CAN HIGH BLOOD PRESSURE ALONE INCREASE ERYTHROCYTIC INTRACELLULAR SODIUM CONCENTRATION?

HIDEO MATSUURA, M.D., TETSUYA OSHIMA, M.D., ICHIRO INOUE, M.D.
TETSUJI SHINGU, M.D., TAKASHI FUJII, M.D., YOSHIRO KATO, M.D.
AND GORO KAJIYAMA, M.D.

In order to clarify the direct effects of high blood pressure on erythrocytic intracellular sodium concentration ($[Na^+]_i$) and sodium transport systems, a static pressure of 2.5 atm was applied to whole blood in plastic syringes at room temperature for 5 and 24 h. In the control samples, 5 h incubation under atmospheric pressure produced a significant decrease in ouabain-sensitive Na^+-K^+ pump activity and plasma pH, but no change in other parameters. After 24 h incubation, $[Na^+]_i$ and mean corpuscular volume were significantly increased and intracellular potassium concentration, ouabain-sensitive Na^+-K^+ pump activity, and plasma pH were decreased. The change in $[Na^+]_i$ during incubation under atmospheric pressure may be due to the increased permeability of the cell membrane and the decrease in ouabain-sensitive Na^+-K^+ pump activity. The pressure load did not increase erythrocytic $[Na^+]_i$ but did decrease it relative to the control. The pressure load had no apparent effects on sodium transport systems, mean corpuscular volume and pH of plasma relative to the control. Although the mechanisms of the effect of pressure load on $[Na^+]_i$ were not determined, we did find that high blood pressure alone was unable to increase erythrocytic $[Na^+]_i$. (*Jpn Circ J* 1992; **56**: 1234–1238)

ALTHOUGH several reports have focused on intracellular cation metabolism abnormalities as causes of essential hypertension,^{1–3} no evidence has been presented which suggests that intracellular sodium metabolism abnormalities are the primary causes of essential hypertension but not the results of blood pressure elevation. There are several factors^{4–6} i.e., renal function, NaCl intake, Na^+ transport systems, intra- and extracellular pH, etc., which modulate intracellular sodium concentration ($[Na^+]_i$). However, there has been no report on whether or not high blood pressure

per se can affect $[Na^+]_i$. Hall et al⁷ reported that pressure loads from 100 ATA to 400 ATA increased Na^+-K^+ pump activity and passive permeability of K^+ , and decreased Na^+-K^+ cotransport in red blood cells. However, since they did not report on changes in $[Na^+]_i$, the possibility that high blood pressure may affect $[Na^+]_i$ still exists. In order to clarify whether or not high blood pressure has a direct effect on $[Na^+]_i$ this in vitro study was performed using whole blood samples under a pressure load of 2.5 atm, which was not as high as in the previous study?

Key words:

Blood pressure
Intracellular sodium concentration
Sodium transport systems
Erythrocytes

SUBJECTS AND METHODS

Subjects

Blood samples were collected from 17

(Received August 19, 1991; accepted May 15, 1992)

The First Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan.

Mailing address: Hideo Matsuura, M.D., The First department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan

Fig.1. Four ml of whole blood in a disposable plastic syringe, 2 cm in diameter, was compressed by a spring balance. The pressure load of 8 Kg/3.14 cm² (2.5 atm) was maintained for 5 h and 24 h at room temperature. As a control, blood in a similar syringe was kept under atmospheric pressure (1 atm) without a pressure load.

healthy, normotensive male volunteers, 30.2 ± 2.7 year of age, working in Hiroshima University Hospital. All volunteers provided 20 ml of heparinized venous blood under fasting conditions in the morning. Blood samples were divided into 5 20 ml plastic disposable syringes, 2 cm in diameter. Each syringe contained 4 ml of blood. Seven of the 17 volunteers provided an additional 80 ml of heparinized venous blood for simultaneous measurement of $[Na^+]_i$, ouabain-sensitive Na^+-K^+ pump activity, furosemide sensitive Na^+ efflux and Na^+-Li^+ countertransport of erythrocytes. For this purpose, blood was additionally divided into 5 20 ml plastic disposable syringes, each containing 20 ml of

blood.

