allowed to stand for 30 min, and then centrifuged at 13000×g for 30 min in a Sorvall RC-5B centrifuge. The pellet was resuspended and washed three times with 15 mM Tris buffer (pH 7.4). The entire procedure was performed at 4°C and protein concentration was determined by the method of Bradford(1976) with bovine serum albumin as a standard.

Na, K-ATPase activity

Na, K-ATPase activity was determined by continuously monitoring the oxidation of red uced nicotinamide adenine dinucleotide(NADH) by a UV/Vis spectrophotometer(Beckman D-65) equipped with a constsnt temperature water bath at a wavelength of 340 nm(linked-enzyme spectrophotometric assay, Schwartz *et al.*, 1969).

In the presence of pyruvate kinase(PK)/lactate dehydrogenase(LDH), phosphoenolpyruvate(PEP) and NADH, as PEP is transformed into pyruvate or lactate. The oxidation of NADH is directly proportional to the ADP hydrolyzed from ATP by ATPase of ghost cell membrane. The compositions of reaction medium were 110 mM NaCl, 10 mM KCl, 5 mM $MgCl_2$, 5 mM Na-ATP(vanadium free, Boeringer), 0.08 mM NADH, 1 mM PEP, 14 units PK /20 units LDH(suspension supplied by Sigma Cemical Co.) and 25 mM L-histidine base(pH 7.4). After preincubation of the reaction medium for 10 min at 37°C, protein of ghost cell membrane(20 μl) was added to a final volume of 0.5 ml. In the absence of ouabain, the decrease in absorbance for 10 min at 340 nm is a measure of total ATPase activity. Na K-ATPase activity was determined by measuring the difference between the total ATPase activity and the ouabain insensitive ATPase activity remained in the presence of ouabain(1 mM).

Rubidium-uptake

Sodium pump activity was estimated from ouabain sensitive Rb-uptake as described previously by Bernstein *et al.*(1970) with some modification. Each erythrocyte fraction obtained accoriding to density gradient was washed three times with phosphate buffer(155 mM NaCl, 5,5 mM glucose, 6.5 mM Na_2HPO_4/NaH_2PO_4, pH 7.4). Packed red cells were preincubated in phosphate buffer(1 ml) for 10 min at 37°C in a water bath and Rb-uptake was started by the addition of RbCl(4, 8 or 16 mM).

After 60 minnutes, the reaction mixture was transferred to conical tube containing 1 ml of dibutyryl phthalate and spun at 3000×g for 5 minutes. The supernatent was removed and the erythrocytes were then hemolyzed by the addition of destilled water. The aliquot of hemolysate was deproteinized by 5% trichloroacetic acid and uptaked rubidium concentration was measured in flameless atomic absorption spectrophotometer(Varian, GTA-96). All determinations were carried out in duplicate. Ouabain-sensitive Rb-uptake(specific) was the difference in values observed in the absence and presence of 1.6×10^{-3} M ouabain.

^{3}H-ouabain binding experiment

^{3}H-ouabain binding was induced in the solution contained 110 mM NaCl, 10 mM KCl, 5 mM $MgCl_2$, 5 mM Na_2ATP, 25 mM L-Histidine, pH 7.4 and 1 mM phosphoenolpyruvate. Ater incubation of each erythrocyte membrane protein(34 μg) for 90 min at 37°C with ^{3}H-ouabain ($0.13, 1, 6, 64\times10^{-6}$M) in the absence or pres-

ence of excess unlabeled ouabain, the reaction mixture was rapidly filtered on filter paper (Gelman, GN-6). The filters were then washed three times with 5 ml of ice cold 180 mM NaCl and incubated at least 12 h in scintillation cocktail(triton 300 ml, toluene 694.5 ml, PPO 5 g, POPOP 0.5 g/1) prior to determination of radioactivity in liquid scintillation counter(Packard, Tri-Crab 300C). Specific binding site was calculated from the difference in values observed in the absence(total binding) and presence of cold ouabain(6.4×10^{-3} M)(nonspecific binding).

Statistical analysis was performed with Student's t test, and the level for significance was taken as a probability less than 5%($p<0.05$). All values reported represent the mean±SE.

RESULTS

Changes of blood pressure in experimental hypertensive rats

Systolic pressure and diastolic pressure measured from sham operated rat and 1-kidney, 1-clip hypertensive rat are given in Table 1. The systolic blood pressure of hypertensive rats was significantly greater(158%) than that of normotensive rats. Also in diastolic pressure, hypertensive rats showed significantly higher blood pressure(141%) than sham operated rats.

