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Lack of Deficiency in Extracellular and Intralymphocyte Free Mg^{2+} in Genetically Hypertensive Rats

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ABSTRACT

Recently it has been suggested that Mg deficiency may play a key role in hypertension and several cardiovascular diseases. In order to investigate the status of Mg in genetic hypertension, the cytosolic free Mg^{2+} concentration ($[Mg^{2+}]i$) in the lymphocytes and serum concentrations of free Mg^{2+} and total Mg were measured in spontaneously hypertensive rats/Izumo (SHR/Izm), stroke-prone spontaneously hypertensive rats/Izumo (SHRSP/Izm), and Wistar-Kyoto rats/Izumo (WKY/Izm). In addition, the basal cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) was assessed in the three strains. Systolic blood pressure was highest in SHRSP/Izm and lowest in WKY/Izm. No significant differences were found in either the serum free Mg^{2+} concentrations or the serum total Mg concentrations among WKY/Izm, SHR/Izm, and SHRSP/Izm. $[Mg^{2+}]i$ in the lymphocytes was significantly higher in SHR/Izm than in WKY/Izm (254±51 versus 201±36μmol/liter, $p<0.05$), but the $[Mg^{2+}]i$ in SHRSP/Izm (211±34μmol/liter) was at the same level as in WKY/Izm. No significant correlation was found between $[Mg^{2+}]i$ in the lymphocytes and systolic blood pressure. Basal $[Ca^{2+}]i$ did not differ among the three strains. Thus, an increase in $[Ca^{2+}]i$ is not obligatory in all cells of genetically hypertensive rats. Mg deficiency may not exist in the intracellular or extracellular space in genetically hypertensive rats.

Key words: *Cytosolic magnesium, Lymphocyte, Mag-fura-2*

There has been increasing evidence of abnormalities in the Na^+ transport across the cell membrane associated with elevated $[Na^+]i$ and abnormal Ca^{2+} handling and increased $[Ca^{2+}]i$ in a wide variety of cells and tissues of essential hypertensive patients and genetically hypertensive rats[21–23]. Furthermore, abnormal cation homeostasis at the cellular level is considered to be involved, at least in part, in the pathogenesis of hypertension[25]. This abnormality may explain why nutritional factors such as Na^+ and Ca^{2+} intakes are associated with blood pressure regulation[20,24].

Another nutritional factor implicated in the pathogenesis of several cardiovascular diseases is Mg deficiency[1,29]. In theory, Mg deficiency may account for alterations in Na^+ transport and Ca^{2+} handling at the cellular level. However, it remains unclear whether this is the case with hypertension. A negative correlation between dietary Mg intake and blood pressure was demonstrated by epidemiological studies[13]. However, there is disagreement concerning the hypotensive effect of oral Mg supplementation[3,9].

The Mg deficiency hypothesis in genetic hypertension has been studied both at the extracellular and intracellular levels. As it is well known that extracellular Mg^{2+} affects Ca^{2+} influx[6,46,47], a reduction in serum Mg^{2+} may lead to increased $[Ca^{2+}]i$. However, the serum total Mg concentration has been reported higher[39], lower[33] or unchanged[30] in patients with essential hypertension. There is little information concerning genetically hypertensive rats.

Intracellular Mg has been mainly studied in the erythrocytes of essential hypertensive patients and spontaneously hypertensive rats using atomic absorption spectrophotometry[15,27,33] or nuclear magnetic resonance[12,16,31,41]. Many of these investigators have reported a reduction in the intracellular Mg content in the erythrocytes[16,27,31,33], whereas a few reported an unchanged[41] or increased concent[15]. Alternatively, lymphocytes have been used as available nucleated cells to evaluate the cellular metabolism of cations[4,21,23]. Especially in Mg^{2+} metabolism, earlier studies reported that the level of Mg in the lymphocytes was a more accurate reflection of its levels in cardiac and skeletal muscle[34] than that in the erythrocytes. Furthermore, it has been demonstrated in genetically hypertensive rats that T cell function is abnormal[5,26,27] and restoration of T cell depression attenuates hypertension[2,14]. Thus, alterations in immunologic func-

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tion including T-lymphocyte dysfunction may contribute to the pathophysiology of high blood pressure in genetically hypertensive rats. In the present study, $[Mg^{2+}]i$ was determined in thymic lymphocytes using a fluorescent Mg^{2+} sensitive dye, mag-fura-2.

