Lipids of Erythrocyte Membrane and Na-K ATPase Activity in Normotensive Adolescents with Hypertension Heredity

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I Introduction

Numerous studies and reports have investigated an important role of lipid in the erythrocyte membrane. About 50% of the lipid composition of erythrocyte membrane consists of phospholipid, 40% is unesterified cholesterol, and 10% is glycolipid. The modified lipid composition of erythrocyte membrane influences the activity of membrane-bound enzyme like Na-K ATPase (Anner, 1985; Cooper *et al.*, 1983; Incerpi *et al.*, 1983; Marcus *et al.*, 1986; Yeagle, 1983).

The relationship between Na-K ATPase activity and hypertension has been investigated (Swarts *et al.*, 1981; Walter, 1982; Wambach *et al.*, 1982; Wocial *et al.*, 1984), since Garay and Meyer (1979) published their observations and estimated the positive association between sodium-potassium cotransport and hypertension. Recently an inhibitor of Na-K ATPase was found in hypertensives, and low activity of Na-K ATPase points to a possible association with essential hypertension (Hamlyn *et al.*, 1982; Hugues *et al.*, 1984; MacGregor *et al.*, 1980).

We have, therefore, investigated the relationship between membrane lipids and Na-K ATPase activity using erythrocyte membranes from normotensive adolescents grouped according to a family history of hyper-

tension.

II Materials and Methods

Adolescents investigated in this present study were all students of the food and nutrition course in Toyama Junior College. They were 71 female aged either 18 or 19. The participants were divided into two groups according to the result of questionnaires provided beforehand to ascertain their family history of hypertension. Thirty-four female adolescents who had parents and/or grandparents with essential hypertension were classified as subjects with a family history of hypertension and 37 others as those without a family history.

Blood pressure was measured with mercury sphygmomanometer on the right arm in the sitting position after a five minute rest. Diastolic blood pressure was determined as 5th-Korotkoff phase. Mean blood pressure was calculated as the sum of diastolic blood pressure and 1/3 of systolic blood pressure. Body mass index (Katsura's method) was determined using Broca's standard body weight adapted to the Japanese (>150 cm; height - 100, <150 cm; $0.9 \times (\text{height} - 100)$).

Fresh blood was obtained from healthy adolescents after measurement of blood pressure. For measurement of erythrocyte mem-

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brane lipids and Na-K ATPase procedure of Doge *et al* (1963). Membrane lipids were extracted in chloroform-methanol-water by the method of Bligh and Dyer (1959). Then cholesterol and phospholipid were analyzed by enzymatic method. The ghosts were also analyzed for Na-K ATPase activity by the method of Swarts *et al* (1981) with a modified method for determination of ATP consumption. Namely, instead of measuring phosphate, ATP concentration was directly measured using firefly luciferinluciferase (Sigma) with Lumino-phorometer TD-4000 (Analytical Luminescence Laboratory Inc.). Erythrocyte membrane protein was determined using the Dye-binding assay kit (BIO-RAD Laboratory).

Serum total cholesterol, phospholipid and triglyceride were analyzed by enzymatic method with a Hitachi-726 autoanalyser. Serum HDL-cholesterol was measured by

precipitation method using dextran sulfate.

Urine specimens were pooled in the same morning when blood pressure measurement and blood sample collection had been carried out, Sodium, potassium and creatinine concentrations were measured by flame photometry and with Hitach-726 autoanalyser respectively.

III Results

Table 1 shows that serum total cholesterol was significantly higher and urinary Na/K ratio was significantly lower in the participants with a positive family history of hypertension. Urinary Na/creatinine ratio was also lower in the groups with a positive family history, although the standard deviations were large in both groups and the difference was not statistically significant. But mean blood pressure, body mass index, other serum lipids except for total cholesterol,

Table 1 Comparison of variables investigated among subjects grouped according to their family history of hypertension.