Hematological characterization of density-seperated erythrocytes

From fractionated erythrocytes by Percoll density gradient centrifugation, Cell counts, hemoglobin content(Hgb) and mean corpuscular volume(MCV) determination were performed by Coulter counter(Table 2). The Erythrocyte count was increased progressively with age from $8.875\pm0.44(\times10^6/mm^3)$ in fraction-a(top layer) to $10.0\pm0.19(\times10^6/mm^3)$ in fraction-c (bottom layer), also hemoglobin concentration (Hgb g/dl) was increased in fraction-c. Mean corpuscular volume(MCV μm^3/ea) became slightly decreased in fraction-c(8%) when compared to the corresponding value of fraction-a. Also the membrane protein(mg) per ml packed cell or per 10^9 RBC was decreased in aged fraction, approximately 10% and 20% respectively.

Na, K-ATPase activity

Changes of Na, K-ATPase activity were examined in the cell membrane of each erythrocyte fraction(Table 3, Table 4). In this experiment, reaction was induced in 110 mM MaCl and 10 mM KCl, and Na, K-ATPase might be

Table 1. Blood pressure in control and 1-kidney, 1-clip hypertensive rats

	Systolic pressure	Diastolic pressure (mmHg)
Control	110.5±3.47	84.5±3.38
Hypertensive	165.5±6.11*	119.0±6.23*

Values represent mean ± SE obtained from 10 experiments.

*: *Significantly different from the corresponding* value of control group($p<0.05$)

Table 2. Hematological characteristics in the erythrocyte fractions of rats

	Fraction a	b	c
RBC count($10^6/mm^3$)	8.875±0.44	9.125±0.37	10.000±0.19
Hgb(g/dl)	16.700±1.02	18.330±1.02	19.170±1.02
MCV(μm^3/ea)	55.450±4.31	54.400±2.57	50.080±1.82
Membrane protein			
(mg/ml packed RBC)	0.847±0.076	0.792±0.084	0.768±0.079
(mg/10^9 RBC)	0.095	0.087	0.077

Values represent mean ± SE obtained from 4 cases. Hgb: hemoglobin
MCV: mean corpuscular volume, ea: each cell

Table 3.ATPase activities in the cell membrane of each erythrocyte fraction of normotensive rats

	Fraction		
	a	b	c
	(μmole Pi/mg protein/hr)		
Total	5.51±0.83	5.64±0.63	4.66±0.52
Ouabain sensitive	2.50±0.35	2.30±0.35	1.80±0.40
Nonspecific	3.01±0.45	3.34±0.45	2.86±0.37

Values represent mean±SE obtained from 5 experiments.

Table 4.ATPase activities in the cell membrane of each erythrocyte fraction of hypertinsive rats

	Fraction		
	a	b	c
	(μmole Pi/mg protein/hr)		
Total	3.80±0.49	2.97±0.42	2.61±0.41
Ouabain sensitive	1.46±0.27*	1.25±0.21*	0.86±0.20*[A]
Nonspecific	1.62±0.28	1.72±0.32	1.75±0.35

Values represent ±SE obtained from 5 experiments.
*: Significantly different from the corresponding value of normotensive group($P<0.05$)
[A]: Significantly different from the value of fraction-a

Table 5. Na, K-ATPase activities in the cell membrane of each erythrocyte fraction of normotensive and hypertensive rats

		μmole Pi/mg protein/hr	μmole Pi/10^9 RBC/hr
Normotensive	F-a	2.50±0.35	0.238±0.033
	F-b	2.30±0.25	0.200±0.022
	F-c	1.80±0.40	0.139±0.031
Hypertensive	F-a	1.46±0.27*	0.139±0.026*
	F-b	1.25±0.21*	0.109±0.018*
	F-c	0.86±0.20*[A]	0.066±0.015*[A]

Values represent mean±SE obtained from 5 experiments. *: Significantly different from the corresponding value of normotensive group($P < 0.05$) [A]: Significantly different from the value of fraction-a($P < 0.05$)

stimulated maximally. In all fractions of the hypertensive rats, total ATPase and ouabain sensitive ATPase activities were lower than those of normotensive rats. In the aged red cell (fraction-c), although Na, K-ATPase activity of normotensive rats was 28% lower than that of fraction-a, Na, K-ATPase activity of hypertensive rats was significantly lower(40%) than that of fraction-a($P<0.05$).

