Increased Na\(^+\) and decreased Mg\(^{2+}\) intracellular concentrations in vascular smooth muscle cells from spontaneously hypertensive rats

Klaus KISTERS, Ernst-Rudolf KREFTING*, Claus SPIEKER, Walter ZIDEK†, Karl Heinz DIETL‡, Michael BARENBROCK and Karl Heinz RAHN

Medizinische Universitäts-Poliklinik, Albert-Schweitzer-Str. 33, D-48149 Münster, Germany, *Institut für Medizinische Physik der Universität Münster, Münster, Germany, †Chirurgische Universitätsklinik, Waldegerstr. 1, D-48129 Münster, Germany, and ‡Medizinische Universitätsklinik Bochum, Bochum, Germany

A B S T R A C T

1. Although in blood cells decreased magnesium concentrations and increased sodium concentrations in essential hypertension have often been described, only sparse data exist on cellular magnesium or sodium content and exchange in vascular smooth muscle cells.

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

3. Electron-probe X-ray microanalysis was used to determine intracellular Mg\(^{2+}\) and Na\(^+\) concentrations in aortic cryosections 3 \(\mu\)m thick. The Mg\(^{2+}\) content was 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 (\(P < 0.05\)). Vascular smooth muscle Na\(^+\) content was 283 ± 59 mmol/kg dry weight in WKY and 402 ± 123 mmol/kg dry weight in SHR aged 3 months (\(P < 0.05\)), and 289 ± 17 mmol/kg dry weight in WKY versus 548 ± 39 mmol/kg dry weight in SHR aged 8–10 months (\(P < 0.05\)).

4. Aortic smooth muscle cells from SHR are characterized by a markedly lower intracellular Mg\(^{2+}\) content in 8–10-month-old animals and increased Na\(^+\) concentrations compared with normotensive cells in 3- and 8–10-month-old rats. The results may be due to genetically determined disturbances in transmembrane Mg\(^{2+}\) and Na\(^+\) transport. Cellular magnesium and sodium handling may be disturbed in SHR aortic smooth muscle as it is in hypertensive blood cells. In addition, it is concluded that vascular smooth muscle cell Mg\(^{2+}\)–Na\(^+\) exchanger can be altered in a subgroup of SHR.

INTRODUCTION

There is important evidence that various nutritional factors may be implicated in the development of essential 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\(^{2+}\) ions in patients with essential hypertension and rats with genetically spontaneously hypertension 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$^{2+}$ intake and blood pressure values [12,13]. In a large population study, Witteman et al. [14] reported a significantly decreased risk of developing hypertension when Mg$^{2+}$ intake was above 300 mg daily. Moreover, some clinical studies have also shown that oral Mg$^{2+}$ supplements may reduce blood pressure values and enhance the hypotensive effect of some antihypertensive drugs [15,16], while others were unable to confirm these findings. From 10 recent clinical trials, Laragh and Brenner [17] concluded an overall lack of response of blood pressure to a magnesium supplementation. The importance of magnesium in blood pressure regulation still remains an open question. Magnesium supplementation in a large dose may sometimes lower blood pressure but this can be due to a diuretic effect.

The Mg$^{2+}$ deficiency hypothesis in essential hypertension has been studied both at extracellular and intracellular levels. At the extracellular level, serum Mg$^{2+}$ values have been reported to be higher, lower or unchanged in hypertensive patients compared with normotensive subjects [18–22]. Nevertheless, several investigators have found a decreased intracellular Mg$^{2+}$ content in erythrocytes from patients and animals with essential hypertension [21,23–29].

One of the most important mechanisms contributing to intracellular Mg$^{2+}$ homeostasis is a Na$^+$-dependent Mg$^{2+}$ efflux through the plasmalemmal membrane. This mechanism has been described by Feray and Garay [30–32] in human and rat erythrocytes. Evidence for a Mg$^{2+}$–Na$^+$ exchanger has been obtained in giant squid axon, and the existence of a similar mechanism in liver cells, thymocytes and myocardiocytes has been suggested [33–36].

The increased Na$^+$ concentration in different hypertensive cells has been previously attributed to a reduced Na$^+$–K$^+$–ATPase activity, an increased activity of the Na$^+$–H$^+$-exchanger and a Na$^+$–Li$^+$-countertransport activity [37].

**Methods**

**Experimental design**

We used aortas from 10 spontaneously hypertensive rats (SHR) and 10 Wistar–Kyoto rats (WKY), aged 8–10 months. At this age the systolic pressure of the SHR was $190.4 \pm 10.1$ mmHg ($P < 0.01$) and that of the WKY was $116.4 \pm 6.2$ mmHg.

