# Age dependent decreased intracellular $Mg^{++}$ concentrations in vascular smooth muscle cells of the abdominal aorta from normotensive (WKY) and spontaneously hypertensive rats (SHR)

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## Zusammenfassung

Es deuten viele Untersuchungen an menschlichen und tierischen Blutzellen darauf hin, daß erniedrigte $Mg^{++}$-Konzentrationen zumindest bei einer Untergruppe von Hypertonikern von pathogenetischer Bedeutung zu sein scheinen. Hinsichtlich glatter Gefäßmuskelzellen liegen jedoch weniger detaillierte Befunde vor. Daher war es von Interesse, intrazelluläre $Mg^{++}$-Konzentrationen in glatten Gefäßmuskelzellen der abdominellen Aorta von 10 spontan hypertonen Ratten (SHR) im Alter von 1 Monat, 3 Monaten und 8–10 Monaten im Vergleich mit je 10 altersgleichen normotensiven Ratten (WKY) zu messen.

Die Messungen wurden mittels Elektronenmikroskop und der Röntgenmikrostrahlanalysetechnik in 3 µm dicken Schnittpräparaten der Aorta durchgeführt.

Die intrazellulären $Mg^{++}$-Konzentrationen betrugen 42 ± 8 mmol/kg Trockengewicht in der SHR-Gruppe versus 44 ± 7 mmol/kg TG bei WKY im Alter von 1 Monat, 47 ± 13 mmol/kg TG bei SHR versus 48 ± 19 mmol/kg TG bei WKY im Alter von 3 Monaten und 37 ± 6 mmol/kg TG bei SHR versus 47 ± 4 mmol/kg TG bei WKY im Alter von 8–10 Monaten ($p < 0,05$).

Glatte Gefäßmuskelzellen von SHR-Tieren zeigen altersabhängig statistisch signifikant erniedrigte $Mg^{++}$-Spiegel im Vergleich zu altersgleichen WKY-Tieren. Diese Beobachtung deutet auf genetisch bedingte Störungen membranöser $Mg^{++}$-Transportsysteme hin.

Zusätzlich zeigte eine Untergruppe junger SHR-Tiere im Alter von 1 Monat und von 3 Monaten, dem Manifestationsalter der Hypertonie bei der SHR, bereits erniedrigte $Mg^{++}$-Konzentrationen in glatten Gefäßmuskelzellen.

Zusammenfassend sind die Beobachtungen der $Mg^{++}$-Veränderungen glatter Gefäßmuskelzellen bei SHR-Tieren analog zu früheren Untersuchungen an Blutzellen essentieller Hypertoniker.



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## Summary

Whereas in blood cells decreased magnesium concentrations in essential hypertension have often been described, only sparse data exist on intracellular magnesium content and exchange in vascular smooth muscle cells.

Therefore in aortic smooth muscle cells from 10 spontaneously hypertensive rats (SHR) of the Münster strain and 10 normotensive Wistar-Kyoto rats (WKY) aged 1 and 3 and 8–10 months, the intracellular magnesium content was measured.

Electron-probe X-ray microanalysis was used to determine intracellular $Mg^{++}$ concentrations in aortic cryosections 3 µm thick. The magnesium ion content was 42 ± 8 mmol/kg dry weight in SHR versus 44 ± 7 mmol/kg dry weight in WKY aged 1 month, 47 ± 13 mmol/kg dry weight in SHR versus 48 ± 19 mmol/kg dry weight in WKY aged 3 months and 37 ± 6 mmol/kg dry weight in SHR versus 47 ± 4 mmol/kg dry weight in WKY aged 8-10 months ($p < 0.05$).

Aortic smooth muscle cells from SHR are characterized by a significantly lowered intracellular magnesium ion content in 8–10 months old animals as compared with aged matched normotensive animals. The results may be due to genetically determined disturbances in transmembrane magnesium transport systems. Additionally, a subgroup of young SHR exists showing low intracellular $Mg^{++}$ concentrations with an age of 1 and 3 months, when hypertension becomes established.

In conclusion, cellular magnesium handling may be disturbed in SHR aortic smooth muscle cells, analogously to alterations in human blood cells from essential hypertensive patients.