Protocol

To study the direct effects of elevated blood pressure upon erythrocytic $[Na^+]_i$ and sodium transport systems, the blood in 2 syringes was kept under a static pressure load of 8 kg/3.14 cm² using a spring balance (Fig. 1), equivalent to 2.5 atm (standard physical atmosphere), at room temperature for 5 and 24 h, respectively. Two other syringes were kept at atmospheric pressure (1 atm) without an additional pressure load for 5 and 24 h as controls. The blood in the last syringe without a pressure load was used for immediate measurement of the basal values for each parameter. The pressure load was applied to whole blood, because the buffer solution used to suspend erythrocytes (75 mM MgCl₂, 85 mM sucrose, 10 mM tris-Mops, 10 mM glucose, pH 7.4) could not maintain a pH above 7 for 5 or 24 h (data not shown).

Measurements

$[Na^+]_i$ and intracellular potassium concentration ($[K^+]_i$) in erythrocytes were measured by a previously described method.⁸ Ouabain-sensitive Na^+-K^+ pump activity was measured by the modified method of Cumberbuch and Morgan.⁹ Na^+-K^+ cotransport was based on furosemide-sensitive Na^+ efflux by the method of Price et al.¹⁰ Na^+-Li^+ countertransport was determined by the method described by Canessa et al.¹¹ Sodium, potassium and lithium concentrations in supernatant were determined by flamephotometry (Hitachi 775-A, Tokyo, Japan). The mean value of triplicate trials was used.

Mean corpuscular volume (MCV) was measured by a blood cell counter S-Plus II (Coulter Electronics, Hialeah, Florida) and plasma pH was determined by a blood gas autoanalyzer ABL 1 (Radiometer, Copenhagen, Denmark) at the basal period, and after 5 and 24 h of incubation.

Statistics

Data are shown as mean $\pm$ SD. Statistical analysis was performed using the Student's paired t-test. Results were considered statistically significant when $p < 0.05$.

TABLE 1 THE EFFECTS OF PRESSURE LOAD ON INTRACELLULAR SODIUM AND POTASSIUM CONCENTRATIONS, SODIUM TRANSPORT SYSTEMS OF ERYTHROCYTES, MEAN CORPUSCULAR VOLUME, AND PLASMA PH

	n	Pressure	Basal	Incubation Time	
				5hrs	24hrs
$[Na^+]_i$ mmol/l cells	17	1 atm	8.90 ± 1.04	8.87 ± 1.02	$9.47 \pm 1.29^{* \#}$
		+2.5 atm		$8.78 \pm 0.98\$!$	$8.97 \pm 1.26 +$
$[K^+]_i$ mmol/l cells	17	1 atm	100.4 ± 3.0	100.7 ± 2.5	$94.1 \pm 4.3^*$
		+2.5 atm		100.2 ± 3.0	$93.9 \pm 3.7^*$
Na^+-K^+ pump h^{-1}	7	1 atm	0.23 ± 0.02	$0.19 \pm 0.03\$$	$0.07 \pm 0.03 \# \$$
		+2.5 atm		$0.19 \pm 0.03\$$	$0.07 \pm 0.05 \# \$$
COT mmol/l cells/h	6	1 atm	0.38 ± 0.22	0.24 ± 0.11	0.30 ± 0.17
		+2.5 atm		0.33 ± 0.26	0.24 ± 0.15
SLC mmol/l cells/h	7	1 atm	0.32 ± 0.17	0.36 ± 0.10	0.39 ± 0.11
		+2.5 atm		0.40 ± 0.07	$0.49 \pm 0.12 \#$
MCV mm^3	17	1 atm	92.7 ± 6.8	92.7 ± 6.7	$94.0 \pm 6.5^* \#$
		+2.5 atm		92.7 ± 6.8	$94.0 \pm 6.5^* \#$
pH of plasma	6	1 atm	7.35 ± 0.03	$7.24 \pm 0.02\$$	$7.00 \pm 0.03 \# \$$
		+2.5 atm		$7.24 \pm 0.03\$$	$6.98 \pm 0.04 \# \$$

Values are mean $\pm$ SD

$[Na^+]_i$ =intracellular sodium concentration

$[K^+]_i$ =intracellular potassium concentration

Na^+-K^+ pump=ouabain-sensitive Na^+-K^+ pump

COT=furosemide-sensitive Na^+ efflux

SLC= Na^+-Li^+ countertransport

MCV=mean corpuscular volume

* $p < 0.01$ vs basal value, # $p < 0.05$ vs value of 5h incubation, \$ $p < 0.05$ vs basal value, ! $p < 0.05$ vs control value (without pressure load) + $p < 0.01$ vs control value.

