Sodium-Potassium Cotransport in Hypertension and Hypotension

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SUMMARY

Furosemide-sensitive Na-K cotransport was measured as zero-trans efflux of each ion from fresh or Na loaded erythrocytes in normotensives (n=34), essential (n=41) or secondary (n=12) hypertensives and essential (n=17) or secondary (n=5) hypotensives.

The stoichiometry of Na and K efflux was 0.9:1 in fresh cells and 1:1 in cells loaded with sufficient Na to give near the maximum velocity (V_{max}) in a combined analysis.

There was a tendency toward a negative correlation between the V_{max} of Na efflux and serum total cholesterol ($p=0.09$) in the whole and it was significant in normotensives ($r=-0.60$). V_{max} of Na efflux also negatively correlated to age ($p<0.01$). After adjustment for age and total cholesterol level, essential hypertensives had a significantly ($p<0.01$) higher mean V_{max} of Na efflux (0.657 ± 0.029 mM/l cells/hr) than normotensives (0.388 ± 0.045), while essential hypotensives had a lower value (0.212 ± 0.047) ($p<0.01$).

Na efflux from fresh cells adjusted for internal Na content was higher in essential hypertensives (0.173 ± 0.018) than in normotensives (0.107 ± 0.026) ($p<0.05$).

Thus, elevated cotransport was linked to essential hypertension and reduced cotransport to hypotension. Whether it is a cause or a result remains to be further investigated.

Additional Indexing Words:

Age Serum total cholesterol Na loaded erythrocyte Fresh cell Zero-trans efflux Japanese

THE classic and current proposition that salt plays a key role in the regulation of blood pressure level has attracted numerous epidemiological, clinical and experimental concerns. A clue to the explanation may be found in a hypothesis proposed by Blaustein¹⁾ that a high cellular Na concentration

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induces an elevated Ca level via Na-Ca exchange, and increases vascular muscle tension leading to high blood pressure. It was also important in reassessing an earlier hypothesis of the natriuretic factor²⁾ and coupling with the concept of a circulating Na pump inhibitor by Haddy.³⁾ Indeed, recent ever-accumulating evidence of the presence of endogenous digoxin-like substances^{4),5)} do provide these ideas with material proof.

In contrast to the possible role of the Na pump, the major Na transport system of cells, the minor pathways such as Na-Na(Li) countertransport^{6),7)} or Na-K cotransport^{8),9)} are less clearly linked to the pathogenesis of hypertension. Although there are considerable controversies about even the basic properties of these minor systems, clinical interests cannot but force us to search for better understanding.

The so-called Na-K cotransport system is a member of the loop diuretics-sensitive chloride transport system¹⁰⁾⁻¹²⁾ which is widespread in the various secretory and absorptive epithelia,^{13),14)} smooth muscle cells,¹⁵⁾⁻¹⁷⁾ blood cells, nerves¹⁸⁾ and even brain capillary endothelial cells.¹⁹⁾

Many studies on the relationship between essential hypertension and this transport system of the erythrocyte membrane, the most representative biomembrane available, have failed to come to an agreement partially because of the assumption of its solely genetic regulation.^{9),20)} Recent reports on several modulators²¹⁾⁻²⁵⁾ urge us to take this aspect into consideration.

In the present investigation, we studied the estimated maximum rate of outward cotransport in erythrocytes from patients with various disorders of blood pressure regulation, adjusting for age, and serum total cholesterol that was expected to indicate membrane cholesterol content to some degree. We chose as subjects not only hypertensives and normotensives but also hypotensives to enhance the contrast by comparing the extremes of the blood pressure spectrum and thereby to ascertain the role of the cotransport system in blood pressure regulation.

SUBJECTS AND METHODS

We studied 41 essential hypertensives (EHT), 12 secondary hypertensives (SHT) (5 renovascular hypertension, 1 idiopathic aldosteronism, 2 Cushing's syndrome and 4 renoparenchymal hypertension), 17 essential hypotensives (eht) and 5 secondary hypotensives (1 case each of Addisonism, Bartter's syndrome, Pseudo-Bartter's syndrome, chronic obstructive lung disease and Shy-Drager syndrome). The control group was 34 normotensives (NT). Age and mean blood pressure (MBP) on the day of blood sampling of each subject group are listed in Table I.