Additionally, 10 SHR (systolic pressure $140.2 \pm 16.0$ mmHg) and 10 WKY (systolic pressure $114.5 \pm 5.4$ mmHg), aged 3 months, were studied.

**Analytical procedures**

The aorta were freed of surrounding connective tissue and immediately frozen in liquid propane cooled with liquid nitrogen at a temperature of about $-190 \degree C$, nearly avoiding electrolyte shifts after the sample was obtained. Next, cryosections with a thickness of 3 µm were made and lyophilized. For the electron-probe microanalysis, an electron microscope with an X-ray detector system was 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.

The Hall equation: $P_x = c_x n_x dI$

where $P_x$ = characteristic radiation, $c = \text{concentration, } n = \text{atoms/cm}^2$, $d = \text{thickness of the probe and } I = \text{stream of incoming electrons}.$

Intracellular sites of measurement were identified: (i) by the morphology obtained by electron microscopy, and (ii) 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 five different sites were calculated. All sites were within smooth muscle cells. The magnification was $5 \times 10000$, so that organelles could be identified.

For the Mg$^{2+}$ and Na$^+$ measurements only sites within the cytoplasm were chosen. The Mg$^{2+}$ and Na$^+$ content was expressed as mmol/kg dry weight of the tissue.

All values are expressed as means and S.D. Statistical evaluations were made by means of the Friedman test (non-parametric 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 3-month-old animals the intracellular Mg$^{2+}$ content in SHR was $47 \pm 13$ mmol/kg dry weight compared with $48 \pm 19$ mmol/kg dry weight in WKY (means ± S.D.) (Figure 1). Vascular smooth muscle cell Mg$^{2+}$ content in 8–10-month-old SHR was $37 \pm 6$ mmol/kg dry weight compared with $47 \pm 4$ mmol/kg dry weight inagematched WKY ($P < 0.05$) (Figure 1). The coefficients of variation for five measurements of intracellular Mg$^{2+}$ content in aortic smooth muscle cells of one animal were $19.8\%$ of the mean value in SHR and $13.2\%$ in WKY. The inter-assay coefficients of variation of cellular Mg$^{2+}$
from five different aortic smooth muscle cell measurements in one animal were 4.6% of the mean value in SHR and 4.4% in WKY.

The Na concentration of vascular smooth muscle cells of 3-month-old WKY was 283 ± 59 mmol/kg dry weight. In age-matched SHR the cellular Na content in vascular smooth muscle cells was significantly increased (402 ± 123 mmol/kg dry weight, means ± S.D., P < 0.05) (Figure 2). The Na concentrations of 8–10-month-old animals were 289 ± 17 mmol/kg dry weight in WKY and 548 ± 39 mmol/kg dry weight in SHR (P < 0.05) (Figure 2).

The coefficients of variation for five measurements of intracellular Na content in aortic smooth muscle cells of one animal were 20.5% of the mean value in SHR and 12.4% in WKY.

The inter-assay coefficient of variation of intracellular Na in five measurements of aortic smooth muscle cell in one animal were 3.8% of the mean value in SHR and 3.3% in WKY.

There was no correlation between cellular Mg and Na concentrations in WKY and SHR vascular smooth muscle cells aged 3 or 8–10 months.

The Mg²⁺/Na⁺ ratio in 10 WKY aged 8–10 months was 0.17 ± 0.05 compared with 0.06 ± 0.08 in age-matched SHR (P < 0.001)

**DISCUSSION**

A role for intracellular Na and Mg²⁺ concentrations in vascular tone has been postulated in primary hypertension [9,10,37,41–44]. However, Na handling in vascular tissues from SHR is still discussed separately, probably due to different analytical procedures [45–47].

In essential hypertension, Resnick et al. [22–25] found decreased intracellular free Mg²⁺ concentrations in erythrocytes as estimated by NMR spectroscopy. Analogous findings were reported in erythrocytes from SHR [48].

On the basis of experimental data, the theoretical mechanisms underlying the Mg²⁺-induced vasodilatation may be: (i) a modification of the response to vasopressor hormones, and (ii) an interaction with cellular Ca²⁺ handling [41]. These possible mechanisms are supported by three lines of evidence. First, the extracellular Mg²⁺ concentration can influence Ca²⁺ metabolism of vascular smooth muscle by changing the Ca²⁺ influx through the plasma membrane. In single myocytes from frog ventricle, the site of interaction between Mg²⁺ and Ca²⁺ was identified as the Ca²⁺ inward current that is dependent on phosphorylation by cyclic adenosine monophosphatase [49]. Second, changes in the extracellular Mg²⁺ concentration induced inverse changes in the Ca²⁺ content of vascular smooth muscle and in exchangeable Ca²⁺ [50,51]. Third, a decrease in the intracellular free Mg²⁺ concentration resulted in diminished membrane Na⁺–K⁺-ATPase and Ca²⁺-ATPase activities [52], and, as a corollary, increased Na⁺–Ca²⁺ exchange and increased intracellular Na⁺ and Ca²⁺ concentrations [52].