In order to determine whether Mg deficiency exists in genetically hypertensive rats, both the $[Mg^{2+}]i$ in the lymphocytes and the serum Mg^{2+} concentrations were measured in both SHR/Izm and SHRSP/Izm, as genetically hypertensive rats, and in Wistar-Kyoto rat/Izm (WKY/Izm) as a normotensive control. Because SHRSP/Izm, a substrain of SHR/Izm, could spoontaneously develop more severe hypertension than SHR[18], comparison of the three strains may help to investigate the relation between Mg deficiency and hypertension. In addition, basal $[Ca^{2+}]i$ and Ca^{2+} handling in mitogen-stimulated T lymphocytes and mitogeninduced proliferative responses were also assessed in the three strains.

MATERIALS AND METHODS

Male WKY/Izm, SHR/Izm and SHRSP/Izm, aged 8 weeks, were obtained from the Disease Model Cooperative Research Association (Kyoto, Japan)[17].

Systolic blood pressure was determined by the tail cuff method. After the animals had been anesthetized with intraperitoneal ketamine (15 mg/100 g of body weight), the thoracic cavity was opened to remove the thymus. The thymus was rinsed in RPMI 1640 medium (Gibco, Rockvill, MD, U.S.A.) and the attached blood vessels and connective tissue were removed. The thymus was minced and the crude suspension was passed through a nylon mesh and layered on Ficoll-metrizoate with a density of 1087 g/liter. It was then centrifuged at 800g for 20 min. The isolated lymphocytes were washed once in RPMI 1640 medium. The cells were counted and suspended in RPMI 1640 medium at a concentration of 5×10^7 cells/ml. The standard HEPES buffer contained NaCl 145mmol/liter, KCl 5mmol/liter, $MgSO_4$ 1mmol/liter, $CaCl_2$ 1mmol/liter, glucose, 5mmol/liter, and HEPES, 10mmol/liter, at pH 7.4.

Measurement of $[Mg^{2+}]i$

A suspension of lymphocytes that contained approximately 5×10^7 cells/ml was loaded with mag-fura-2/acetoxymethyl ester, 2μmol/liter, (Molecular Probes, Eugene, OR, U.S.A.) together with 0.02% Pluronic F-127 (Molecular Probes) for 30 min at 37°C. Extracellular mag-fura-2/acetoxymethyl ester was removed by centrifugation at 2000g for 5 min at room temperature. The lymphocytes were resuspended at approximately 1×10^7 cells/ml in the HEPES buffer described above. These suspensions were re-incubated for 7 min at 37°C before fluorescence was recorded to ensure complete de-esterification of the mag-fura-2/acetoxymethyl ester. For measurements of the fluorescence, 2.5 ml aliquots of the cell suspension were transferred to a quartz cuvette maintained at 37°C in a spectrofluorometer (SPEX Fluorolog; SPEX Industries, Edison, NJ, U.S.A.). The fluorescence signals were monitored at 510nm with alternate excitation with UV light at 340 and 380nm. $[Mg^{2+}]i$ was calculated from the ratio of the fluorescence at the two excitation wavelengths according to the formula[28]:

$$[Mg^{2+}]i = K_D \times (R-R_{min.}/R_{max.}-R) \times (S_{f2}/S_{b2})$$

where the K_D (dissociation constant) was 1.5mmol/liter, R was the fluorescence ratio at the excitation wavelengths (340/380nm) of the samples, and $R_{max.}$ was the fluorescence ratio at the excitation wavelengths (340/380nm) under Mg^{2+}-saturated conditions. In the preliminary study, we confirmed that the fluorescence ratio under Ca^{2+}-saturated conditions was equal to that under Mg^{2+}-saturated conditions. $R_{min.}$ was the fluorescence ratio at the excitation wavelengths (340/380nm) with zero levels of Mg^{2+} and Ca^{2+}. S_{f2} and S_{b2} were the fluorescence intensities at 380nm for mag-fura-2 with zero Mg^{2+} and excess Mg^{2+}, respectively.