Table I. Age and Mean Blood Pressure (MBP) of Each Group

Group	NT	EHT	SHT	eht	sht
n	34	41	12	17	5
male	22	28	6	11	4
female	12	13	6	6	1
age (yrs)					
range	16-60	18-68	23-43	22-47	25-75
mean (SE)	36.7 (2.1)	44.7 (2.1)	34.7 (2.2)	37.2 (2.2)	48.2 (8.7)
MBP (mmHg)					
mean (SE)	85.8 (1.4)	108.0 (2.4)	108.4 (4.9)	71.0 (1.3)	70.2 (3.3)

None of the subjects except 1 SHT (chr. glomerulonephritis) had severe renal impairment ($\text{GFR} < 40 \text{ ml/min}$). All hypertensives were inpatients on mild salt restriction (diet NaCl 6 g/day). Three of them were receiving anti-hypertensive drugs (β -blocker, Angiotensin I converting enzyme inhibitor and Ca antagonist). Hypotensives and normotensives were on a free diet. None of the NT, SHT, eht or sht had family histories of hypertension in first-degree relatives. All subjects were Japanese.

Hypertension was defined as ambulatory diastolic blood pressure above 95 mmHg and hypotension as systolic blood pressure below 100 mmHg.

Preparation of erythrocytes

Venous blood, collected in heparinized tubes after an overnight fast, was immediately centrifuged at 1,080 g for 10 min and the plasma and buffy coat were aspirated. Erythrocytes were then washed twice with an isotonic choline chloride medium containing choline chloride 150 mM/l, glucose 10 mM/l and imidazole-HCl 20 mM/l (pH 7.4) at 4°C. A known volume of the washed cells was set aside to measure intracellular Na, K and hemoglobin concentrations.

Na loading

Intracellular Na and K contents were exchanged using the PCMBS (2,5-p-chloromercuribenzenesulfonate) method described by Garrahan and Rega.²⁶⁾ Washed cells were suspended and kept in a Na loading medium to give a hematocrit of 5% for 20 hrs at 4°C. The loading medium contained NaCl 150 mM/l, MgCl_2 1 mM/l, sodium phosphate 2.5 mM/l (pH 7.4), PCMBS 0.02 mM/l and EGTA [ethyleneglycol-bis (beta-amino-ethyl ether) N,N'-tetra-acetic acid] 1 mM/l (pH 7.4 with Trizma base). It was renewed once after 6-8 hrs.

Sealing step

PCMBS treated cells were spun down and washed at 4°C twice with the isotonic choline chloride medium and once with a sealing medium containing NaCl 145 mM/l, KCl 5 mM/l, sodium phosphate 5.4 mM/l (pH 7.4, 37°C),

MgCl₂ 1 mM/l, glucose 10 mM/l, inosine 3 mM/l, adenine 2 mM/l and cysteine 4 mM/l, adjusted to final pH 7.4 with Trizma base. Then the cells were suspended in the sealing medium to give a hematocrit of 10%, and incubated at 37°C for 1 hr.

Assay for Na-K cotransport

Scaled cells (or fresh cells) were washed 3 times with the isotonic choline chloride medium and resuspended in 0.1 mM/l ouabain-added isotonic choline chloride medium (MgCl₂-sucrose medium was not chosen because of the inhibitory effect of Mg⁺⁺ on this transport system)²⁷⁾ with or without 1 mM/l furosemide (pH 7.4 with Trizma base) to give a hematocrit of 5%, and then incubated for 0, 0.5, 1.5 and 2.5 hrs at 37°C.

After each incubation period, cells were spun down at 4°C at 1,080 g for 10 min and the Na, K and hemoglobin concentrations of the supernatant and cells were measured. Ouabain-insensitive, furosemide-sensitive Na and K effluxes were calculated from the difference between each flux in the absence and presence of furosemide. It is generally accepted that PCMBs does not affect cotransport.²⁸⁾

Na and K concentrations were measured by flame photometry (Japan Spectroscopic Co. Ltd., Flame-30). Hemoglobin was estimated as oxyhemoglobin by measuring light absorption at 541 nm.

PCMBs, ouabain and cysteine were bought from Sigma Chemical Company (St. Louis, Missouri). Furosemide was a gift from Hoechst Japan Pharmaceutical, Inc. (Tokyo, Japan).

Measurement of other parameters

The following parameters were also measured for some subjects: plasma renin activity (PRA) and plasma aldosterone concentration (PAC) by radioimmunoassay^{29),30)} before and after intravenous administration of 40 mg of furosemide; plasma catecholamines by trihydroxyindole (THI) method using high performance liquid chromatography;³¹⁾ and serum total cholesterol by the enzymatic method.³²⁾

Statistical methods

Analysis of covariance was applied when adjustment for covariants was needed. Statistical analysis was done using the Statistical Analysis System (SAS).³³⁾

Since data were not always available for each subject, the number of subjects varied with the analysis.