## Introduction

Various nutritional factors may be implicated in the development of primary hypertension. An excess of sodium intake and a defective calcium intake are both directly correlated with blood pressure values in the general population [1, 2]. In the last 25 years, a greater number of studies showing different abnormalities in transmembrane movements of $Na^{+}$ and $Ca^{++}$ ions in essential hypertensive patients and genetically spontaneously hypertensive rats have contributed to a better understanding of the relations between these cation imbalances and essential hypertension [3–10].

Another nutritional factor recently implicated in the pathogenesis of some forms of essential hypertension is a possible defect in magnesium intake [11]. Epidemiological studies have shown an inverse relation between $Mg^{++}$ intake and blood pressure values [12, 13]. In a large population study, *Witteman* et al. reported a significantly decreased risk of developing hypertension when $Mg^{++}$ intake was above 300 mg daily [14]. Moreover, some clinical studies have also shown that oral $Mg^{++}$ supplements may reduce blood pressure values and enhance the hypotensive effect of some antihypertensive drugs [15, 16], while other autors were unable to confirm these findings. From ten recent clinical trials a lack of response of blood pressure to a magnesium supplementation was concluded [17]. The importance of magnesium in blood pressure regulation still remains an open question. Magnesium supplementation in large

dose may sometimes lower blood pressure but this can be due to a diuretic effect.

The $Mg^{++}$ deficiency hypothesis in essential hypertension has been studied both at extracellular and intracellular levels. At the extracellular level, serum $Mg^{++}$ values have been reported higher, lower, or unchanged in hypertensive patients compared with normotensive subjects [18–22]. Nevertheless, several investigators have found a decreased intracellular $Mg^{++}$ content in erythrocytes from essential hypertensive patients and animals [21, 23–29]. One of the most important mechanisms contributing to intracellular $Mg^{++}$ homeostasis is a $Na^{+}$-dependent $Mg^{++}$ efflux through the plasmalemmal membrane. This mechanism was recently described by *Feray* and *Garay* in human and rat erythrocytes [30–32]. Evidence for a $Mg^{++}/Na^{+}$ exchanger has been obtained in giant squid axon, and the existance of a similar mechanism in liver cells, thymocytes, and myocardiocytes has been suggested [33–37].

## Materials and methods

We used aortae from 10 SHR and 10 WKY (systolic pressure 116.4 ± 6.2 mmHg, mean ± s.d.) aged 8–10 months. The SHR had reached a systolic pressure of 190.4 ± 10.1 mmHg at this age ($p < 0.01$). Additionally 10 SHR (systolic pressure 125.2 ± 11.4 mmHg) and 10 WKY (systolic pressure 115.0 ± 5.8 mmHg) aged 1 month and 10 SHR (systolic pressure 140.2 ± 16.0 mmHg) and 10 WKY (systolic pressure 114.5 ± 5.4 mmHg) aged 3 months were studied. Feed for the entire study was from the same production lot that provided 0.5 mg Mg/g (15–20 g/d) diet and pure water. The aorta were freed of surrounding connective tissue and immediately frozen in liquid propane cooled with liquid nitrogen at a temperature of about −190°C, nearly avoiding electrolyte shifts after the sample was obtained. Then cryosections with a thickness of 3 µm were made and lyophilized. For the electronprobe microanalysis, an electron microscope with an X-ray detector system is used [38,39]. 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 an X-ray photon with a 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* [40] was used. Intracellular sites of measurement were identified: (1.) by the morphology obtained by electron microscopy, and (2.) 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, mean values of at least five intracellular measurements at 5 different sites were calculated. All sites were within smooth muscle cells. The magnification was 5 x 10000, so that organelles could be identified.

For the $Mg^{++}$ measurements only sites within the cytoplasm were chosen. The $Mg^{++}$ content was expressed in mmol/kg dry weight of the tissue.

All values are expressed as median and s. d. Statistical evaluations were made by means of the Friedman test (nonparametric analysis of variance for repeated measurements), using the Bonferroni adjustment.

A P-value of less than 0.05 was accepted to indicate statistical significance.