To determine $R_{max.}$, the cells were lysed with 50μmol/liter digitonin (Sigma Chemical Co., St. Louis, MO, U.S.A.) in the presence of 1mmol/liter Mg^{2+} and 1mmol/liter Ca^{2+}. $R_{min.}$ was then obtained by chelating Mg^{2+} and Ca^{2+} with 6mmol/liter EDTA and by adjustment of pH to 8.3 with 7mmol/liter Tris. We subtracted the autofluorescence of the unloaded lymphocytes, the test agents, and the medium from the measured values.

Because some leakage of mag-fura-2 occurs from the lymphocytes, it can form complexes with extracellular Mg^{2+} and Ca^{2+}[28]. Therefore, a correction for the extracellular leakage of mag-fura-2 is needed to avoid an overestimation of $[Mg^{2+}]i$ in the buffer containing Mg^{2+} and Ca^{2+}. A correction was performed using EDTA similar to the correction for extracellular fura-2 using EGTA[22]. To calculate the extracellular mag-fura-2, we determined the change in the fluorescence of mag-fura-2 after cheating the extracellular Mg^{2+} with 3mmol/liter EDTA at pH 7.4. A rapid drop in the fluorescence signal at 340nm after the addition of EDTA was considered to reflect the contribution of the extracellular leakage of mag-fura-2 from the cells. We also added EDTA after the lysis of the cells to estimate the total fluorescence associated with mag-fura-2. The ratio of fluorescence change after EDTA in a suspension of intact cells to that in the total dye was regarded as the percentage of the dye that leaked from the cells. Measurements were performed in duplicates. The intra-assay coefficient of variation for $[Mg^{2+}]i$ was 2.2%.

Measurement of $[Ca^{2+}]i$

To measure $[Ca^{2+}]i$, the lymphocytes were incu-

Table 1. Systolic blood pressure, heart rate, body weight, and serum creatinine and serum concentration of free Mg^{2+} and total Mg in the three rat strains.

Parameter	WKY/Izm (n=20)	SHR/Izm (n=20)	SHRSP/Izm (n=20)
Systolic BP, mmHg	117±3	157±6*	183±10* +
Heart rate, bpm	383±25	389±31	421±23* +
Body weight, g	234±12	220±6*	202±12* +
Serum creatinine, mmol/liter	0.019±0.006	0.019±0.005	0.017±0.005
Serum free Mg^{2+} concentration, mmol/liter	0.50±0.04	0.52±0.08	0.51±0.11
Serum total Mg concentration, mmol/liter	1.29±0.11	1.33±0.22	1.31±0.21

Values are expressed as means ± SD. * $p<0.05$ vs WKY/Izm; + $p<0.05$, SHRSP/Izm vs SHR/Izm.

bated with 2μmol/liter fura-2/acetoxymethyl ester (Molecular Probes, Eugene, OR, U.S.A.). Fluorescence was recorded at an emission wavelength of 510nm and excitation wavelengths of 340nm and 380nm. Corrections were applied for extracellular fura-2 leakage with 10mmol/liter EGTA[22] and for autofluorescence by subtracting the fluorescence of the unloaded lymphocytes. $R_{max.}$ was obtained by lysing the cells with 50μmol/liter digitonin in the presence of 1mmol/liter Ca^{2+} and 1mmol/liter Mg^{2+}. Ten mmol/liter EGTA was then added at pH 8.3 to obtain the $R_{min.}$ $[Ca^{2+}]i$ was calculated using a general formula[7]. Concanavalin A (Sigma Chemical Co., St. Louis, MO, U.S.A.) was used as a lymphocyte agonist at concentrations of 30 and 100 μg/ml.

Concanavalin A induced lymphocyte proliferative responses

To assess the extent of mitogen-induced cell proliferation, the lymphocytes were cultured in the absence and the presence of varying doses of concanavalin A, 1.25–10 μg/ml, for 3 days at 37°C. Cells (2×10^5) in 1 ml of medium were placed in each well of a multi-well plate. During the last 16 h of culture, the lymphocytes were pulsed with 0.5 μCi/well of [^{3}H]thymidine. Cells were then harvested onto glass-fiber filter disks using a PHD cell harvester for the incorporated radioactivity, and the disks were counted using a scintillation counter.

