Effect of Magnesium Deficiency on Autonomic Circulatory Regulation in Conscious Rats

Yoshinobu Murasato, Yuji Harada, Masaharu Ikeda, Yasuhide Nakashima, Yoshiaki Hayashida

Abstract—A close relationship between magnesium and cardiovascular function has been reported; however, the effect of magnesium deficiency on autonomic cardiovascular regulation has not been clarified. We investigated the effect of magnesium deficiency on the autonomic regulation of oscillations of the R-R interval, arterial blood pressure (BP), and renal sympathetic nerve activity (RSNA) by using the maximum entropy method in conscious rats. Its effect on baroreflex control of RSNA and heart rate were also investigated with a logistic function curve. Mean BP in magnesium-deficient rats was higher than that in control rats (mean ± SE, 114.0 ± 4.3 versus 101.6 ± 3.4 mm Hg; P < 0.05), and urinary excretion of catecholamine was increased by 2.4-fold. The fraction of low-frequency oscillation of RSNA was reduced (31.7 ± 0.9% versus 36.2 ± 1.5%, P < 0.05) and the correlation between low-frequency oscillations of BP and RSNA was weakened in magnesium-deficient rats. There was no difference in high-frequency oscillation of the R-R interval, which is related to vagal tone, whereas sympathetic tone became dominant (square root of low-frequency/high-frequency ratio of R-R interval, 1.00 ± 0.05 versus 0.67 ± 0.05, P < 0.0001) in magnesium-deficient rats. The maximal gain in the BP–RSNA relation tended to be reduced in magnesium-deficient rats (−7.7 ± 1.1% versus −12.2 ± 1.9%/mm Hg, P = 0.07); however, that in the BP–heart rate relation was increased (−8.1 ± 0.7 versus −4.5 ± 0.5 bpm/mm Hg, P < 0.01). These results suggest that magnesium deficiency induces sympathetic excitation, which results in hypertension but attenuates the baroreflex-related response of sympathetic nerves, whereas magnesium deficiency enhances the sensitivity of the sinus node to autonomic regulation. (Hypertension. 1999;34:247-252.)

Key Words: magnesium deficiency • baroreflex • sympathetic nerve • autonomic nervous system • spectral analysis

Magnesium (Mg) is an essential element of circulatory regulation and is the most common intracellular cation after potassium.1–3 It has been reported that Mg plays a role in numerous enzymatic processes in the cardiovascular system, such as in kinases, cyclases, and adenosine triphosphatase or guanine triphosphatase;2 and is a naturally occurring antagonist to calcium.4 Thus, Mg affects the pivotal cardiac functions, including cardiac contraction,5 beating rhythm,6,7 vasomotor control,4,9–10 and proliferation of smooth muscle cells in vessels.9,11 Mg deficiency contributes to the pathogenesis of several cardiovascular diseases, eg, hypertension,12 vasospastic angina,13 ventricular arrhythmia,14 and mitral valve prolapse.15

Recently, Fiset et al16 reported a high incidence of sudden death in Mg-deficient rats, and this result is consistent with epidemiological reports of a high incidence of sudden death in areas with low levels of Mg in drinking water17 and also with clinical reports that hypomagnesemia is frequently observed in patients with heart failure in whom sudden death occurs.14,18 Animal studies have suggested that there are 2 mechanisms of sudden death related to Mg deficiency: arrhythmogenesis and coronary vasospasm.14,4,19 Moreover, the effect of Mg deficiency on the autonomic nervous system may act as a neurogenic trigger of a fatal event. Fiset et al16 reported that fatal arrhythmia occurred after seizures in Mg-deficient rats, whereas there was a small effect on repolarization of ventricular myocytes. The authors implied that the abnormal response of the autonomic nervous system to auditory stress led to a fatal event.