## Results

In 1 month old SHR intracellular $Mg^{++}$ content was 42 ± 8 mmol/kg dry weight versus 44 ± 7 mmol/kg dry weight in age matched WKY. In 3 months old animals the intracellular $Mg^{++}$ content in SHR was 47 ± 13 mmol/kg dry weight compared with 48 ± 19 mmol/kg dry weight in WKY (means ± s.d.). Vascular smooth muscle cell $Mg^{++}$ content in 8–10 months old SHR was measured 37 ± 6 mmol/kg dry weight versus 47 ± 4 mmol/kg dry weight in age matched WKY ($p < 0.05$) (figure 1 and 2).

The coefficients of variation for 5 measurements of intracellular $Mg^{++}$ content in aortic smooth muscle cells of one animal were 19.6% of the mean value in SHR and 13.4% in WKY. The between assay coefficient of variation of cellular $Mg^{++}$ from 5 different aortic smooth muscle cell measurements of one animal were 4.5% of the mean value in SHR and 4.2% in WKY.

Additionally, there was no correlation between intracellular $Mg^{++}$ concentrations and blood pressure values in WKY and SHR vascular smooth muscle cells aged 1 or 3 or 8–10 months.

## Discussion

A role for intracellular $Mg^{++}$ concentrations in vascular tone has been postulated in primary hypertension [41–47].

In essential hypertensives, *Resnick* et al. found decreased intracellular free $Mg^{++}$ concentrations in red blood cells as estimated by nuclear magnetic resonance spectroscopy [22–25]. Analogous findings were reported in red blood cells from spontaneously hypertensive rats [48].

On the basis of experimental data, the theoretical mechanisms underlying the $Mg^{++}$-induced vasodilation may be: (a) a modification of the response to vasopressor hormones, and (b) an interaction with cellular $Ca^{++}$ handling [41]. 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 phosphoylation by cyclic adenosine monophosphatase [49]. Second, changes in the extracellular $Mg^{++}$ concentration induced inverse changes in the $Ca^{++}$ content

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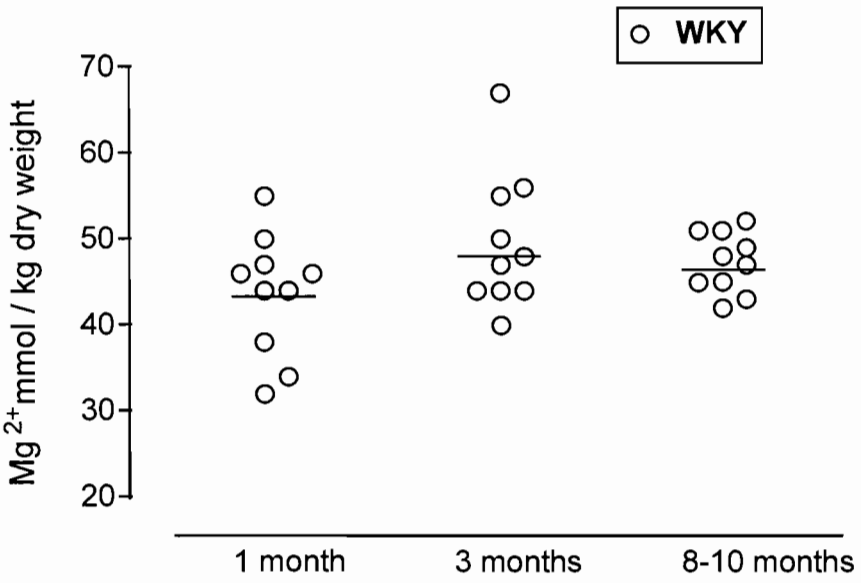


Fig. 1.: $Mg^{++}$ content in vascular smooth muscle cells of 10 normotensive rats (WKY) aged 1, 3 and 8 – 10 months (means ± s.d.).

of vascular smooth muscle and in exchangeable $Ca^{++}$ [50,51]. Third, a decrease in the intracellular free $Mg^{++}$ concentration results in diminished membrane $Na^+$, $K^-$-adenosine triphosphatase and $Ca^{++}$ ATPase activities [52], and, as a corollary, increased $Na^+$-$Ca^{++}$ exchange and increased intracellular $Na^+$ and $Ca^{++}$ concentrations [52].