Decrease in enzyme activity by red cell aging was slightly increased when calculations for all subsequent data were based on erythrocyte number(10^9 RBC) rather than on membrane protein amount(Table 5). In fraction-c of nor-

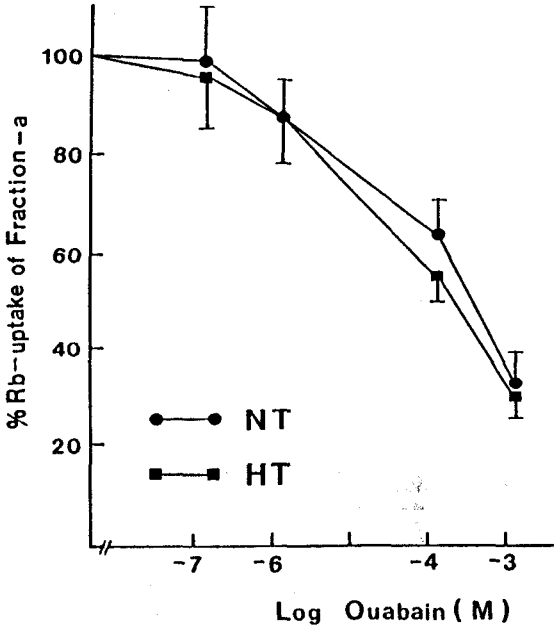

Fig. 2. Effect of various ouabain concentration on the uptake of Rb in the red blood cells(Fraction-a). The specific Rb-uptake was estimated in the indicated concentration of ouabain. Values are expressed as percent of the control activity for fraction-a. Each value represents the mean of five experiments.
NT: normotensive rat, HT: hypertensive rat

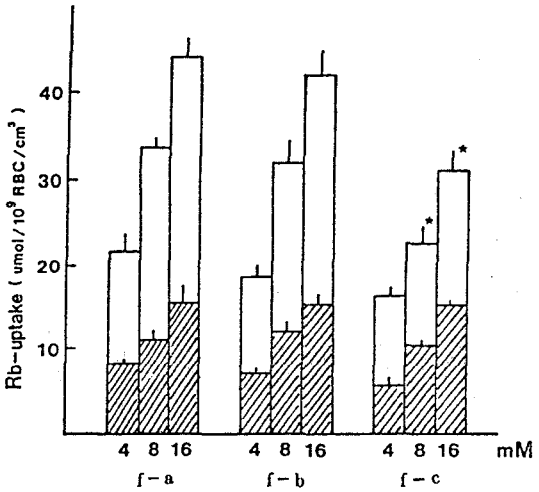

Fig. 3. Rb-uptake in erythrocyte of normotensive rats. Na-pump activity was estimated from the ouabain sensitive Rb-uptake observed in the presence of 4, 8 and 16 mM RbCl in phosphate buffer at 37°C with an 60 min incubation. Shaded bars represent nonspecific Rb-uptake observed in the presence of 1.6×10^{-3} M ouabain. The total bars represent the Rb-uptake observed in the absence of ouabain. Openbars therefore represent the ouabain sensitive(specific) Rb-uptake, i.e. the difference in values observed in the absence and presence of 1.6×10^{-3} M ouabain. n=4. [A]: significantly different from the corresponding value of fraction-a($P<0.05$).

motensive rats and hypertensive rats, Na K-ATPase activity compared that in fraction-a decreased approximately 40% and 50% respectively

Rb-uptake in various ouabain cencentration

Functional aspects of Na, K-ATPase were studied from the ouabain sensitive Rb-uptake in the red blood cells. Rb-uptake in the medium contained 8 mM RbCl was examined in the absence or prsence of ouabain(0.0016, 0.016, 1.6 and 16×10^{-4} M)(Fig. 2). In the fraction-a-erythrocyte of normotensive and hypertensive rat, Rb uptake in the absence of ouabain was inhibited about 13, 40 and 70% in the presence of 1.6×10^{-6}, 1.6^{-4} and 1.6×10^{-3} M ouabain respectively.