To assess autonomic nervous activity, oscillations of the R-R interval (RRI) and blood pressure (BP) have generally been investigated by spectral analysis.20–28 These oscillations consist of high-frequency (HF), low-frequency (LF), and very-low-frequency components. The HF component of RRI variability is believed to be mediated by cardiac parasympathetic tone, whereas the LF component is mediated by both cardiac sympathetic and parasympathetic tones, which are regulated by the baroreflex. Hence, the ratio of LF power to HF power (LF/HF) is an index of cardiac sympathovagal balance.20,24,26 The LF component of BP variability parallels baroreflex-related sympathetic nerve activity.21,26,27 It has also been reported that the baroreflex is a sensitive marker of sudden death and malignant ventricular arrhythmia.29

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However, the effect of Mg deficiency on the autonomic nervous system has not been clarified. In this study, we investigated the effect of Mg deficiency on the autonomic regulation of oscillations of RRI, BP, and renal sympathetic nerve activity (RSNA) using the maximum entropy method. Next, the correlations between baroreflex-related parameters and the LF component of RSNA variability were investigated. Finally, we investigated the changes in RSNA and heart rate (HR) in response to changes in BP induced by drug administration and analyzed the effect of Mg deficiency on baroreflex sensitivity.

**Methods**

**Animals and Diets**

This study was performed in accordance with the guidelines specified for institutional animal care and use of the University of Occupational and Environmental Health, Kitakyushu, Japan. Seventeen male Wistar rats, weighing 180 to 200 g, were housed in stainless-steel cages with a constant temperature (24°C), constant humidity (55%), and a daily 12-hour light/dark cycle. They were divided into 2 groups, the control group (n=8), and Mg-deficient (MGD) group (n=9), and fed a synthetic diet (Oriental Kobo) that contained either 3.79 mmol Mg/100 g (control diet) or 0.19 mmol Mg/100 g (MGD diet). The composition of the 2 diets were the same except for the amount of Mg. The MGD diet contained 39.7% corn starch, 20.0% casein, 13.2% α-corn starch, 10.0% sucrose, 7.0% soybean oil, 5.0% cellulose, 3.5% mineral mixture, 1.0% vitamin mixture, 0.3% l-cysteine, and 0.3% choline bitartrate. All rats were given deionized water.

**Urine Sampling**

At 3 weeks of dietary treatment, each rat was weighed and placed in another cage so that a 24-hour urine sample could be collected. Urine was collected for individual rats, and Na, K, and Cl were measured with an autoanalyzer (Hitachi 7170, Hitachi Seisakusho). One rat, which had a BUN nitrogen (BUN), and creatinine were measured with an autoanalyzer (Hitachi 7170, Hitachi Seisakusho). Nitrogen (BUN), and creatinine were measured with an autoanalyzer (Hitachi 7170, Hitachi Seisakusho). The neutral- and noradrenaline were measured by high-performance liquid chromatography (HPLC-725CA, Tosoh). The nitrate plus nitrite (NOx) concentration was determined with an autoanalyzer (TCL-NOx 1000, Tokyo Kasei Kogyo). The neutral- and noradrenaline were measured by high-performance liquid chromatography (HPLC-725CA, Tosoh). The nitrate plus nitrite (NOx) concentration was determined with an autoanalyzer (TCL-NOx 1000, Tokyo Kasei Kogyo). The neutral-

**Animal Preparation and Data Recording**

Two or 3 days after they were returned to their home cages, the rats were anesthetized with pentobarbital (50 mg/kg IP) and prepared for injections of phenylephrine (20 μg/kg) and nitroprusside (20 μg/kg) using a syringe pump (CFV-2100, Nihon Kohden) at a rate of 5 μL/s. The interval between administration of phenylephrine and nitroprusside was at least 10 minutes, during which MBP, HR, and RSNA returned to the baseline levels. To quantify the RSNA response percentage changes in the integrated RSNA during the experiment were calculated, taking the mean value before drug administration as 100%. Data for the MBP-RSNA and MBP-HR relations after phenylephrine and nitroprusside administration were fitted to a logistic functional curve with a logistic equation: RSNA or HR= P4+P1/(1+exp[P2(MBP−P3)]), where P1 is the range of RSNA or HR (maximum value−minimum value), P2 is the slope coefficient, P3 is MBP at the midrange of the curve, and P4 is the minimum RSNA or HR. Baroreflex sensitivity was defined as the maximum gain of the logistic function curve. The maximum gain was calculated as −P1×P2×P3/2.

