Vascular function of MGH and MGL mice, two strains which differ by a genetic variation of magnesium metabolism

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Abstract. Mg deficiency is considered as a risk factor of cardiovascular disorders like hypertension and atherosclerosis. MGH and MGL mice, selected for high and low Mg status, are animal models which present variations of Mg metabolism of genetic origin. The cardiovascular functions of these mice have never been studied. In this study, the arterial blood pressure of MGH and MGL strains was measured by plethysmography. Morphology and reactivity to vasoconstrictor agents were also investigated by a pressurized and perfused system in mesenteric resistance artery. It is shown that: (1) MGH mice presented a higher plasma Mg concentration than MGL; (2) arterial blood pressure and heart rates were similar between the two groups; (3) media thickness, media cross-sectional area, and internal and external diameters were smaller in pressurized mesenteric resistance arteries from MGH mice than in those from MGL mice; (4) the vasoconstriction induced by vasopressin (but not norepinephrine) was higher in the mesenteric arteries from MGH mice than in those from MGL ones. In summary, MGH mice as compared to MGL mice present differences in arterial geometry and higher reactivity to vasopressin without repercussions on arterial blood pressure. The real repercussion of these observations on the cardiovascular system of the MGH and MGL models is at present unknown. More experiments are needed to clarify the influence of differences in Mg metabolism of genetic origin on cardiovascular function.

Key words: magnesium, blood pressure, genetics, mouse

For 40 years, numerous epidemiological, clinical and experimental studies have shown a relationship between magnesium (Mg), metabolism disorders and different pathological states. Mg seems to be particularly linked to cardiovascular disorders [1, 2] and the role of Mg in the pathogenesis and treatment of hypertension is receiving increased attention [3, 4]. There is an inverse correlation between plasma Mg concentration and blood pressure [5]. In rats, Mg deficiency results in an increase of blood pressure [6-9]. Hypomagnesaemia and decreased tissue content of Mg have also been demonstrated in various experimental models of hypertension, especially in spontaneously hypertensive rats (SHR) [10]. Moreover, in this model, an oral Mg supplementation attenuates blood pressure elevation [11, 12], but it seems to depend on the age of the animals [13].
Recent dietary surveys have shown that the average Mg intake in western countries is often below the Recommended Dietary allowances (RDA = 6 mg/kg/day) [14, 15]. In fact, in developed countries, the consumption of dietary sources rich in Mg, like legumes, leafy green vegetables or cereals has been declining since the beginning of the 20th century. Although this “nutritional deficit” is the principal origin of Mg deficiency in western populations, there are some inter-individual differences of Mg homeostasis which have a genetic origin [16-18]. This hypothesis was subsequently confirmed by study of mono and dizygotic twins [19] and by the study of different inbred strains in the mouse [16]. The genetic system involved in this control is certainly polygenic and presents a large polymorphism. This hypothesis is consistent with the significant correlation found by Henrotte et al. [16, 17] between blood Mg level and human (HLA) or mouse (H-2) tissues antigens, two antigenic systems coded by the major histocompatibility complex (MHC), one of the most polymorphic genetic systems described to date. The same association is also found between MHC or non MHC genes and levels of Mg in different tissues [17].

To further investigate the mechanism and the biological significance of the variations of Mg metabolism of genetic origin, Henrotte et al. [20] selected MGL (Mg Low) and MGH (Mg High) mice. These two strains present some genetic differences in Mg homeostasis: MGH mice have higher red blood cell and plasma Mg concentrations than MGL mice [21-25].

The purpose of the present work was to examine some cardiovascular hemodynamic and mechanical factors and vascular reactivity to agonists in MGH and MGL mice.

Material and methods

Mice
MGL and MGH male mice aged 8 weeks were used: (INRA, Clermont-Ferrand Theix, France). These strains were obtained from a bidirectional selective breeding which had been carried out for 18 generations, from a heterogenous outbred population constituted of F2 segregant hybrid between 4 inbred strains: C57BL/6, DBA/2, C3H/EB, AKR [20].

Upon arrival, the mice were housed in stainless steel cages at a constant temperature of 22 ± 2°C and a daily 12-h light/dark cycle. Food (regular mouse food A04, UAR, France) and distilled water were distributed ad libitum. Before starting the experiments, one week of adaptation was observed.

All experiments were performed according to the Institute Ethics Committee, in accordance with decree no. 87-848.