Measurement of serum total Mg and serum free Mg^{2+}

A blood sample was obtained from the right atrium when the thoracic cavity was opened. The serum total Mg was measured by atomic absorption spectrophotometry. The serum ionized Mg^{2+} was measured by a Mg^{2+} ion-selective electrode (Nova 8; Nova Biomedical, Waltham, MA)[32].

Statistical analysis

Values are expressed as means ± SD. Data were analyzed with one-factor ANOVA followed by Fisher's protected least significant difference test as a multiple comparison procedure. The correlation between two variables was studied by the linear regression method and Speaman's rank order correlation test. A level of $p<0.05$ was considered statistically significant.

RESULTS

Systolic blood pressure was significantly higher in SHR/Izm than in WKY/Izm and higher in SHRSP/Izm than in SHR/Izm and WKY/Izm (Table 1). Body weight was highest in WKY/Izm and lowest in SHRSP/Izm.

SHRSP/Izm had a significantly higher heart rate than SHR/Izm and WKY/Izm. The serum creatinine levels measured by the alkaline picrate method did not differ significantly among the three strains.

Table 1 shows the serum total Mg concentration and serum free Mg^{2+} concentration. The serum free Mg^{2+}, an active fraction of Mg, was closely correlated with the serum total Mg concentration (Fig. 1: $R=0.554$, $p<0.05$). No significant differences were found in either the serum free Mg^{2+} concentration or the serum total Mg concentration among WKY/Izm, SHR/Izm, and SHRSP/Izm. No significant correlation was found between the systolic blood pressure and serum free Mg^{2+} concentration (Fig. 2: $R=0.004$) or serum total Mg concentration ($R=0.07$).

The intracellular concentrations of mag-fura-2 and fura-2 were similar in the three groups (Table 2). Thus, the lymphocytes were loaded with the dye to a similar extent. In addition, $R_{max.}$ and $R_{min.}$ of mag-fura-2 and fura-2 were similar in all the groups, indicating that the ester hydrolysis of dyes was similar in the three groups. Thus, we can safely compare the calculated values of $[Mg^{2+}]i$ and $[Ca^{2+}]i$.

Figure 3 shows the $[Mg^{2+}]i$ in lymphocytes from the three strains. The $[Mg^{2+}]i$ in SHR/Izm was significantly higher than in WKY/Izm (254±51 versus 201±36μmol/liter, $p<0.05$), but the $[Mg^{2+}]i$ in SHRSP/Izm (211±34μmol/liter) was at the same

level as in WKY/Izm. The $[Mg^{2+}]i$ in the lymphocytes did not correlate with the serum Mg^{2+} level or blood pressure in each group or in the combined three groups (serum Mg^{2+}: R=0.01, systolic blood pressure: R=0.06).

Figure 4 shows the resting level of $[Ca^{2+}]i$ in lymphocytes from the three strains. No significant differences were found in basal $[Ca^{2+}]i$ among WKY/Izm, SHR/Izm, and SHRSP/Izm (54.1±5.6, 52.9±2.9, and 49.4±4.5nmol/liter, respectively). Concanavalin A-stimulated $[Ca^{2+}]i$ rises in the absence and presence of extracellular Ca^{2+} are shown in Figures 5 and 6, respectively. The $[Ca^{2+}]i$ response to concanavalin A in the absence of extracellular Ca^{2+}, indicating a release from internal Ca^{2+} stores, was significantly less in SHRSP/Izm than in WKY/Izm and SHR/Izm (55.0±4.9, versus 62.0±8.2, and 59.2±3.9nmol/liter, respectively, $p<0.05$). No significant difference existed between WKY/Izm and SHR/Izm. The $[Ca^{2+}]i$ response to concanavalin A in the presence of extracellular Ca^{2+} was significantly less in

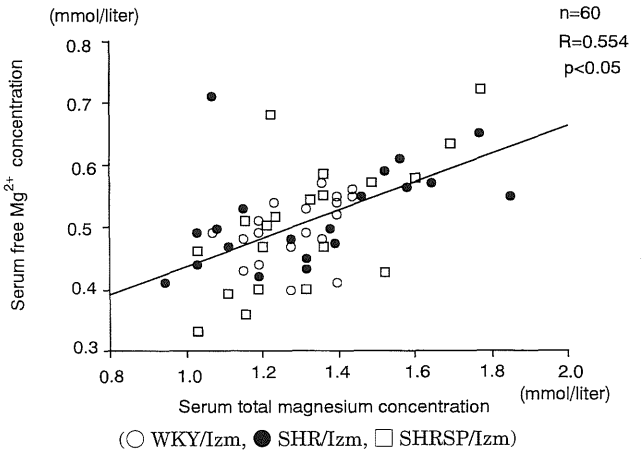

Fig. 1. Relationship between serum total magnesium concentration and serum free Mg^{2+} concentration.