RESULTS

All data are presented in Table I.

1) The effect of the pressure load on erythrocytic $[Na^+]_i$

Basal $[Na^+]_i$ was 8.90 ± 1.04 mmol/l cells. $[Na^+]_i$ under the control condition did not change after 5 h of incubation, but had significantly increased at 24 h. Under the pressure load, $[Na^+]_i$ decreased relative to the control after both 5 and 24 h incubation.

2) The effect of the pressure load on erythrocytic $[K^+]_i$

The time course of $[K^+]_i$ under atmospheric pressure and under the static pressure load showed very similar patterns. After 5 h, there was no change in $[K^+]_i$. However, $[K^+]_i$ had significantly decreased after 24 h. The pressure load had no apparent effect on $[K^+]_i$ after 5 and 24 h incubation.

3) The effects of the pressure load on 3 erythrocytic sodium transport systems

Ouabain-sensitive Na^+-K^+ pump activity

decreased with an increase in the duration of incubation, but Na^+-Li^+ countertransport and furosemide-sensitive Na^+ efflux were not affected by incubation. The pressure load enhanced Na^+-Li^+ countertransport activity at 24 h but showed no effect on ouabain-sensitive Na^+-K^+ pump activity or furosemide-sensitive Na^+ efflux.

4) The effects of the pressure load on MCV and plasma pH

After 24 h incubation, the increase in MCV was slight, but significant, in both groups. However, MCV was apparently not affected by the pressure load. Plasma pH decreased significantly with an increase in incubation period in both groups, but was not affected by the pressure load.

DISCUSSION

Although this study was performed under nonphysiological conditions, i.e., 24 h in a plastic syringe under high static pressure at

room temperature and in an anaerobic environment, because of limitations of the experimental system, the results indicate that high pressure alone decreased, rather than increased $[Na^+]_i$ in erythrocytes. These findings support the hypothesis^{1,3,12} that abnormalities in the membrane transport of sodium and $[Na^+]_i$ may cause hypertension.

Under the control condition, there were no changes in erythrocytic $[Na^+]_i$, $[K^+]_i$ or MCV after 5 h incubation. After 24 h incubation, however, there were significant increases in $[Na^+]_i$ and MCV and a decrease in $[K^+]_i$. These results suggest that the activity of the ouabain-sensitive Na^+-K^+ pump was suppressed during incubation in excess of 5 h due to the room temperature, pH or pump damage and that the passive diffusion of Na^+ was increased. In fact, the activity of the ouabain-sensitive Na^+-K^+ pump was significantly suppressed with incubation time. Since the Na^+-K^+ pump exchanges 3 intracellular sodium ions for 2 extracellular potassium ions, it is difficult to explain the changes in $[Na^+]_i$ and $[K^+]_i$ after 24 h incubation solely in terms of the inhibition of the ouabain-sensitive Na^+-K^+ pump. Another possible cause of the $[Na^+]_i$ change is acidosis. Actually, plasma pH significantly decreased with an increase in the duration of incubation. However, it is unlikely that acidosis is a factor in these $[Na^+]_i$ and $[K^+]_i$ changes, since erythrocytic $[Na^+]_i$ significantly decreases with a decrease of pH of the medium⁵. Although we did not measure the passive Na^+ influx, it is likely that the permeability of erythrocytic membranes increased during incubation for 24 h. There was no evidence whether or not the decrease in ouabain-sensitive Na^+-K^+ pump activity and the increase in membrane permeability were caused by the same mechanism, such as membrane damage.