Blood pressure and heart rate measurements
Systolic blood pressure and heart rate were non invasively measured in 8 unanaesthetized and prewarmed mice from each group by the tail-cuff method, using a piezoelectric transducer (Pulse sensor, Kent Scientific, Torrington, CT, USA) connected to a Maclab/8s system and Chart v. 3/4 software (PHYMEP, Campo Formio, France), as previously described [26].

Animals followed a training to get used to the protocol over 15 days. Then, 10 measures of blood pressure and heart rate were performed and the mean of each parameter was calculated.

Mg determination
Nine mice from each group were anaesthetized by an intraperitoneal injection of sodium pentobarbital (60 mg/kg). A maximum amount of blood was collected at the abdominal aorta level and immediately centrifuged at 4 000 g for 10 minutes at 4°C. Plasma was then drawn and frozen at - 80°C until analysis. Moreover, the kidneys and heart were dissected, weighed and frozen at - 80°C.

Samples of organs were defrosted, weighed then dried at 105°C during 28 hours. They were weighed again and dissolved in nitric acid (65%) at 80-120°C until total dissolution. Solutions were preserved at 4°C until analysis. Mg determinations were carried out by atomic absorption spectrometry (Perkin Elmer 3300, Norwalk, USA), with a range prepared from a standard solution (Merck standard, Darmstadt, Germany) and with an appropriate dilution for each matrix in a solution of lanthanum oxide (0.1%).

Study of mesenteric arteries

Dissection and artery settings
Five mice from each group were killed by neck elongation and the first mesenteric handle was removed and placed in cold physiological salt solutions (PSS) of the following compositions (mmol/L): NaCl, 120; NaHCO3, 25; KCl, 4.7; KH2PO4, 1.2; MgSO4, 1.2; CaCl2, 1.6; glucose, 11; ethylenediaminetetraacetic acid (EDTA), 0.026. One segment of the third-order branch of a mesenteric artery was used per mouse and was carefully dissected and cleaned of all adherent connective tissues under a dissecting microscope.

An arterial segment (2-3 mm in length) was slipped onto two glass microcannulae in a servo-controlled
pressurized flow chamber [27] which contained PSS. PSS was bubbled with 95% O₂–5% CO₂ and maintained at 37°C as previously described [28]. One cannula was fixed, whereas the other could be positioned as appropriate. Both ends of the arterial segment were secured to the microcannula with nylon ties. The axial length of the arterial segment was adjusted by carefully moving the cannula until the vascular walls were parallel without any stretch. Intraluminal flow was initiated using a peristaltic pump (Living system instrumentation, Burlington, USA). Preheated and pregassed PSS was perfused to maintain an intraluminal pressure equal to 40 mmHg. This pressure was chosen in preliminary studies since arterial segments elicited a maximal decrease in lumen diameter with 10⁻⁴ mol/L norepinephrine when exposed to this intravascular pressure (data not shown). After intravascular pressure and flow had been established, the arterial segments were checked for leaks, which were identified by a reduction in the preset intraluminal pressure. The arterial segments were then equilibrated for 1 h.

**Experimental protocol**

Morphological measurements (external diameter, lumen diameter, media thickness) in vessels were made from the transillumination image with a microcomputer-based video imaging system at 4 points along a portion of each vessel, and the mean value was calculated. The media cross-sectional area (CSA) was calculated by subtraction of the luminal cross-sectional area from total cross-sectional area: CSA = π(D_e²-D_l²)/4, where D_e was the external diameter and D_l was the lumen diameter of blood vessel. Then, the viability of the segment was evaluated. An arterial segment was considered viable if it constricted and developed tone in response to an extraluminal application of 10⁻⁴ mol/L norepinephrine in PSS and then to an extraluminal of high potassium PSS (NaCl, 1.8; NaHCO₃, 25; KCl, 100; KI, 1.2; MgSO₄, 1.2; CaCl₂, 1.6; glucose, 11; EDTA, 0.026). The integrity of the vascular endothelium was verified by dilation in response to an extraluminal application of acetylcholine (1⁻⁴ mol/L) in PSS containing 10⁻⁴ mol/L norepinephrine. After each activation, the arterial segment was perfused with PSS and allowed to regain its resting diameter. Finally, cumulative concentration-response curves to norepinephrine (3⁻¹⁸ – 3⁻¹⁵ mol/L) and vasopressin (1⁻¹⁰ – 3⁻¹⁰⁻⁷ mol/L) were performed by extraluminal application of the drugs. The mesenteric artery was stimulated at each concentration until the maximal decrease in lumen diameter was obtained. The maximal contraction (E_max) induced by the two vaso-active agents was expressed as the greatest percentage decrease in lumen diameter: E_max = 100 (D_r

**Data and statistical analysis**

All values indicated in the tables, figures and texts are presented as means ± SEM. Statistical evaluation of the data was performed by unpaired Student t-test (GraphPad Instat, GraphPad Software Inc).