**Spectral Analysis**

Data sampling and spectral analysis were performed by use of a modification of the method described previously. 2 The RRI, BP, and RSNA waveforms were replayed, and 210-second sections consisting of consecutive sinus beats were selected during the control periods. These sections were digitally sampled by an analog-to-digital converter every 30 ms, and RRI, BP, and RSNA were analyzed and stored on a microcomputer. Ten data sets of 1000 points that overlapped one-third of the preceding data set were processed on a computer system. The time series of data were analyzed by the maximal entropy method with high resolution (MemCalc, Suwa Trust). The magnitude of power was integrated in each band and then averaged within the group. The very-low-frequency band ranged between 0.034 and 0.27 Hz; the LF band, between 0.27 and 0.75 Hz; and the HF band, between 0.75 and 3.5 Hz. Each LF power and its square root, LF/P2 of BP and RRI variabilities and the normalized unit of LF power of RSNA variability were investigated to evaluate the baroreflex, where the normalized unit represents the valued power as a percentage of the total power. Because the voltages of RSNA showed a wide range (20 to 200 μV), it is not appropriate to compare the absolute power of RSNA variability, HF power and its square root, LP/P2 of RRI variability were assessed to evaluate parasympathetic nerve activity. LF/HF and its square root, (LP/HF)2/P2 of RRI variability were assessed to evaluate sympathovagal balance.

**Analysis of Baroreflex Function**

The analysis was performed by use of a modification of a method described in a previous report. 30 After the control period, the mean RSNA, BP, and HR were simultaneously converted into digital data every 2 ms. These digital values were averaged every 1 second and stored on a computer (DOS/V machine, software; Visual Designer, Intelligent Instrumentation). The relationship between mean BP (MBP) and RSNA and that between MBP and HR were investigated when BP was increased and decreased by alternating intravenous injections of phenylephrine (20 μg/kg) and nitroprusside (20 μg/kg) using a syringe pump (CPV-2100, Nihon Kohden) at a rate of 5 μL/s. The interval between administration of phenylephrine and nitroprusside was at least 10 minutes, during which MBP, HR, and RSNA returned to the baseline levels. To quantify the RSNA response percentage changes in the integrated RSNA during the experiment were calculated, taking the mean value before drug administration as 100%. Data for the MBP-RSNA and MBP-HR relations after phenylephrine and nitroprusside administration were fitted to a logistic functional curve with a logistic equation: RSNA or HR= P4+P1/(1+exp[P2(MBP−P3)]), where P1 is the range of RSNA or HR (maximum value−minimum value), P2 is the slope coefficient, P3 is MBP at the midrange of the curve, and P4 is the minimum RSNA or HR. Baroreflex sensitivity was defined as the maximum gain of the logistic function curve. The maximum gain was calculated as −P1×P2×P3/2.

**Blood Sampling**

At least 24 hours later, blood samples were obtained with an arterial catheter. Plasma electrolytes (Mg, Ca, Na, K, and Cl), blood urea nitrogen (BUN), and creatinine were measured with an autoanalyzer (Hitachi 7170, Hitachi Seisakusho). One rat, which had a BUN concentration of >21.4 mmol/L and a Mg concentration of 0.83 mmol/L, was excluded from the MGD group because of dehydration.