In the blood vessels, erythrocytes are exposed to pulsating pressure. In this study, however, they were exposed to a static pressure load of 8 kg/3.14 cm², equivalent to 2.5 atm. Since a blood pressure of 200 mmHg/100 mmHg, for example, is equivalent to a mean blood pressure of 133 mmHg, (0.175 atm), the pressure load used in this study was rather high. The pressure load of 2.5 atm was used to obtain effects from a pressure load during the very short ex-

perimental period which are comparable to those from a pressure load of long duration in patients with essential hypertension. This high static pressure load did not affect $[K^+]_i$ and MCV but decreased $[Na^+]_i$ after 5 h incubation and suppressed the elevation of $[Na^+]_i$ after 24 h incubation. Hall et al⁷ reported that pressure loads of 100, 200, 300 and 400 atm affected human red blood cell K^+ influx component. However, these pressure levels, were extremely high and ouabain-sensitive Na^+-K^+ pump activity and passive K^+ influx were not affected at 100 atm. The causes of $[Na^+]_i$ change with a pressure load should be attributed to the changes in Na^+ transport systems caused by the pressure load. Our data show that a static pressure load failed to change the activity of the ouabain-sensitive Na^+-K^+ pump and furosemide-sensitive Na^+ efflux. These transport systems which could modify $[Na^+]_i$ were resistant to the pressure load used in this study. Na^+-Li^+ counter-transport also cannot explain the decrease in $[Na^+]_i$, because this transport system has no influence on $[Na^+]_i$.

In summary, although the mechanisms of decreased erythrocytic $[Na^+]_i$ due to a static pressure load in vitro could not be elucidated in this study, it was clearly demonstrated that elevated blood pressure alone did not increase erythrocytic $[Na^+]_i$.

Acknowledgement

We thank Miss Yuko Omura for her assistance with the preparation of this manuscript.

REFERENCES

1. BLAUSTEIN MP: Sodium ions, calcium ions, blood pressure regulation and hypertension: a reassessment and a hypothesis. *Am J Physiol* 1977; **232**: 165–173
2. HADDY F, PAMNANI M, CLOUGH D: The sodium-potassium pump in volume-expanded hypertension. *Clin Exp Hypertens* 1978; **1**: 295–336
3. DE WARDENER HE, MACGREGOR GA: Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: its possible role in essential hypertension. *Kidney Int* 1980; **18**: 1–9
4. DE WARDENER HE, MACGREGOR GA: The natriuretic hormone and essential hypertension. *Lancet* 1982; **1**: 1450–1454
5. KIDO K, MATSUURA H, MATSUMOTO K, FUJII H, OSHIMA T, OTSUKI T, WATANABE

- M, KAJIYAMA G, MOTONAGA K: Regulation mechanism of intracellular sodium concentration in patients with chronic renal failure on maintenance hemodialysis. *Jpn J Nephrol* 1988; **30**: 1147–1152
6. EDMONDSON RPS, MACGREGOR GA: Leucocyte cation transport in essential hypertension: its relation to the renin-angiotensin system. *Br Med J* 1981; **282**: 1267–1269
 7. HALL AC, ELLORY JC, KLENIN RA: Pressure and temperature effects on human red cell cation transport. *Membrane Biol* 1982; **68**: 47–56
 8. OSHIMA T, MATSUURA H, KIDO K, MATSUMOTO K, OTSUKI T, SHINGU T, INOUE I, KAJIYAMA G: Intracellular sodium and potassium concentrations in erythrocytes of healthy male subjects. *Jpn J Nephrol* 1988; **30**: 1095–1101
 9. KIDO K, MATSUURA H, OTSUKI T, MATSUMOTO K, SHINGU T, OSHIMA T, INOUE I, KAJIYAMA G: Sodium chloride sensitivity, intracellular sodium concentration in erythrocytes and lymphocytes, and renin profile in essential hypertension. *Jpn Circ J* 1989; **53**: 101–107
 10. PRICE M, HANNAERT P, DAGHER G: Interaction of internal and external K with the erythrocyte Na⁺, K⁺ cotransport system in essential hypertension. *Hypertension* 1984; **6**: 352–359
 11. CANESSA M, ADRAGNA M, SALOMON HS: Increased sodium-lithium countertransport in red cells of patients with essential hypertension. *N Engl J Med* 1980; **302**: 772–776
 12. AVIV A, LASKER N: Proposed defects in membrane transport and intracellular ions as pathogenic factors in essential hypertension. In: Raragh JG, Brenner BM eds. *Hypertension: Pathophysiology, Diagnosis, and Management*, New York, Raven Press, 1990, p.923–937