**Results**

**Mg concentrations, blood pressure and heart rate**

In plasma, the Mg concentration was significantly higher in MGH than in MGL mice (table 1). The Mg level in kidney was also higher in the MGH group than in the MGL group; however, it was not significantly different between these two groups for the heart (table 1). Blood pressure and heart rates were similar between the two groups (table 2).

**Table 1. Magnesium concentration in plasma and dried organs of MGH and MGL mice.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>MGH</th>
<th>MGL</th>
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<tbody>
<tr>
<td>Plasma (nmol/L)</td>
<td>0.79 ± 0.02</td>
<td>0.68 ± 0.03**</td>
</tr>
<tr>
<td>Heart (µmol/g)</td>
<td>45.67 ± 1.35</td>
<td>45.78 ± 1.07</td>
</tr>
<tr>
<td>Kidney (µmol/g)</td>
<td>24.34 ± 0.36</td>
<td>22.91 ± 0.40*</td>
</tr>
</tbody>
</table>

Means ± SEM of 9 animals per group. * p < 0.05, ** p < 0.01 – significant differences between groups.

**Table 2. Arterial pressure and heart rate of MGH and MGL mice.**

<table>
<thead>
<tr>
<th></th>
<th>MGH</th>
<th>MGL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial pressure (mmHg)</td>
<td>127 ± 7</td>
<td>115 ± 10</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>590 ± 14</td>
<td>627 ± 24</td>
</tr>
</tbody>
</table>

Means ± SEM of 8 animals per group. Differences between groups were not significantly different p > 0.05.
Mesenteric arteries study

Mesenteric artery morphologies
MGH mice presented some significant differences as compared with the MGL group, i.e. smaller media thickness, smaller lumen diameter and external diameter and smaller media cross-sectional area (CSA). The media/lumen ratio was no different between the two groups (table 3).

Norepinephrine-induced constriction
Extra-luminally applied norepinephrine induced a concentration-dependent constriction of the isolated mesenteric arteries (figure 1A). The constriction (higher maximal response E\textsubscript{max}: 54.40 ± 4.30% for MGL and 54.23 ± 2.97% for MGH) and the sensitivity (pD\textsubscript{2}) (5.59 ± 0.09 for MGL and 5.67 ± 0.05 for MGH) were not significantly different between the two studied strains.

Vasopressin-induced constriction
Extra-luminally applied vasopressin induced a concentration-dependent constriction of the isolated mesenteric arteries (figure 1B). The constriction was significantly greater in MGH than in MGL mice. The E\textsubscript{max} to vasopressin was significantly higher in MGH than in MGL mice (39.33 ± 4.80% for MGL vs. 55.56 ± 3.65% for MGH, p < 0.05) whereas the pD\textsubscript{2} value tended to be higher in MGH than in MGL mice (pD\textsubscript{2}: 8.73 ± 0.04 for MGH and 8.50 ± 0.10 for MGL, p < 0.06).

Discussion
The metabolic variation between MGH and MGL mice was accompanied by modifications of the vascular morphology of mesenteric resistance arteries and higher vasopressin-induced vasoconstriction. Despite these differences, the arterial blood pressure was similar between the two groups. The MGL mice had a lower Mg plasma concentration than MGH mice. Mg status cannot be only determined with magnesemia. 1% of total Mg of the body is located in plasma, while the rest is found in organs (45%) and bones (54%) [29]. A high proportion of blood Mg is also located in erythrocytes [30]. We have previously shown that MGH mice also presented a higher Mg concentration in the kidney. This difference for the kidney was also observed in the present work. In addition, previous studies [21, 22, 25] showed less Mg urinary excretion in the MGH strain as compared to MGL ones.

