

Journal of Clinical and Basic Cardiology

An Independent International Scientific Journal



Journal of Clinical and Basic Cardiology 1999; 2 (1), 120-122

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Intracellular Mg^{++} concentrations in vascular smooth muscle cells and heart muscle cells in spontaneously hypertensive rats

K. Kisters, E. R. Krefting¹, G. Westermann, N. Senninger², K. H. Rahn, K. H. Dietl²

Decreased intracellular Mg^{++} concentrations seem to be involved in the pathogenesis of primary hypertension. Of special interest is the smooth muscle cell with its electrolyte metabolism in primary hypertension, but also heart muscle cells and their Mg^{++} concentrations are of growing interest. Therefore in aortic smooth muscle cells and striated heart muscle cells (left ventricle) from 10 spontaneously hypertensive rats (SHR) of the Muenster strain and 10 normotensive Wistar-Kyoto rats (WKY), the intracellular Mg^{++} content was measured. Electron-probe X-ray microanalysis technique was used to determine intracellular Mg^{++} concentrations in aortic cryosections 3 μ m thick and striated heart muscle cells 4 μ m thick (Camsan CS 24 apparatus). Vascular smooth muscle Mg^{++} content was 0.90 ± 0.15 g/kg dry weight in SHR versus 1.15 ± 0.10 g/kg dry weight in WKY ($p < 0.05$). In striated heart muscle cell Mg^{++} concentrations, there was no significant difference in SHR and WKY (81.3 ± 6.7 versus 79.0 ± 8.3 mmol/kg dry weight).

In conclusion the present study has revealed that genetic hypertension in the spontaneously hypertensive rat is accompanied by decreased intracellular Mg^{++} concentrations in vascular smooth muscle cells. These results are similar to those obtained in human blood cells. This Mg^{++} decrease may be due to transmembrane defects or disturbed Mg^{++} transport systems, eg, Mg^{++}/Na^{+} antiport or Mg^{++}/Ca^{++} exchange. In striated heart muscle cells, Mg^{++} content was not significantly different in SHR and WKY. As described earlier, free Mg^{++} content in hypertensive heart muscle cells is decreased, total heart muscle Mg^{++} content as shown here being not significantly different, a transport defect in hypertensive heart muscle cells has to be assumed.

In conclusion, Mg^{++} handling is different in vascular smooth muscle and striated heart muscle cells in WKY and SHR ($p < 0.01$). *J Clin Basic Cardiol* 1999; 2: 120–2.

Key words: Mg^{++} , hypertension, vascular smooth muscle cells, heart muscle cells, SHR

Changes in Mg^{++} metabolism have been implicated in the pathogenesis of hypertension [1–5]. Whereas plasma Mg^{++} concentrations have often been investigated, comparatively few data on intracellular Mg^{++} concentrations are available [6, 7]. Furthermore, the role of intracellular Mg^{++} content in essential hypertension is also controversially discussed [8–11]. Decreased intracellular free Mg^{++} concentrations in erythrocytes of essential hypertensive patients have been described [12], whereas other authors were unable to confirm these findings concerning total erythrocytic Mg^{++} concentrations [13–15]. These different results in intracellular Mg^{++} content in essential hypertension may be due to various analytical techniques. Most of the results showing a decreased cellular magnesium ion content in primary hypertension were obtained from blood cells, but only sparse data exist concerning intracellular total magnesium ion concentrations in intact vascular smooth muscle cells or intact striated heart muscle cells in hypertension [6, 16, 17]. Therefore, it was of interest to develop a method to determine total Mg^{++} content in intact vascular smooth muscle and intact striated heart muscle cells of spontaneously hypertensive and normotensive rats.

Methods

We used aortae and left ventricle from 10 SHR and 10 WKY (systolic pressure 117.3 ± 6.1 mmHg, means $\pm$ SD) aged 9 months. The SHR had reached a systolic blood pressure of 192.5 ± 10.5 mmHg at this age. The dry heart (g) to body weight (kg) ratio of the SHR was greater than that for the WKY rats (1.7 ± 0.2 versus 1.1 ± 0.2 , $p < 0.01$) indicating significant hypertrophy of the SHR hearts.

For determination of intracellular magnesium, smooth muscle cells from abdominal rat aorta and striated heart muscle cells of the left ventricle were investigated.

The aortae and the left ventricle were freed of surrounding connective tissue and immediately frozen in liquid propane cooled with liquid nitrogen at a temperature of about -190°C . Then, cryosections with a thickness of 3 μ m for aortae (Figure 1) and 4 μ m for heart muscle cells (Figure 2) were made and thereafter lyophilized. Then, magnesium measurements were performed in each sample by electronprobe microanalysis technique as described earlier [18, 19].