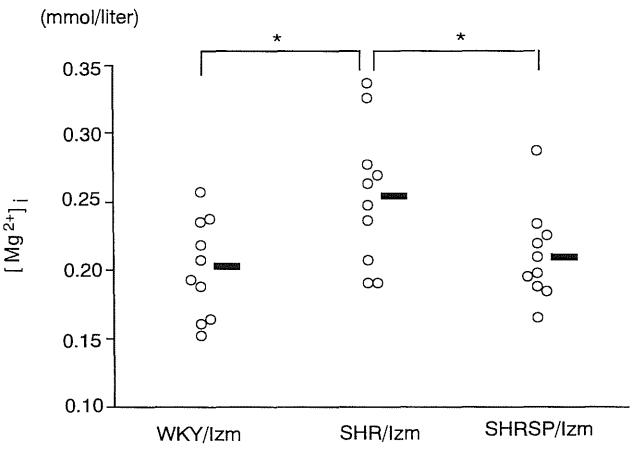

Fig. 3. Intracellular free Mg^{2+} concentration $[Mg^{2+}]i$ in the three strains.
*$p<0.05$ The bar indicates mean value of $[Mg^{2+}]i$ in each strain.

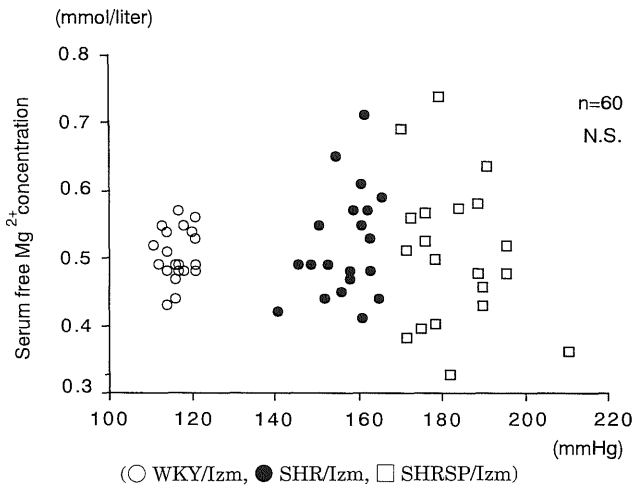

Fig. 2. Relationship between systolic blood pressure (SBP) and serum free Mg^{2+} concentration.

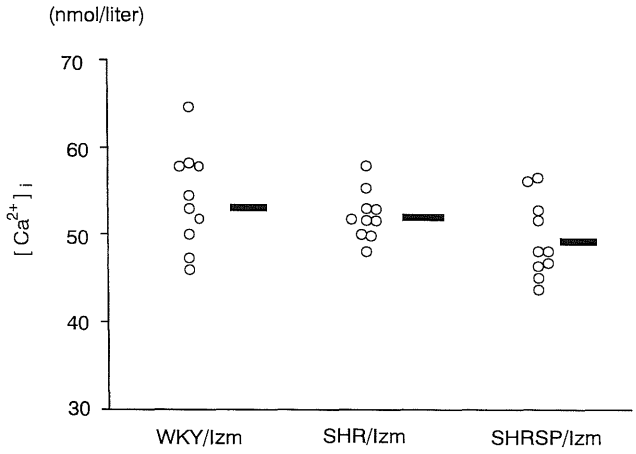

Fig. 4. Resting level of $[Ca^{2+}]i$ in lymphocytes in the three strains.

Table 2. Intracellular metabolism of mag-fura-2 and fura-2 in lymphocytes of the three rat strains.