The results obtained in this study show significantly increased Na⁺ concentrations and decreased total intracellular Mg²⁺ concentrations in vascular smooth muscle cells of SHR compared with WKY aged 8–10 months (P < 0.05), the change being more marked for sodium, and additionally significantly increased in 3-month-old SHR compared with WKY (P < 0.05). The findings are similar to those in erythrocytes of patients with essential hypertension or in SHR. Magnesium is largely bound, mainly to phosphate-containing molecules, including ATP. A decrease in total magnesium content is not a direct proof of a decreased Mg²⁺ ion concentration. It could in particular reflect an alteration of 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 Mg²⁺ efflux system in erythrocytes, it was suggested that patients with essential hypertension...
hypothesis could have increased activity of this Mg\(^{2+}\)–Na\(^{+}\) exchange transport system, which could explain the reduced intra-erythrocytic Mg\(^{2+}\) content [30–32]. This hypothesis was investigated by Gunther et al. [34,35], who found no differences between a small group of patients with essential hypertension and normotensive subjects. Nevertheless, in a larger group of patients with essential hypertension, Picado et al. found an acceleration of the Mg\(^{2+}\)–Na\(^{+}\) exchanger, with almost half of the patients showing values higher than the normal upper limit of normotensive control subjects [10].

The kinetic conditions for the assay of the \(V_{\text{max}}\) of Mg\(^{2+}\)–Na\(^{+}\) exchanger have been previously examined by Feray and Garay [30]. These authors measured Mg\(^{2+}\) efflux as a function of intracellular Mg\(^{2+}\) and extracellular Na\(^{+}\) and found that Mg\(^{2+}\) efflux was saturable and followed Michaelis–Menten kinetics. The apparent \(K_{m}\) was 2.6 mmol/l for intracellular Mg\(^{2+}\) and 20.5 mmol/l for extracellular Na\(^{+}\). The coupling of the Mg\(^{2+}\) extrusion to the Na\(^{+}\) influx has been suggested by Gunther et al. [54], who found a correlation between Na\(^{+}\) influx and Mg\(^{2+}\) efflux in chicken erythrocytes.

In the present study the Mg\(^{2+}\)/Na\(^{+}\) ratio in SHR aged 8–10 months with established hypertension was found to be significantly decreased compared with age-matched WKY (\(P < 0.001\)). In conclusion, increased Na\(^{+}\) and lowered Mg\(^{2+}\) concentrations in vascular smooth muscle cells of SHR may contribute to the development of primary hypertension. The importance of magnesium in blood pressure regulation still remains an open question; it is not known if a Mg\(^{2+}\) deficiency is a cause or a consequence of primary hypertension. Similar to investigations in human blood cells a subgroup with essential hypertension exists, showing a Mg\(^{2+}\) deficiency to be involved in the pathogenesis of hypertension. In these cases an altered Na\(^{+}\)–Mg\(^{2+}\) exchanger is hypothesized. From the data presented here no evidence is given that the decreased magnesium and the increased sodium contents are linked through a Na\(^{+}\)–Mg\(^{2+}\) exchanger as, in young rats, before hypertension becomes well established, no correlation between intracellular Mg\(^{2+}\) and Na\(^{+}\) concentrations was found. Probably a subgroup of SHR exists showing a decreased Mg\(^{2+}\)/Na\(^{+}\) ratio as compared with WKY, similar to studies in erythrocytes from those with Mg\(^{2+}\)-deficient essential hypertension.

REFERENCES


Received 2 April 1998; accepted 10 June 1998

cellular Na\(^+\) and Mg\(^{2+}\) in spontaneously hypertensive rats

587

1987 Cellular ions in hypertension, diabetes and obesity: a nuclear magnetic resonance spectroscopy study. Hypertension 17, 951–957


36 Vormann, J. and Gunther, T. (1987) Amiloride-sensitive net Mg\(^{2+}\) efflux from isolated perfused rat hearts. Magnesium 6, 220–224


46 Friedeman, S. M. (1979) Evidence for enhanced sodium transport in the tail artery of the spontaneously hypertensive rat. Hypertension 1, 572–582