RESULTS

Since the separate investigations for males and females in each of the

Table II. Means and Standard Errors of Cation Contents of Erythrocytes Prepared for Assay of Cotransport

Group	Fresh cells (mM/l cells)			Loaded cells (mM/l cells)		
	n	Na	K	n	Na	K
NT	8	8.9±0.4	101.9±1.7	34	54.3±1.5	52.7±1.8
EHT	18	9.9±0.4	103.2±1.4	41	52.4±1.3	53.4±1.3
SHT	6	8.8±0.7	101.9±2.8	11	48.7±1.7	57.5±1.6
eht	1	8.9	108.3	17	49.8±1.9	58.4±2.6
sht	—	—	—	5	52.4±5.0	51.7±3.8

Fig. 1. The relationship between Na and K efflux from fresh cells (left), and Na loaded cells (right) in each group. The linear regressions among the respective 4 and 5 groups were not significantly different in terms of either the slope or the intercept, and the data from all the groups were combined.

following analyses confirmed no major differences between sexes, the combined data of both sexes are presented.

Cation content

Table II shows the cation contents of Na loaded cells from each group. No significant difference was observed among them. Those of some fresh cells for which cation efflux was measured directly without Na loading are also presented.

Stoichiometry of Na and K efflux

Fig. 1 shows the relationship between Na and K effluxes from fresh cells (left) and Na loaded cells (right). Analysis of covariance showed no significant difference among the linear regressions of the groups in terms of the slope ($p=0.66$ and 0.53 , respectively) and the intercept ($p=0.83$ and 0.79 , respectively). The data of all the groups were, therefore, combined to obtain the

Fig. 2. The relationship between intracellular Na content and Na efflux from fresh cells. Linear regression coefficients were not significantly different among the 3 groups ($p=0.90$). The pooled regression coefficient was 0.0246 ($p<0.01$).

Table III. Means and Standard Errors of Furosemide-Sensitive Na Efflux from Fresh Cells Adjusting for Intracellular Na

Group	n	Na efflux (mM/l cells/hr)	p^a	p^b
NT	8	0.107 ± 0.026	—	—
EHT	18	0.173 ± 0.018	<0.05	—
SHT	6	0.117 ± 0.030	0.81	0.12

^a NT vs EHT or SHT; ^b EHT vs SHT.

regression line.

The stoichiometry of Na and K efflux was 0.9:1 at low Na content of fresh cells and 1:1 at sufficient Na content to ensure the maximum activation of loaded cells.

Cotransport in fresh cells

Fig. 2 shows the relationship between intracellular Na content and Na efflux from fresh cells. Although the mode of the activation of outward cotransport by intracellular Na is not fitted by a linear fashion,³⁴⁾ we regarded it as linear in the narrow range of Na represented in our study. When Na effect was compared among NT, EHT and SHT by analysis of covariance, linear regression coefficients were not significantly different among the groups ($p=0.90$), and the pooled regression coefficient was statistically significant (0.0246, $p<0.01$).

Table III shows internal-sodium-adjusted means of Na efflux from fresh cells for each group. Mean Na efflux in EHT (0.173 ± 0.018 mM/l cells/hr)

Fig. 3. The relationship between $V_{\max}$ of Na efflux and serum total cholesterol. Linear regression coefficients were not significantly different among the groups ($p=0.96$). The pooled regression coefficient was -0.0009 ($p=0.09$).

was significantly higher than that in NT (0.107 ± 0.026) ($p < 0.05$). There was no statistically significant difference between SHT (0.117 ± 0.030) and NT or EHT.

Cotransport in loaded cells

Furosemide-sensitive Na and K efflux rates from loaded cells, all of which contained about 50 mM/l cells of both Na and K, were regarded as giving near the maximum velocity ($V_{\max}$).^{34), 35)}

As shown in Figs. 3 and 4, $V_{\max}$ of Na efflux seemed to be related to both serum total cholesterol and age. Confirming the homogeneity of regression slopes among the groups, the pooled regression coefficients were evaluated. In all cases examined, the pooled regression coefficient between the $V_{\max}$ of Na efflux and serum total cholesterol did not reach a significant level (-0.0009 , $p=0.09$), but there was a significant correlation in NT ($n=16$, $r=-0.60$, $p < 0.05$). The pooled regression coefficient between the $V_{\max}$ of Na efflux and age was statistically significant (-0.0054 , $p < 0.01$).