Parameter	WKY/Izm	SHR/Izm	SHRSP/Izm
[mag-fura-2]i, μmol/liter	408±36	410±32	408±28
Rmax of mag-fura-2	33±4	34±3	33±3
Rmin of mag-fura-2	0.75±0.04	0.75±0.03	0.76±0.04
[fura-2]i, μmol/liter	510±44	516±40	520±38
Rmax of fura-2	34±6	36±6	34±4
Rmin of fura-2	0.83±0.04	0.82±0.03	0.80±0.04

[mag-fura-2]i: intracellular mag-fura-2 concentration [fura-2]i: intracellular fura-2 concentration

SHRSP/Izm than in WKY/Izm and SHR/Izm (81.5±6.8, versus 97.6±7.2, and 91.9±5.6nmol/liter, respectively, $p<0.05$). We assessed the concanavalin A-induced Ca^{2+} influx by subtraction of the concanavalin A-induced $[Ca^{2+}]i$ rises in the absence of extracellular Ca^{2+} from that in the presence of extracellular Ca^{2+}. The Ca^{2+} influx was significantly less in SHRSP/Izm than in WKY/Izm and SHR/Izm (26.5±9.2, versus 35.6±8.0 and 32.7±5.0nmol/liter, respectively, $p<0.05$).

Table 3 shows concanavalin A induced lymphocyte proliferative responses in the three strains.

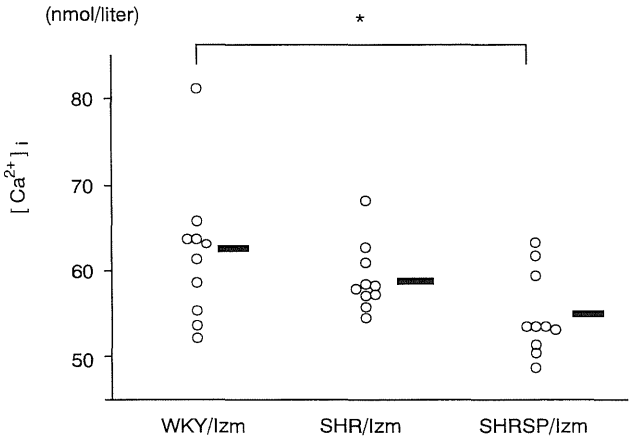

Fig. 5. $[Ca^{2+}]i$ response to concanavalin A in the absence of extracellular Ca^{2+}.

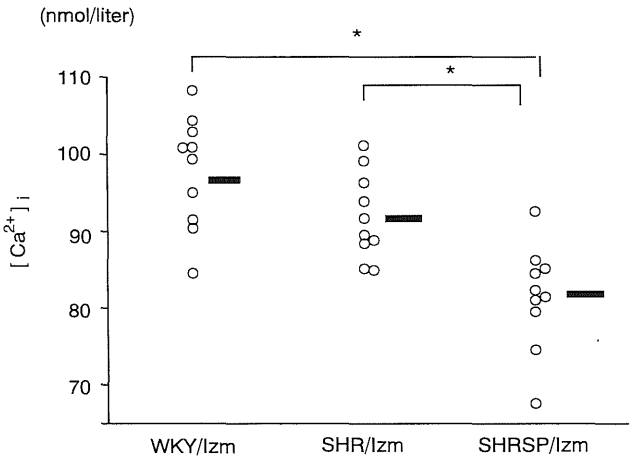

Fig. 6. $[Ca^{2+}]i$ response to concanavalin A in the presence of extracellular Ca^{2+}.

The proliferative responses were significantly less in both SHR/Izm and SHRSP/Izm than in WKY/Izm.