Rb-uptake in various RbCl concentration

In order to assess the capacity of the sodium pump for the low or high concentration of RbCl in the incubation medium, total or ouabain sensitive Rb-uptake was examined in the various Rb concentration(Fig. 3, Fig. 4). Nonspecific Rb-uptake in the presence of ouabain(1.6×10^{-3} M) was increased, as RbCl is increased 4 mM to 8 or 16 mM, but increased with almost same ratio in each fraction. Total Rb-uptake in fraction-a or fraction-b of normotensive rat was increased about 60% and 110%, and in fraction-c, increased about 40% and 90% with increasing RbCl 4 mM to 8 and 16 mM respectively. However in hypertensive rats, it was increased about 56% and 59% in fraction-a and about 30% and 60% in fraction-c.

Ouabain sensitive Rb-uptake was also decreased in fraction-c compared to that in frac-

tion-a in both group(Fig. 5). At high RbCl content(16 mM), ouabain sensitive Rb-uptake in hypertensive rat was less than normotensive rat in each fraction. The patterns in the changes of these Rb-uptake values were almost same as those of the value of Na, K-ATPase activity observed with spectrophotometry. However ouabain sensitive Rb-uptake of hypertensive rat at low RbCl concentration(4 mM), was slightly increased(fraction-a and b) or slightly decreased (fraction-c) as it was compared to same fraction of normotensive rat. And difference between Rb-uptake in fraction-a and fraction-c of hypertensive rat was significant($p<0.05$). From

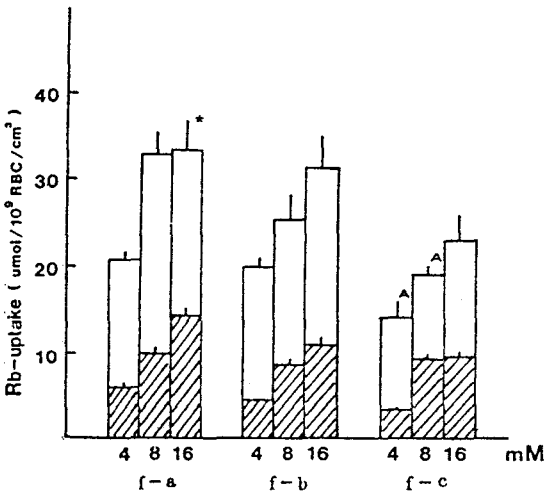

Fig. 4. Rb-uptake in erythrocyte of hypertensive rats. Details are the same as Fig. 3. *: significantly different from the corresponding value of normotensive rat($P<0.05$). ^{A}: Significantly different from the corresponding value of fraction-a($P<0.05$).

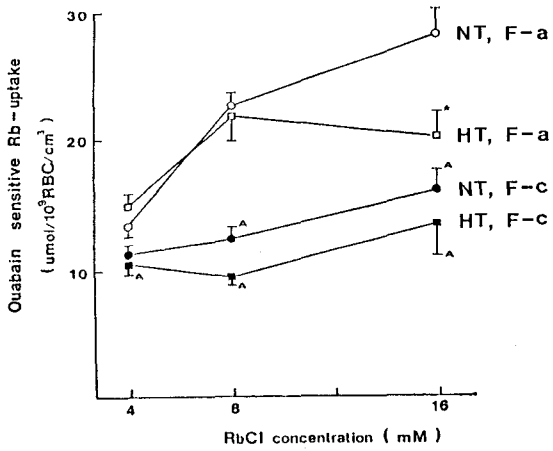

Fig. 5. Ouabain sensitive Rb-uptake in each fraction of normotensive and hyperten-sive rats. Each point represents the mean of four experiments±SE. Normotensive rat(NT); hypertensive rat(HT); fraction-a(F-a); fraction-c(F-c). *: significantly different from the corresponding value of normotensive rat($P<0.05$). ^{A}: significantly different from the corresponding value of the fraction-a($P<0.05$).

Table 6. Specific binding of ^{3}H-ouabain to cell membrane of young and aged erythrocyte fraction of normotensive and hypertensive rats

^{3}H-ouabain($\times 10^{-6}$ M)	^{3}H-ouabain binding(pmol/mg protein)			
	0.13	1	6	64.1
Normotensive rat				
fraction-a	0.23±0.02	0.66±0.08	10.50±1.19	90.8± 6.01
fraction-c	0.18±0.03	0.56±0.07	6.00±3.70	81.0±11.40
Hypertensive rat				
fraction-a	0.20±0.02	0.64±0.08	5.00±1.70*	46.6± 9.80*
fraction-c	0.14±0.03^{A}	0.41±0.02^{A}	1.77±1.44*	41.7±14.90*

Values represents mean ± SE obtained from 4 experiments.
*: Significantly different from the corresponding value of normotensive rats.
^{A}: Siginficantly different from the corresponding value of fraction-a

this Rb-uptake experiment it was suggested that in hypertensive rat, capacity of Na-pump for higher stimulation is less than that of normotensive rat but in normal or understimulation Na-pump is not inhibited in rather, increased in young erythrocytes.