Since age and serum total cholesterol were different among the groups, both factors were controlled as covariates in comparing the $V_{\max}$ of Na efflux among the groups. The age- and cholesterol-adjusted mean of the $V_{\max}$ of Na efflux was significantly higher ($p < 0.01$) in EHT (0.657 ± 0.029 mM/l cells/hr) than in NT (0.388 ± 0.045), while lower ($p < 0.01$) in eht

Fig. 4. The correlation between V_{max} of Na efflux and age. Linear regression coefficients were not significantly different among the groups ($p=0.27$). The pooled regression coefficient was statistically significant (-0.0054 , $p<0.01$).

Table IV. Means and Standard Errors of Na Efflux from Na Loaded Cells Adjusting for Age and Serum Total Cholesterol

Group	n	Na efflux (mM/l cells/hr)	p^a	p^b	p^c
NT	17	0.388 ± 0.045	—		
EHT	40	0.657 ± 0.029	<0.01	—	
SHT	11	0.374 ± 0.056	0.85	<0.01	
eht	15	0.212 ± 0.047	<0.01		—
sht	4	0.288 ± 0.091	0.33		0.46

^a NT vs others; ^b EHT vs SHT; ^c eht vs sht.

(0.212 ± 0.047). EHT showed a higher mean V_{max} than SHT (0.374 ± 0.056) ($p<0.01$). There was no significant difference between sht (0.288 ± 0.091) and NT or eht (Table IV).

There was no significant correlation between the V_{max} of Na efflux and PRA, PAC, epinephrine or norepinephrine in any group.

DISCUSSION

The loop diuretics-sensitive, chloride-dependent Na-K transport system in human erythrocytes appears to have somewhat complicated aspects. It mediates bidirectional 1:1 mutually-dependent Na-K (and perhaps 2 Cl which is difficult to demonstrate owing to enormous flux) fluxes driven by the transmembrane gradient of each co-ion (even against its own), with or without³⁶⁾ net effects. In the latter case the idea of 1:1 exchange diffusion of

both Na for Na and K for K could be introduced.^{37),38)} The ouabain-resistant, furosemide-sensitive zero-trans efflux of Na and K that we are discussing is one of the simplest aspects of this system, and is usually called furosemide-sensitive outward cotransport.

According to Garay et al,³⁹⁾ the activation of this system by intracellular Na is fitted by a sigmoidal fashion, and the rate of Na transport is described by Hill's equation:

$$V = \frac{V_{\max}}{1 + [K_{50\%} / (Na_i^+)]^n}$$

where: $V_{\max}$ is the maximum rate of outward Na-K cotransport; $K_{50\%}$ represents the intracellular Na concentration required for half-maximum outward cotransport stimulation and n is a cooperativity index (Hill's n).

The cotransport rate at 20–25 mM/l cells of both inner Na and K was first reported to have been reduced in essential hypertensives by Garay et al,⁴⁰⁾ who attributed it to reduced $V_{\max}$ in their earlier reports. More recently, they have pointed out abnormally high $K_{50\%}$ and compensatory increases in $V_{\max}$ in some hypertensives.^{39),41)}

Adragna et al³⁵⁾ found an elevated cotransport rate for inner Na and K at 50 and 60 mM/l cells respectively, which ensure at least 80–90% of the maximum activation, in hypertensives and normotensives with hypertensive relatives. However, except for an agreement that major racial differences seem to exist and blacks have lower cotransport rate than whites independent of the blood pressure level,^{42),43)} many other reports are still controversial as to not only the outward cotransport rate at various inner cation compositions^{44),45)} but also the inward one.^{46),47)}

It seems that, besides their belief in its genetic regulation they have taken no account of several other factors which may modulate this transport system. In the light of recent investigations, even if the density of the transport units is determined genetically, this system is also under the influences of cellular Ca^{++} ,²¹⁾ cyclic AMP,²¹⁾ membrane cholesterol^{22)–24)} and cellular ATP,²⁵⁾ with three of the former acting as inhibitors and the latter as a stimulator in human erythrocytes. In our study, erythrocytes were depleted of cyclic AMP and repleted with ATP in proportion to each reproducing capacity, and the influence of intracellular Ca^{++} on the physiological level was assumed to be negligible, if any, compared with the drastic effect of Ca ionophore. Then only membrane cholesterol would not have been controlled.