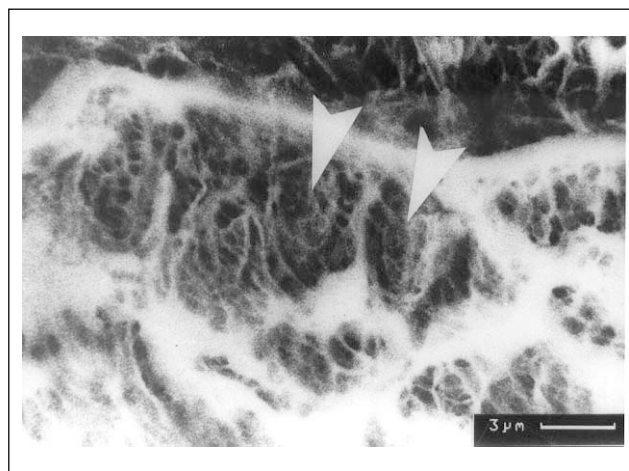


Figure 1. Cryosection of rat aorta of about 3 μ m length showing vascular smooth muscle cells (white arrows)

Received December 17th, 1998; accepted: December 23rd, 1998.

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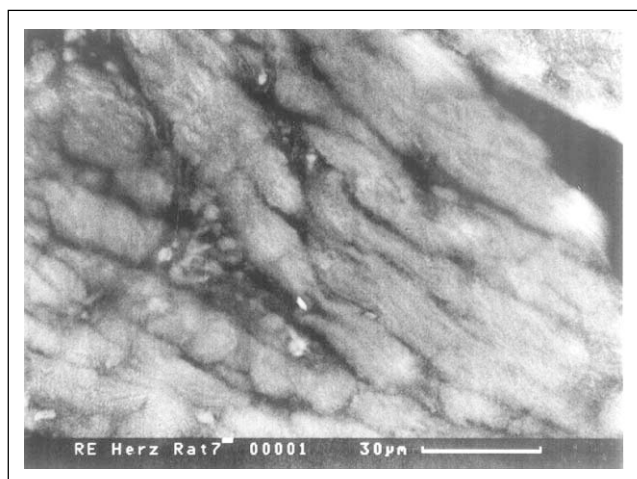


Figure 2. Cryosection of the left ventricle from a spontaneously hypertensive rat showing striated muscle cells (about 4 μm length)

Briefly, for the electronprobe microanalysis an electron microscope with X-ray detector system was used (Camsan CS 24 apparatus). When the electrons of the incoming beam strike an atom in the specimen they can knock an electron out of the kernel. If this hole is in an inner shell, it is filled with an electron of a higher shell, and a X-ray photon with discrete energy corresponding to the difference between the two atomic shells is emitted simultaneously. The energy of these X-rays is characteristic for each element. For quantification, the continuum method developed by Hall was used [20, 21].

Intracellular sites of measurements were identified by the morphology obtained by electron microscopy, and by simultaneous measurements of sulphur and phosphorus, the concentrations of which were markedly elevated in the intracellular compared with the extracellular space.

In each aorta and left ventricle mean values of at least 5 intracellular measurements at different sites were calculated. All sites were within smooth or striated muscle cells. The magnification was $5 \times 10,000$ so that the intracellular organelles could be identified. For the magnesium measurements only sites within the cytoplasm were chosen.

Mg^{++} content was then expressed in mmol/kg or g/kg dry weight of the tissue. Data are means $\pm$ SD. P values below 0.05 were considered to be significant. Statistical analysis was performed by ANOVA.

Results

Vascular smooth muscle Mg^{++} content was 0.90 ± 0.15 g/kg dry weight in SHR versus 1.15 ± 0.10 g/kg dry weight in WKY (Figure 3, $p < 0.05$). In total striated heart muscle cell Mg^{++} concentrations, there was no significant difference between SHR and WKY (81.3 ± 6.7 versus 79.0 ± 8.3 mmol/kg dry weight). All values are means $\pm$ SD.

There was no correlation between smooth muscle cell magnesium content or striated heart muscle cell magnesium concentrations and blood pressure values in the normotensive or spontaneously hypertensive rats.

Furthermore, there was a significant increase in heart muscle to body weight ratio in SHR versus WKY rats ($p < 0.01$).

In addition no correlation between intracellular Mg^{++} concentrations, blood pressure values or total heart muscle weight was found. Intracellular Mg^{++} handling is different in vascular smooth muscle cells and striated heart muscle cells in WKY and SHR ($p < 0.01$).