Variables		Positive family history (n=34)	Negative family history (n=37)	P value ^b
Serum	Body mass index (Katsura, %)	-0.24±9.42	-0.93±11.30	NS
	Mean blood pressure (mmHg)	82.7± 8.0	82.0± 9.0	NS
	Total cholesterol (mg/dl)	192.7±37.9	174.9±28.9	<0.05
	Phospholipid (mg/dl)	192.2±28.5	180.2±24.3	NS
	Cholesterol/Phospholipid	1.00±0.09	0.97±0.09	NS
	HDL-cholesterol (mg/dl)	60.1±12.0	60.9±11.3	NS
Urinary	Tryglyceride (mg/dl)	73.8±34.6	74.1±35.3	NS
	Na/Creatinine (mEq/g)	158.7±60.6	190.0±81.9	<0.10
	Na/K	3.32±1.01	4.11±1.66	<0.05
Erythrocyte membrane				
	Na-K ATPase (mU/mg protein)	12.82 (1.75) ^a	13.34 (1.43) ^a	NS
	Cholesterol (mg/mg protein)	0.26±0.10	0.25±0.08	NS
	Phospholipid (mg/mg protein)	0.28±0.11	0.27±0.08	NS
	Cholesterol/Phospholipid	0.94±0.07	0.94±0.08	NS

Numbers in the table are means±standard deviations.

a : The numbers in brackets are SD of geometric mean.

b : Significance was determined by Student's t test, NS; not significant ($p \geq 0.10$).

Table 2 The correlations of erythrocyte membrane lipids and other variables investigated among subjects grouped according to their family history of hypertension.

Variables	Positive family history		Negative family history	
	Membrane cholesterol	Membrane phospholipid	Membrane cholesterol	Membrane phospholipid
Body mass index	0.017	0.000	-0.161	-0.135
Mean blood pressure	-0.055	-0.004	-0.336*	-0.319
Serum Total cholesterol	0.155	0.168	-0.399*	-0.280
Phospholipid	0.115	0.148	-0.222	-0.159
HDL-cholesterol	0.009	-0.050	0.138	0.071
Tryglyceride	0.294	0.414*	-0.126	-0.072
Urinary Na/creatinine	-0.116	-0.100	-0.038	-0.068
Na/K	0.214	0.224	0.306	0.260
Erythrocyte membrane Na-K ATPase	0.577***	0.587***	0.217	0.166

The supercript symbols indicate the statistical significance (* $p < 0.05$; *** $p < 0.001$).

Fig. 1 The correlation between erythrocyte membrane cholesterol and Na-K ATPase activity of erythrocyte. ● Subjects having relatives with hypertension ($n=34$, $r=0.577$, $p<0.001$). ○ Subjects having relatives without hypertension ($n=37$, $r=0.217$, not significant).

Na-K ATPase activity of erythrocyte membrane, and membrane lipids of erythrocyte were virtually identical within both groups.

The relationships between membrane lipids of erythrocyte and other variables examined are shown in Table 2. In the group

Fig. 2 The correlation between erythrocyte membrane phospholipid and Na-K ATPase activity of erythrocyte. ● Subjects having relatives with hypertension ($n=34$, $r=0.587$, $p<0.001$). ○ Subjects having relatives without hypertension ($n=37$, $r=0.166$, not significant).

with a positive family history of hypertension, significant positive correlations were observed between membrane cholesterol and Na-K ATPase activity of erythrocyte, and between membrane phospholipid and Na-K ATPase activity, whereas no significant relationship of these variables existed in the group with a negative family history (Fig. 1, Fig. 2). In addition to these, a significant positive correlation was demonstrated between membrane phospholipid and serum triglyceride in the group with a positive family history of hypertension. On the other hand, in the group with a negative family history, significant negative correlations existed between membrane cholesterol and

serum total cholesterol.

Except for associations with membrane lipids, Na-K ATPase activity of erythrocyte membrane was only significantly correlated with urinary Na/K ratio in the participants with a positive family history of hypertension ($r=0.406$, $p<0.05$).

IV Discussion

Modification of the cholesterol content of erythrocyte membrane can cause modulation of ATPase activity of erythrocyte membrane. A decrease of cholesterol/phospholipid ratio of erythrocyte membrane, as a result of cholesterol depleted in vitro has been shown to activate Na-K ATPase activity of erythrocyte

(Anner, 1985; Cooper *et al.*, 1975; Girurad *et al.*, 1976). In the case where membrane cholesterol is enriched in vitro and, therefore, cholesterol/phospholipid ratio of erythrocyte membrane increases, conflicting results have been reported. Yeagle (1983) have demonstrated that high cholesterol inhibited the ATPase activity of enzyme of erythrocyte membrane and low membrane cholesterol activated that enzyme activity. Conversely, Incirpi *et al* (1983) have pointed out that increased membrane cholesterol induced an increase of Na-K ATPase activity.