**Statistical Analysis**

All values are mean±SE. Statistical analysis was performed with StatView software (Abacus Concepts). Comparisons between the 2 groups were analyzed with an unpaired Student t test. Correlation coefficients were calculated and tested using Fisher’s z transformation. Significance was established at P<0.05.
Results

Baseline Characteristics

After 2 weeks, rats in the MGD group began to exhibit signs of hyperactivity, irritability, and a skin rash on the neck and face where their fur fell out. As shown in Table 1, growth retardation was observed in the MGD group. Baseline MBP in the MGD group was greater than that in the control group. There was no difference in mean HR. There was no difference in mean voltage of RSNA between LF 1/2 of BP variability and the normalized unit value (LF/HF) 1/2 was less than that for LF/HF.

Neurohumoral factors, NOx, and electrolytes in urine are shown as the amount excreted per kilogram of body weight (BW). Values in urine excretion were quantified as the amount excreted per kilogram of body weight (BW).

Spectral Analysis

Table 2 shows the mean and SE of parameters that reflect autonomic nervous activity in 70 data sets for each group. There was no difference in LF power or LF 1/2 of BP and RRI variabilities, whereas the normalized unit value of LF power of RSNA variability decreased in MGD group. There was no difference in HF power and HF 1/2, which are related to vagal activity. Both LF/HF and (LF/HF) 1/2 were increased in the MGD group, and the P value for (LF/HF) 1/2 was less than that for LF/HF.

As shown in Figure 1a, there was a positive correlation between LF 1/2 of BP variability and the normalized unit value.
of LF power of RSNA variability in each group (control, $r=0.80$, $P<0.0001$; MGD, $r=0.41$, $P=0.0004$). However, the $r$ value and its significance in the MGD group were less than those in the control group. As shown in Figure 1b, there was no correlation between the normalized unit value of LF power of RSNA variability and LF $^{1/2}$ of RRI variability. As shown in Figure 1c, there was a positive correlation between the normalized unit value of LF power of RSNA variability and (LF/HF) $^{1/2}$ of RRI variability in the MGD group ($r=0.44$, $P=0.0001$), whereas there was no correlation in the control group, in which (LF/HF) $^{1/2}$ of RRI variability remained $<1.6$.

**Analysis of Baroreflex Function**

**MBP-RSNA Relation**

The means and SEs of the 4 parameters of the logistic function curve are given in Figure 2a. There was a decrease in P1 and an increase in P3 in the MGD group. The maximal gain in the MGD group was smaller than that in the control group, but this difference was not statistically significant (MGD, $-7.7\pm1.1\%$/mm Hg; control, $-12.2\pm1.9\%$/mm Hg, $P=0.07$). Figure 2b shows the correlation curve derived from mean parameters P1 through P4. The curve in the MGD group shifted to the right (white arrow), and the range of RSNA was lower (black arrow) in the MGD group than in the control group.

**MBP-HR Relation**

The means and SEs of the 4 parameters are given in Figure 2c. Both P2 and maximal gain were increased in the MGD group. Figure 2d shows the correlation curve derived from mean parameters P1 through P4; note that the slope of the curve in the MGD group increased (white arrow).

**Discussion**

The most important finding of this study is that Mg deficiency causes discrepant baroreflex gains for sympathetic nerve activity and HR, ie, attenuation of RSNA and enhancement of HR. The spectral analysis revealed a decrease of the LF component of RSNA variability and loss of correlation between LF components of BP and RSNA variabilities in the MGD group. The blunted baroreflex control of sympathetic nerve activity might be caused by the diminished oscillatory capability of the sympathetic neural modulation under sympathetic overactivity. RRI variability exhibited a sympathetic dominance in proportion to the increase in the LF component of RSNA variability in the MGD group, suggesting an enhancement of the sensitivity of sinus node to sympathetic nerve activity.

**Neurohumoral Factors and NO Release**

Catecholamine secretion was increased in the MGD group (Table 1). This is consistent with previous reports that Mg deficiency caused an increase in noradrenaline release because of the increased level of serotonin$^{13}$ and the increased uptake of catecholamine at the terminal of the sympathetic nerves.$^{34}$ Clinically, hypomagnesemia and over-release of catecholamine are observed in patients with mitral valve prolapse, and catecholamine release is improved by Mg supplementation.$^{15}$ Mg deficiency induces hyperactivity, irritability, growth retardation, and skin lesions with itching,$^{16}$ all of which were observed in this study. These stressful conditions may also lead to a secondary increase in catecholamine secretion.