The unesterified cholesterol (UEC) of erythrocytes is in equilibrium with that of plasma LDL and there is a positive correlation between the ratio of UEC to phospholipid of erythrocytes and that of plasma LDL.⁴⁸⁾ Although

in our study only values of serum total cholesterol were available, a crude estimation of the amount of UEC of LDL is possible, and further, a similar estimation of that of erythrocytes may also be possible. Therefore, a tendency toward a negative correlation between serum total cholesterol and Na cotransport rate would be compatible with the inhibitory effect of erythrocyte UEC enriched in vivo^{23),24)} or in vitro.^{22),23)}

Another correlation between age and cotransport rate may be due to the well-known relationship between age and LDL-cholesterol⁴⁹⁾ in part, but other factors could also have an influence. What is more interesting is that it reminds us of a report on the negative correlation between Na-Li countertransport and age.⁵⁰⁾ In spite of persistent counterarguments, the idea that these two processes represent different modes of a single, highly complex transport system has been firmly supported.^{12),51)} Hence, our result may suggest that they hold several modulators in common.

It is quite apparent that all these modulating factors should be controlled to compare the cotransport activity among various groups. Now that we have shown that cotransport is higher in essential hypertensives and lower in hypotensives after both age and cholesterol were adjusted, what does this mean?

Although the physiological role of this system in human erythrocytes has not been fully elucidated, its bidirectional operation is believed to cause net fluxes of ions driven by the net chemical potential represented as below:⁵¹⁾

$$\mu_{\text{net}} = RT \ln \frac{[\text{Na}_e^+][\text{K}_e^+][\text{Cl}_e^-]^2}{[\text{Na}_i^+][\text{K}_i^+][\text{Cl}_i^-]^2}$$

where: R is gas constant;

T is absolute temperature;

[Na⁺], [K⁺] and [Cl⁻] are the respective internal (i) and external (e) concentrations.

Physiological compositions of internal and external Na and K are so critical for the direction of the net driving force that this system may function as a rapid regulator of intra or extracellular homeostasis; the higher the transport rate, the more rapid the approach to a new steady-state.

However, it seems unlikely that this function is directly related to blood pressure regulation. Therefore, we must assume that the cotransport activity is under a dynamic regulatory mechanism directed toward the minimum perturbation of the internal milieu, changing the number and/or the turnover rate of transport units. In this instance the elevated or reduced cotransport activity in vitro may indicate a compensatory response to the respective reduced or elevated activity in vivo, or to other environmental changes requir-

ing correction, although we cannot identify any such particular change which has influence on blood pressure. Also, it seems that an elevated $V_{\max}$ in essential hypertensives does not necessarily indicate a compensation for an abnormally high $K_{50\%}$ as proposed by Garay since essential hypertensives also showed an elevated cotransport rate at physiological cellular cation composition. In addition, one may question whether hydrostatic pressure per se has any thermodynamic effect on the transport activity. It is quite reasonable, but the effect of physiological pressure is thought to be almost negligible.⁵²⁾ Actually, the difference in the cotransport activity between essential and secondary hypertensives in whom blood pressures were almost equal would refute the pressure effect.

On the other hand, there may be another interpretation based on the assumption that the cotransport system has the same kinetic behavior in different cell systems under final regulation by genetic factors. The function of the loop diuretics-sensitive chloride transport system is so differentiated, depending upon its specific localization in each tissue, as to allow it to participate in electrolyte absorption in Henle's loop,⁵³⁾ salt uptake to the brain via the blood brain barrier,¹⁹⁾ and maintenance of the higher than equilibrium Cl-activity in vascular smooth muscle which may be related to muscle tension.^{16),17)} If the properties of this system in erythrocyte membranes are universal throughout the body, and fitted to these tissues, it might suggest the predisposition to salt retention, neuronal excitability^{53),54)} and vascular tension. While this generalization is fascinating, further study is needed for its clarification.

It is not clear whether the present findings reflect constitutional phenomena or responsive ones, but it seems to be meaningful that essential hypertensives and hypotensives showed contrasting behavior. Since we found a high incidence of elevated PRA, which was attributed to subclinical salt depletion, among essential hypotensives,⁵⁵⁾ we have speculated that patients with essential hypotension have a genetic predisposition to somewhat impaired salt handling and therefore that this condition is a true counterpart of essential hypertension in its pathogenesis as far as salt metabolism is concerned. On the other hand, a spontaneously hypotensive strain of rats⁵⁶⁾ was developed by successive matings of the animals with the lowest systolic pressure for generations as were several genetically hypertensive strains. Most studies of the cotransport system do not address hypotension, but deal only with pathological hypertension. However, the clinical significance of salt handling may have varied with the change of salt intake, and essential hypotensives, probable beneficiaries in a salt-surfeited modern world, would have had a distinct handicap in salt-poor prehistoric times. Thus, essential hypertension and hypo-

tension should be viewed as two deviations from the normal and both should be studied.

The relationship between the cotransport system and blood pressure regulation is still not clearly understood today, but the contrasting behaviors of the extremes of essential hypertension and hypotension as found in the present investigation may suggest a relation between them.

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