DISCUSSION

As Mg^{2+} is a necessary cofactor for many enzymes and signal transaction proteins and regulates ion transport, increasing attention has been directed to alterations in Mg homeostasis and its role in abnormal cellular processes. One could propose the hypothesis that Mg deficiency may be involved in the pathogenesis of hypertension. In the present study, both intracellular and extracellular free Mg^{2+} concentrations were investigated in two kinds of genetically hypertensive rats, SHR/Izm and SHRSP/Izm, in comparison with WKY/Izm as a normotensive control. Although it has been reported, as a possible important role of Mg^{2+} in vascular tone, that extracellular Mg^{2+} affects Ca^{2+} influx[6,46,47], there was no available data on the serum Mg^{2+} concentration in genetically hypertensive rats. Because ionized Mg^{2+} was an active fraction of Mg, not only the serum total Mg concentration but also the serum free Mg^{2+} concentration were measured by atomic absorption spectrophotometry and by a Mg^{2+} ion-selective electrode[32], respectively. The serum free Mg^{2+} concentration was closely correlated with the serum total Mg concentration. No significant difference was found in either the serum total Mg concentration or the serum free Mg^{2+} concentration among WKY/Izm, SHR/Izm, and SHRSP/Izm. No significant correlation was found between the serum free Mg^{2+} concentration and blood pressure. Therefore, our data cannot support the hypothesis that a reduction in extracellular Mg^{2+} might contribute to the development of hypertension in genetically hypertensive rats.

This study is the first report on thymic lymphocyte $[Mg^{2+}]i$ in genetically hypertensive rats. The $[Mg^{2+}]i$ in the lymphocytes was increased in SHR/Izm, and that in SHRSP/Izm was similar to WKY/Izm. These results are in contrast with the hypothesis that Mg deficiency in the intracellular space is involved in the pathophysiology of hypertension. In patients with essential hypertension, many investigators have reported a decrease in the intracellular total Mg content in the erythrocytes using atomic absorption spectrophotome-

Table 3. Concanavalin A induced lymphocyte proliferative responses in the three rat strains.

Concanavalin A	[^{3}H] thymidine uptake, counts/min		
μg/ml	WKY/Izm (n=10)	SHR/Izm (n=10)	SHRSP/Izm (n=10)
0	531±382	531±451	343±87
1.25	3236±2499	1693±1141	2101±1560
2.5	11477±7412	6553±5136	6457±4040
5.0	29614±8105	17563±8027*	19698±9247*
10.0	44892±17395	24850±13177*	28055±15895*

Values are expressed as means ± SD. * $p<0.05$ vs WKY/Izm

try[15,27,33], whereas a few investigators have reported unchanged[41] or increased[15] contents. However, free Mg^{2+} serves as a cofactor in a variety of intracellular enzymatic processes. The measurement of intracellular total Mg content may not always reflect the actual status of ionized Mg^{2+} in the intracellular space. In the platelets of hypertensive patients, the $[Mg^{2+}]i$, determined by mag-fura-2, was reported to be decreased[38] or increased[10]. In genetically hypertensive rats, there have been a relatively small number of reports concerning $[Mg^{2+}]i$. Previous studies about $[Mg^{2+}]i$ in SHR reported a decrease in the erythrocytes[16] and cardiomyocytes[12], or no difference in the thoracic aorta[11]. Differences in the technical approach adopted for measuring $[Mg^{2+}]i$ or cell selection may contribute to the discrepancy between previous reports[11,12,16] and the present results. In the present study, a fluorescent Mg^{2+} sensitive dye was used where previous investigators[11,12,16] used nuclear magnetic resonance in order to measure $[Mg^{2+}]i$. In addition, using mag-fura-2, the $[Mg^{2+}]i$ in the lymphocytes of patients with essential hypertension was also reported not to be decreased[4]. It is possible that the Mg^{2+} homeostasis may differ between T-lymphocytes and other cells. Indeed, we have reported that a Na^+/Mg^{2+} exchange regulates $[Mg^{2+}]i$ in both the erythrocytes and platelets[44] but not in the lymphocytes[35]. However, earlier studies reported that the level of Mg in the lymphocytes was a more accurate reflection of its levels in cardiac and skeletal muscle than was its level in the erythrocytes[34].

SHRSP/Izm, a substrain of SHR/Izm, spontaneously develop more severe hypertension than SHR/Izm and die of massive cerebral hemorrhage or infarction[18], and the systolic blood pressure in SHRSP/Izm was already highest at the age of 8 weeks among the three strains in this study. Therefore, if an abnormality observed in SHR/Izm is involved in the pathophysiology of hypertension, the abnormality should be augmented in SHRSP/Izm. Although the $[Mg^{2+}]i$ in SHR/Izm was significantly higher than in WKY/Izm in this study, the $[Mg^{2+}]i$ in the lymphocytes did not differ between WKY/Izm and SHRSP/Izm. Thus, one could speculate that an increase in $[Mg^{2+}]i$ may not be involved in the pathogenesis of genetic hypertension. Another interpretation is that increased $[Mg^{2+}]i$ may play a compensatory rather than causative role in high blood pressure in SHR/Izm. However, this compensatory mechanism may not work in SHRSP/Izm, resulting in more severe hypertension and stroke.