The lack of a difference in the serum level or excretion of Na and K (Table 1) suggests that the Na/K exchange system in the kidney, including the renin-angiotensin-aldosterone system, was maintained within the normal level in the MGD group. This is consistent with previous findings in Mg-deficient rats.$^{10}$ However, it has been clinically observed that plasma renin activity was enhanced in hypertensive patients with low serum Mg.$^{12}$ It has also been reported that hypokalemia often occurs in patients with moderate to severe Mg deficiency and that K is not repleted until Mg is administered concomitantly with K.$^{2}$ In this study, hypokalemia was observed concomitantly with hypomagnesemia (Table 1). Mg deficiency leads to secondary hypoparathyroidism because Mg is a cofactor of adenylate cyclase, which produces cAMP, which in turn is required for the exocytosis of parathyroid hormone.$^{35}$

NO release was enhanced in the MGD group (Table 1). Mak et al$^{16}$ also showed NO overproduction during Mg deficiency and that it participated in oxidative depletion of red blood cell glutathione. Because we did not evaluate the response of endothelium-derived NO but rather the total
amount of NO release in this study, the induction of inducible NO synthase in multiple skin lesions may have caused the increase in NOx. It has been reported that endothelium-dependent vasodilatation induced by acetylcholine is attenuated in the isolated artery of Mg-deficient animals. Because the present and previous results indicate that Mg deficiency does not impair NO synthesis, atherosclerotic change induced by Mg deficiency might attenuate the response of endothelium-derived NO to shear stress.

The present result that Mg-deficient rats exhibited hypertension coincides with results reported by Altura et al[7] but not with results of a study that showed no effect on BP. This discrepancy may be due to differences in methodology. In the latter report, the researchers used the tail-cuff method, which requires that the rats be restrained. The measured BP may be influenced by the stress induced by restraint. On the other hand, intraarterial pressure was measured directly with rats in an anesthetized state by Altura et al but not with a study that showed no effect on BP. This discrepancy may be due to differences in methodology. In the latter report, the researchers used the tail-cuff method, which requires that the rats be restrained. The measured BP may be influenced by the stress induced by restraint. On the other hand, intraarterial pressure was measured directly with rats in an anesthetized state by Altura et al and with rats in a conscious, unrestrained state in this study. The present study also showed that hypertension in Mg-deficient rats was not due to either the enhanced renin-angiotensin system or impairment of NO release but probably to excessive catecholamine release and an abnormal response of vessels to catecholamine.

Baroreflex

It has been reported that the LF oscillations of BP and RRI increase when sympathetic activity rises, such as with vasodilatation, tilting, and mental stress, and that LF oscillation of muscle sympathetic nerve activity increases when nitroprusside is administered in healthy human subjects. However, the present study showed that Mg deficiency increased catecholamine release (Table 1) but reduced LF oscillation in RSNA variability (Table 2) and weakened the correlation between LF oscillation in BP variability and in RSNA variability (Figure 1a). These results indicate that the high sympathetic drive caused by Mg deficiency blunted the baroreflex-related sympathetic oscillation in response to BP oscillation. This coincides with a report that LF oscillation of muscle sympathetic nerve activity was absent in patients with severe heart failure, who had high sympathetic tone. The blunted baroreflex-related response of RSNA strongly suggested that the sympathetic excitation caused by Mg deficiency was so tonic that it impaired LF oscillation. Another possible mechanism may include the central effect of excessive catecholamine release.