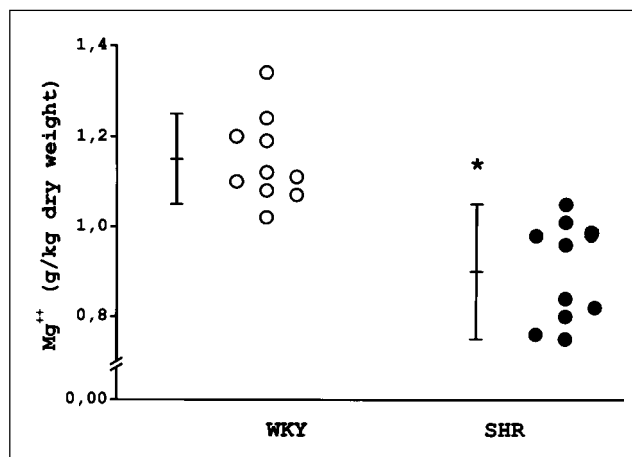


Figure 3. Intracellular magnesium ion content in aortic smooth muscle cells from normotensive rats (WKY) and SHR (means $\pm$ SD, $p < 0.05$)

Discussion

To assess intracellular Mg^{++} stores appropriately still remains difficult. Red blood cells are not a generally accepted indicator of cellular Mg^{++} stores. In human studies, only blood cells can routinely be used to measure intracellular Mg^{++} concentrations. Mg^{++} measurements in lymphocytes and platelets are complicated by the fact that the volume of these cells is difficult to assess. On the other hand, measurements of cytosolic free Mg^{++} by the fluorescent dye mag fura II circumvent this difficulty, but can only be done in fresh material, thus obviating the possibility to measure a greater number of stored samples.

A role for cellular Mg^{++} concentration in vascular tone has been postulated in hypertension [1–5, 10]. In essential hypertensives Resnick et al. found decreased intracellular free Mg^{++} concentrations in red blood cells as estimated by nuclear magnetic resonance spectroscopy [12].

Analogous findings were reported in the spontaneously hypertensive rat [6, 10, 22, 23].

Whereas from investigations in blood cells, a magnesium deficiency in primary hypertension seems likely, comparatively few data exist on intracellular electrolyte concentrations in vascular smooth muscle cells or in striated heart muscle cells [6, 7, 16, 17].

An intracellular Mg^{++} deficiency and, possibly, a defect in intracellular Mg^{++} transport could play a pathogenetic role in the development of primary hypertension [6]. On the basis of experimental data the mechanisms underlying the Mg^{++} induced vasodilation may be:

- a modification of the response to vasopressor hormones, and
- an interaction with cellular Ca^{++} handling [2].

These possible mechanisms are supported by 3 lines of recent evidence.

First, the extracellular Mg^{++} concentration can influence Ca^{++} metabolism of vascular smooth muscle by changing the Ca^{++} influx through the plasma membrane. Recently, in single myocytes from frog ventricle the site of interaction between Mg^{++} and Ca^{++} was identified as the Ca^{++} inward current that is dependent on phosphorylation by cyclic adenosine monophosphatase [24].

Second, changes in the extracellular Mg^{++} concentration induced inverse changes in the Ca^{++} content of vascular smooth muscle and in exchangeable Ca^{++} [25, 26].

Third, a decrease in the intracellular free Mg⁺⁺ concentration results in diminished membrane Na⁺, K⁺ adenosine triphosphatase and Ca⁺⁺ ATPase activities [27], and, as a corollary, increased Na⁺-Ca⁺⁺ exchange and increased intracellular Na⁺ and Ca⁺⁺ concentrations [28–30].

The results obtained in our study show significantly lower total intracellular Mg⁺⁺ concentrations in vascular smooth muscle cells of SHR as compared to WKY ($p < 0.05$). The findings are similar to those in red blood cells of essential hypertensives or in spontaneously hypertensive rats [31, 32].

As the present results show, a cellular magnesium deficiency in vascular smooth muscle cells seems likely in the development and pathogenesis of primary hypertension, although the present results cannot evaluate the relative roles of the possible mechanisms.

As described earlier, free magnesium ion content in hypertensive striated heart muscle cells is decreased, total heart muscle magnesium content as shown in the presented study here was not found significantly different. For these reasons, magnesium handling seems to be different in smooth muscle and striated heart muscle cells, due to eg, different transport mechanisms or transmembrane defects ($p < 0.01$).

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft for supporting this study with a research grant.

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