There is considerable evidence of abnormalities in the cellular metabolism of Na^+ and Ca^{2+} in hypertension. Blood cells frequently have been used for analysis of cellular cation metabolism because they are readily available and easy to handle. However, concern has been raised about using circulating blood cells because of their exposure to the mechanical shear forces in the circulatory system. Therefore, there is a possibility that abnormal cation metabolism in circulating cells taken from hypertensive rats could be secondary to high blood pressure. However, as thymocytes are non circulating cells that are shielded from circulating shear stresses, the findings in the present study may not result from disturbances induced by high blood pressure.

Both an increase of $[Ca^{2+}]i$ and abnormal Ca^{2+} handling have been reported in various cells of patients with essential hypertension and of genetically hypertensive rats[20–25]. However, in the present study, no significant difference was found in basal $[Ca^{2+}]i$ among WKY/Izm, SHR/Izm, and SHRSP/Izm. This result is consistent with a previous study that basal $[Ca^{2+}]i$ was increased in the platelets but unchanged in the thymocytes in SHR[23]. This may be due to the difference between different cell types. In this study both the Ca^{2+} release from internal Ca^{2+} stores and the Ca^{2+} influx induced by concanavalin A were significantly less in SHRSP/Izm than WKY/Izm, whereas no significant difference was found between SHR/Izm and WKY/Izm. The response of $[Ca^{2+}]i$ in platelets to thrombin was attenuated in SHRSP and enhanced in SHR[19]. Therefore, Ca^{2+} handling differs between SHR substrains. The increase in $[Ca^{2+}]i$ may not be obligatory in all cells of genetically hypertensive rats.

As recent studies have suggested that inflammation plays an important role in the pathophysiology of cardiovascular disease[36], and that lymphocytes are involved in the atherosclerotic process[8], several investigators have reported that alterations in immunologic function can contribute to the pathophysiology of hypertension[5]. In fact, in genetically hypertensive rats T-lymphocyte dysfunction has been demonstrated[5,26,37] and the restorations of T cell depression by the graft or removal of the thymus may delay the onset and attenuate the degree of hypertension[2,14]. In this study, an attenuation of lymphocyte proliferative responses to concanavalin A, a T-lymphocyte selective mitogen, was confirmed both in SHR/Izm and SHRSP/Izm. However, this T-lymphocyte abnormality in genetically hypertensive rats cannot be explained by alterations in the cellular homeostasis of Ca^{2+} and Mg^{2+}.

It has been reported that intracellular Mg^{2+} could have an affect on Ca^{2+} influx and Ca^{2+} release from intracellular stores[40,42,43,45]. In cardiac myocytes, intracellular Mg^{2+} may inhibit Ca^{2+} influx[40,42,43], whereas an increase in $[Mg^{2+}]i$ increases both the Ca^{2+} discharge from intracellular stores and the Ca^{2+} influx in vascular smooth muscle cells[45]. In SHR/Izm, Ca^{2+} handling was normal and $[Mg^{2+}]i$ was increased, and in SHRSP/Izm, the $[Ca^{2+}]i$ response was attenuated and $[Mg^{2+}]i$ was normal.

If $[Mg^{2+}]i$ is a major factor for regulating Ca^{2+} handling in T-lymphocytes, $[Mg^{2+}]i$ might increase the response of $[Ca^{2+}]i$.

In conclusion, neither extracellular Mg^{2+} nor intracellular Mg^{2+} was decreased in either SHR/Izm or SHRSP/Izm. Our results suggested that a deficiency of Mg^{2+} did not exist in genetically hypertensive rats. In addition, basal $[Ca^{2+}]i$ did not differ among the three strains, but both the Ca^{2+} release from internal Ca^{2+} stores and the Ca^{2+} influx induced by mitogen were significantly less in SHRSP/Izm, suggesting that an increase in $[Ca^{2+}]i$ is not obligatory in all cells of genetically hypertensive rats.

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