These results obtained by spectral analysis also coincide with results of direct evaluation of the MBP-RSNA relation, ie, Mg deficiency caused a decrease in the range of RSNA, whereas maximal gain tended to decrease (Figure 2a and 2b). This indicates that sympathetic excitation was saturated because it reached its ceiling and reduced its LF oscillation in MGD group. Blunted baroreflex sensitivity in the MBP-RSNA relation has been reported under high sympathetic tone, such as in heart failure and in hypertensive animals. Blockade of angiotensin II receptor or brain ouabain prevented impairment of the baroreflex in heart failure. Hirakawa et al[30] showed that both RSNA and baroreflex sensitivity increased in proportion to arterial CO₂ during hypoxia, suggesting an interaction between the baroreflex and chemoreflex. Although there is no report available concerning the effect of Mg deficiency on brain ouabain, angiotensin, or the interaction between the baroreflex and chemoreflex, excessive catecholamine release might influence them centrally.

Mg deficiency did not significantly affect LF power of BP or RRI variability (Table 2). Akselrod et al[31] reported a decrease in LF oscillation of BP in spontaneously hypertensive rats, which have high sympathetic tone, and suggested the saturation of alpha-receptors. LF oscillation of RRI was decreased in patients with heart failure, and the mechanism was thought to be limitation of the sinus node responsiveness to high sympathetic drive and beta-receptor downregulation. Because Mg deficiency increased calcium influx into myocytes and vascular smooth muscle cells and enhanced the responsiveness to sympathetic excitation, LF oscillations of BP and RRI were maintained regardless of the low LF oscillation of sympathetic activity in this study.

The present results obtained by direct evaluation of the MBP-HR relation also showed that Mg deficiency caused an increase in maximal gain (Figure 2c and 2d), which is opposite of the results obtained for the MBP-RSNA relation (Figure 2a and 2b). This suggests that the responsiveness of the sinus node to autonomic regulation is enhanced despite the blunted response of sympathetic activity to a change in BP. One possible mechanism is that the increased calcium influx caused by Mg deficiency may lead to partial depolarization, enhanced automaticity, or increased sensitivity to catecholamine in the sinus node. Another mechanism may be the result of a modification of Na/K adenosine triphosphatase, whose cofactor is Mg. Thus, this may lead to an increase in intracellular sodium and thereby calcium by Na/Ca exchange. This suggests arrhythmogenic vulnerability in response to sympathetic excitation during Mg deficiency.

Sympathovagal Balance

With regard to RRI variability, the parameters that represented the sympathovagal balance were increased in the MGD group, whereas there was no difference in vagus nerve-related parameters between the 2 groups (Table 2). This suggests that sympathetic tone is elevated, because there was no change in vagal tone. We previously demonstrated that LF/HF decreased despite sympathetic excitation when both sympathetic and vagus nerves were activated during hypercapnic hypoxia. Thus, LF/HF does not always represent sympathetic tone but rather sympathovagal balance.

There was no correlation between LF of RRI variability and sympathetic tone in either group (Figure 1b), and the sympathovagal balance was maintained and independent of sympathetic tone in the control group (Figure 1c). Pagani et al[32] reported that there was a close correlation between the normalized unit value of the LF component in the variability of muscle sympathetic nerve activity and LF/HF or LF power of RRI variability in healthy human subjects. The difference between our results in the control group and theirs may be due to the fact that we examined the spontaneous oscillation of sympathetic tone for 30 seconds, whereas they studied the oscillation of 512 beats, probably for >5 minutes, induced by drug administration. Another explanation is the difference in cardiac innervation between species, because rats have a HR that is 5-fold higher than that in humans.
In the MGD group, the dominance of sympathetic tone was augmented in proportion to sympathetic excitation (Figure 1c). This suggests that the short-term stability of the sympato-thalamic balance was impaired by Mg deficiency. This may contribute to the increased gain in the MBP-HR relation.

In summary, Mg deficiency induces excessive catecholamine release and results in hypertension. High sympathetic tone induced by Mg deficiency impairs baroreflex control of sympathetic nerve and reduces its baroreflex-related oscillation. Mg deficiency augments baroreflex control of HR, suggesting a modulatory effect on the sinus node. The results suggest that Mg deficiency leads to a blunted baroreflex and enhances HR response to the stress.

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