^{3}H-Ouabain binding studies

Erythrocyte ghosts from each fractions were reacted with ^{3}H-Ouabain, and we ascertained that the Na-pump sites or specific ouabain binding sites on red blood cell were changed in aged erythrocyte of normotensive and hypertensive rats. In rat, there are two different classes of Na, K-ATPase with respect to their different affinities for ouabain(Adams *et al.*, 1982; Lee *et al.*, 1984). One is characterized by a low apparent affinity for ouabain(Kd, 30×10^{-6}M), represents a large proportion of total sites, another is a higher affinity site for ouabain(Kd, 0.3×10^{-6}M), which may represent a small proportion of total sites. In this experiment(Table 6) the oldest cell fraction(fraction-c) of normotensive rat showed slight decrease in specific ouabain binding site at low or high ouabain concentration compared to the youngest cell (fraction-a). In the fraction-c of hypertensive rat, ouabain binding sites at high ouabain concentration were also slightly decreased compared to the fraction-a. However at the low concentration of ^{3}H-ouabain(high affinity site) in the fraction-c of hypertensive rat, it was significantly decreased($P<0.05$). And ouabain binding site at high concentration of ^{3}H-ouabain(low affinity site) in hypertensive rats, there were less ouabain binding sites than normotensive rats($P<0.05$).

DISCUSSION

We can know the close relationship between the development of hypertension and Na, by seeing the facts that we have to inhibit Na ingestion or stimulate Na excretion during the treatment of hypertension, and that the blood vessel contractility will be increased by the increased administration of Na. For the contraction of blood vessel, Ca-uptake from the extracelluar matrix is important because the blood vessel smooth muscle has less developed sarcoplasmic reticulum than skeletal muscle. So, when the Na-pump of blood vessel smooth muscle is inhibited, the quantity of Ca-uptake will be increased in the intracellular fluid and this will make increase of the blood vessle contractility. And it will contribute to the hypertension development.

Many scholars reported that in the cell membrane of erythrocyte or leukocyte which is not directly related with elevation of blood pressure, there is a change of Na-pump. But there were different opinions among the scholars, some scholars like Poston *et al.*(1982) and Edmondson *et al.*(1975) insisted the inhibition of Na-pump, and some scholars like Swartz *et al.* (1981) and Duhm*et al.*(1983) insisted that there was no change or increase of Na-pump. Furthermore, Na-pump can be stimulated by increased intracellular Na(Katano *et al.*, 1985; Jones *et al.*, 1979).

In this experiment, ouabain sensitive Rb-uptake of hypertensive group(fraction-a), when there was 4 mM of RbCl in the reaction medium, wasn't inhibited in rather slightly increased compared to the control group. But the Na-pump activity(in the solution contained 110 mM NaCl and 10 mM KCl, Table 5) or Rb-uptake in 16 mM RbCl contained solution(Fig 5), of hypertensive group erythrocyte was significantly decreased compared with the control group. And by 16 mM RbCl, Rb-uptake in fraction-a-erythrocyte was increased markedly in control group(110%) but less increased in hypertensive group(30~45%) compared to that by 4 mM RbCl(Fig. 5). ^{3}H-ouabain binding quantity at 0.13 or 1×10^{-6}M ^{3}H-ouabain concentration, in the fraction-a of hypertensive rat was not make a significant difference with the control group. But at 6 or 64×10^{-6}M ^{3}H-ouabain concentration, it was significantly decreased ($P<0.05$)(Table 6).

These results in young erythrocyte of hypertensive rat, suggest that although decreased pump activity at strong stimulation may be due to decreased pump sites(low affinity site), increased pump activity at weak stimulation may be a result of compensatory increase in molecular activity for the decreased pump sites.

On the other hand, Katano(1984, 1985) and Kennedy and Seifen(1988) reported the number or activity of Na-pump in the cardiac muscle of

an aged rat was reduced, and Yang *et al.*(1988) and Blostein *et al.*(1990) also reported the reduce of Na-pump activity in the aged erythrocyte cell membrane of human, rabbit or sheep. So the Na-pump in the hypertensive rat erythrocyte cell membrane could be affected by the aging of cell.