In the present study, blood pressure was not different between the MGH and MGL mice. Some authors have already studied the effect of hypermagnesemia on blood pressure, with some epidemiological studies showing that a diet rich in Mg is inversely correlated to a decrease in blood pressure in normotensive subjects [31]. In the same way, Altura et al. [32] reviewed some epidemiological studies which

Table 3. Morphologic parameters of pressurized mesenteric resistances arteries of MGH and MGL mice.

<table>
<thead>
<tr>
<th></th>
<th>Weight (g)</th>
<th>Media thickness (µm)</th>
<th>Lumen diameter (µm)</th>
<th>Vessel diameter (µm)</th>
<th>Media/lumen ratio (%)</th>
<th>CSA (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGH</td>
<td>32 ± 1</td>
<td>7.4 ± 0.4</td>
<td>151.0 ± 5.9</td>
<td>165.9 ± 5.8</td>
<td>9.95 ± 0.80</td>
<td>3693 ± 230</td>
</tr>
<tr>
<td>MGL</td>
<td>39 ± 1</td>
<td>9.4 ± 0.5*</td>
<td>200.9 ± 11.8**</td>
<td>219.6 ± 12.7**</td>
<td>9.35 ± 0.27</td>
<td>6283 ± 720**</td>
</tr>
</tbody>
</table>

Means ± SEM of 5 animals per group. * p < 0.05, ** p < 0.01 – significant differences between groups.
showed that a diet rich in Mg decreased the frequency of hypertensive diseases. In animal models of hypertension, oral supplementation of Mg results in a decrease in the elevation of blood pressure in secondary hypertension (DOCA-salt hypertension) and in SHRs [12, 33]. However, in normotensive rats, oral Mg supplementation has no effect on blood pressure [34]. In the present study we have shown no relationship between magnesemia (genetically determined) and blood pressure.

Knowing that catecholamines are important in the regulation of blood pressure, the absence of altered vascular reactivity to norepinephrine in the isolated and perfused mesenteric arteries between MGH and MGL supports the fact that the vascular contractile function is not related to the genetically determined Mg status in these mice. In a same way, it has been demonstrated that increasing blood pressure is associated with an increase of the media/lumen ratio [35]. In our study, the media/lumen ratio was not different between these two strains. All these findings seem indicate that MGH and MGL mice present no alteration of their cardiovascular functions despite the well-known cardiovascular effects of Mg.

Some differences, however, appear between MGH and MGL mice in vasopressin-induced vasoconstriction and the morphology of mesenteric arteries. In fact, the effect of vasopressin was more important in the mesenteric arteries from MGH mice than in those from MGL mice. The difference between MGH and MGL mice lies in the maximum effect \( (E_{\text{max}}) \) and not in the sensitivity \( (pD_2) \). This may be a consequence in the differences of Mg metabolism between MGH and MGL mice. However, the variations of response to vasopressin have no consequence on the arterial blood pressure. The real impact of this observation on the cardiovascular system of these mice is still unknown. \( \text{In vitro} \), high extracellular Mg levels decrease vasopressin-induced contraction in rat aorta [36]. In resistant mesenteric arteries, however, high extracellular Mg concentration decreases vasopressin-induced vasoconstriction in hypertensive but not normotensive rats [28].

The mesenteric arteries of MGH mice present morphological differences as compared to MGL mice, with a smaller lumen and total diameter of the vessel, a smaller media thickness and cross-sectional area. Because blood pressure was measured at only one point and this experiment was performed in young mice, the real impact of this vascular remodelling on the cardiovascular system between these mice strains is actually unknown. During the aging process, blood pressure and cardiovascular function change, with an increase of the risks of cardiovascular diseases, like atherosclerosis and hypertension [37]. Mg may protect against these pathologies by preventing the structural modifications of the arteries observed during cardiovascular diseases (i.e., increase of the vessel diameter or media thickness) or by an anticalcic action [32]. Because MGH mice present less important vascular remodeling, they might be protected against atherosclerosis and hypertension during aging. Some other experiments in these strains are expected to clarify this theoretical hypothesis.

In conclusion, our study focuses on the vascular function of MGH and MGL mice, models of genetic variations of magnesium status. The two strains present some variations in the morphology and reactivity of the mesenteric arteries which are not associated with changes in blood pressure levels. More studies in MGH and MGL strains are needed to characterize the cardiovascular functions of this model, and the effect of the variations of Mg metabolism of a genetic origin.

References