The results obtained in this study show significantly lowered total intracellular $Mg^{++}$ concentrations in vascular smooth muscle cells of SHR as compared to WKY aged 8 – 10 months ($p < 0.05$). The findings are similar to those in red blood cells of essential hypertensives or in spontaneously hypertensive rats. Another aspect is that magnesium is bound for a large part, mainly to phosphate-containing molecules, including ATP, therefore influencing phosphate or ATP metabolism. The plasma phosphate level is lower in SHR than in WKY, and defects in regulation of the mitochondrial ATP synthase have also been described in SHR [53].

After the recognition of a $Mg^{++}$ efflux system in erythrocytes, it was suggested that patients with essential hypertension could have increased activity of this $Mg^{++}/Na^+$ exchange transportsystem, which could explain the reduced intraerythrocytic $Mg^{++}$ content [30–32]. This hypothesis was investigated by *Günther* et al., who found no differences between a small group of essential hypertensive patients and normotensive subjects [34, 35].

Nevertheless, in a larger group of essential hypertensive patients, *Picado* et al. found an acceleration of the $Mg^{++}/Na^+$ exchanger, with almost half of the patients showing values of this transport system higher than the normal upper limit of normotensive control subjects [10]. The kinetic conditions for the assay of the $V_{max}$ of $Mg^{++}/Na^+$ exchanger have been previously examined by *Feray* and *Garay* [30]. These authors measured $Mg^{++}$ efflux as a function of intracellular $Mg^{++}$ and extracellular $Na^+$ and found that $Mg^{++}$ efflux was saturable and followed Michaelis-Menten kinetics. The apparent Km was 2.6 mmol/L cell for intracellular $Mg^{++}$ and 20.5 mmol/L for extracellular $Na^+$. The coupling of the $Mg^{++}$ extrusion to the $Na^+$ influx has been suggested by *Günther* et al., who found a correlation between $Na^+$ influx and $Mg^{++}$ efflux in chicken erythrocytes [54].

In conclusion, lowered $Mg^{++}$ concentrations in vascular smooth muscle cells in a subgroup of SHR may contribute to the development of primary hypertension. Additionally, this $Mg^{++}$ deficiency becomes well established with increasing age in SHR. Similar to investigations in human blood cells a subgroup of essential hypertensives exists, showing a $Mg^{++}$ deficiency being involved in the pathogenesis of human hypertension.

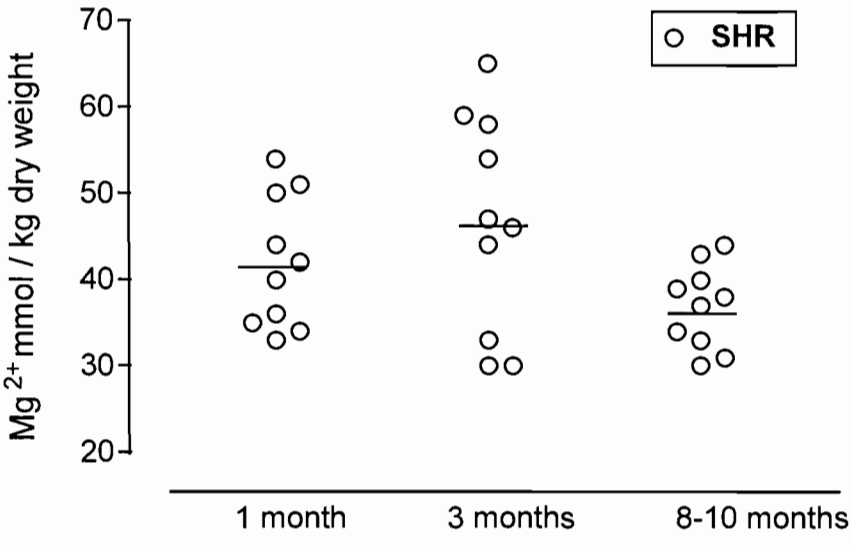


Fig. 2.: $Mg^{++}$ content in vascular smooth muscle cells of 10 spontaneously hypertensive rats (SHR) aged 1, 3 and 8 – 10 months (means ± s.d.).

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