In our experiment, separated aged erythro cyte showed increase of hemoglobin content, the reduce of mean courpuscular volume (MCV), the increase of erythrocyte number per packed RBC volume(ml) and the reduce in the cell membrane protein amount per RBC, which were in accord with the report of Cohen *et al.* (1976).

In aged erythrocyte membrane(fraction-c), the Na-pump activity was reduced about 40% in the control group and about 50% in the hypertensive group compared with fraction-a of each group(Table 5). And the ouabain sensitive Rb-uptake in fraction-c in low RbCl concentration was slightly reduced in normotensive rat but reduced about 30% in hypertensive rat, and in high RbCl concentration, reduced about 30-40% in both group, compared with that in fraction-a of each group(Fig. 5).

^{3}H-ouabain binding sites in aged cell were slightly decreased in 0.13 or 1×10^{-6} M and in 6 or 64×10^{-6} M ^{3}H-ouabain concentration in both group except that significantly decreased in hypertensive rat in low ^{3}H-ouabain concentration, compared to that in fraction-a of each group(Table 6). Because Na-pumps in rat are mainly low affinty sites but in this experiment they were slightly decreased in number with aging, it is possible that decreased Na-pump activity by aging might be due to change in molecular activity. However Na-pump sites in aged erythrocyte of hypertensive rat were significantly decreased at low and high ^{3}H-ouabain concentration compared to that those in normotensive rat.

From these results of aged erythrocyte, it is suggested that 1) in normotensive rat, inhibited Na-pump may be related to the change in molecular activity of pump, 2) and in hypertensive rat, it may be related to the decrease in the number of high and low affinity site as well as the change in molecular activity.

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= 국문초록 =

고혈압쥐 노화 적혈구에서의 Na, K-ATPase에 관한 연구

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고혈압백서(1-kidney, 1-clip-hypertensive rat)의 적혈구에서 노화 과정에 따른 Na, K-ATPase의 변동을 관찰하고저 노화적혈구를 분리한다음 세포막에서의 Na-pump 활성도 및 ouabain의 결합실험과 Rb의 세포내 유입실험을 시행하여 다음과 같은 결과를 얻었다.

1. 본 실험에 사용한 고혈압 백서의 혈압은 수축기 및 이완기 혈압이 165.5/119.0 mmHg로 유의하게 증가 하였다. 노화 적혈구의 평균용적(MCV)과 세포막 단백질 함량은 감소되고 혈색소치는 증가 되었다.

2. 110 mM NaCl 및 10 mM KCl 존재하에서의 적혈구 세포막 Na, K-ATPase 활성도는 대조군에 비해 고혈압군에서 억제 되었으며 양군 모두에서 노화에 의해 그활성도가 감소되었다.

3. 4 mM RbCl존재하에서 Ouabain에 의해 억제되는 Rb의 유입은 정상 및 고혈압군의 노화 적혈구에서 약간 감소되었으며 고혈압군의 young erythrocyte에서는 오히려 약간 증가 되었다.

4. 16 mM RbCl 존재하에서 Ouabain에 의해 억제되는 Rb의 유입은 양군의 노화 적혈구에서는 각군의 young erythrocyte에 비해 약 30-50% 감소되었으며, 고혈압군에서는 특히 young erythrocyte에서 정상군의 young erythrocyte에 비해 유의하게 감소되었다.

5. 0.13×10^{-6} M과 1×10^{-6} M에서의 ouabain binding은 정상군의 노화적혈구에서는 young erythrocyte에 비해 약간 감소되었으나 고혈압군의 노화적혈구에서는 유의하게 감소되었다.

6. 6×10^{-6} M과 64×10^{-6} M 에서의 ouabain binding은 양군의 노화 적혈구에서는 약간 감소되었지만 유의성은 없었으며 고혈압군의 young erythrocyte 및 노화적혈구에서는 정상군의 young erythrocyte및 노화 적혈구에 비해 유의하게 감소되었다.

이상의 결과로부터 ① 고혈압쥐의 young erythrocyte에서는 low affinity의 Na-pump수의 감소및 molecular activity의 증가, ② 정상쥐의 노화 적혈구에서는 molecular activity의 저하, ③ 고혈압쥐의 노화적혈구에서는 molecular activity의 저하 및 high affinity와 low affinity의 Na-pump수의 저하등에 의하여 Na-pump의 기능이 변동될 수 있을 것으로 추측된다.