The modulation of Na-K ATPase activity in vitro is thought to be given by the wide-ranging changes of cholesterol content and/or cholesterol/phospholipid ratio of erythrocyte membrane. The lipid composition of erythrocyte membrane can be altered by varying dietary supplementation (Popp-Snijders *et al.*, 1986; Schick *et al.*, 1972). Vajreswari *et al* (1983) have demonstrated that in male rat fed high erucic acid mustard oils, cholesterol/phospholipid ratio of erythrocyte membrane was increased and the enhancement of cholesterol/phospholipid ratio increased Na-K activity of erythrocyte, compared with those in male rats fed low erucic acid rapeseed. In the present study, the distinct increases of Na-K ATPase activity were observed according to increased cholesterol or increased phospholipid in erythrocyte membrane of normal female adolescents with a family history of essential hypertension, whereas there was no relationship of these in erythrocyte membrane of normal subjects without the same family history.

Cooper *et al* (1983) have shown that there was a significant difference in the sum of net Na transport mediated by Na-K pump and Na-

K cotransport between high school students with a family history of hypertension and those without a family history. In both hypertensives and normotensives with a family history of hypertension, the significant elevations of Na-K ATPase activity inhibitor have been demonstrated, compared with that in normotensives without a family history (Hugues *et al.*, 1984). These results suggest that a substantial genetic component influences Na-K ATPase activity, although the present study showed that there was no difference in Na-K ATPase activity between two groups.

On the other hand, Na-K ATPase activity is inhibited among obese persons (Luise *et al.*, 1980). Kagaminori *et al* (1985) have pointed out that the correlations for sodium pump existed not only between parent and offspring but also between husband and wife. The effect of different dietary supplements on Na-K ATPase activity of membrane have been investigated (Abeywarden *et al.*, 1984; Vajreswari *et al.*, 1983). In addition to these, the present study showed a significant positive correlation between urinary Na/K ratio as a index of salt intake and Na-K ATPase activity in the subjects with a family history of hypertension. Based on these results, an environmental factor was suggested as playing a possible role in Na-K ATPase activity, apart from the genetic effect.

Na-K ATPase activity maintains intercellular sodium and potassium concentrations. For this reason, Na-K ATPase activity has been thought to be one of the predisposing factors in essential hypertension. It is considered that the positive correlation between urinary Na/K ratio and Na-K ATPase activity of erythrocyte membrane in normotensives

with a family history of hypertension, as the present study showed, might indicate that the high salt intake may cause a higher intercellular sodium concentration and activated Na-K ATPase activity could be a consequence of higher intercellular sodium concentration (Kagaminori *et al.*, 1986). The present study also showed that Na-K ATPase activity of membrane was related to membrane cholesterol and membrane phospholipid, and it was only demonstrated in the cases with a family history of hypertension. Both membrane lipids and Na-K ATPase activity of membrane are affected by dietary factors, such as fat and salt, as mentioned above. Therefore it would be possible that three correlations between erythrocyte membrane lipids (cholesterol and phospholipid) and Na-K ATPase activity of erythrocyte, and between Na-K ATPase activity of erythrocyte and urinal Na/K ratio are due to a functional disorder of cell membrane through the intermediary of history of hypertension. Though the mechanism that the change of membrane composition causes in the modulation of sodium pump activity has not been solved, this phenomenon is important in the search for the predisposition to essential hypertension.

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〔原 著〕

高血圧家族歴を有する青年期女子における赤血球膜中の脂質と
Na-K ATPase 関連について

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Na ポンプに対する赤血球膜脂質の影響を検討するために、青年期女子を対象に赤血球膜中のコレステロール、磷脂質および Na-K ATPase, その他の測定を行い、高血圧の家族歴の有無別に比較した。高血圧家族歴(+)の34人と家族歴(-)の37人の比較では、家族歴(+)群に血清コレステロールが有意に高く、早朝1回尿中 Na/K 比が有意に低かった。高血圧家族歴(+)群には赤血球膜中コレステロールと磷脂質は膜中の Na-K

ATPase 活性と統計的に有意な正相関(それぞれ $r=0.58$, $r=0.59$; $p<0.001$)が認められたが、これは家族歴(-)群にはみられなかった。また家族歴(+)群で赤血球膜 Na-K ATPase は尿中 Na/K 比と有意な正相関($r=0.41$; $p<0.05$)が認められた。これらのことは膜中脂質が膜中 Na-K ATPase 活性と高血圧の関連に何らかの役割を果たしていることを推測させるものであった。

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