Early onset of a decreased intracellular magnesium and phosphate concentration in smooth muscle cell of SHR

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Abstract. A decrease in total magnesium content is not a direct proof of a decreased magnesium ion concentration. It could reflect a phosphate alteration or an ATP metabolism disorder. Plasma phosphate levels are lower in spontaneously hypertensive rats (SHRs) than in Wistar-Kyoto rats (WKYs), and defects in membrane regulation or mitochondrial ATP synthase occur. Only sparse data exist concerning cellular magnesium and phosphate concentrations in hypertensive cells. In aortic smooth muscle cells from 10 SHRs of the Münster strain and 10 age-matched normotensive WKY rats, the intracellular phosphate and magnesium content was measured by electron probe X-ray microanalysis (Camscan CS 24 apparatus, Cambridge, U.K.). The Mg²⁺ content was 0.09 ± 0.15 g/kg dry weight in SHRs versus 1.15 ± 0.10 g/kg dry weight in WKY rats (p < 0.01). Vascular smooth muscle phosphate content was 23.6 ± 0.79 g/kg dry weight in WKY rats versus 15.81 ± 1.22 g/kg dry weight in SHRs (p < 0.01). In aortic smooth muscle cells of one month old SHRs intracellular magnesium was measured as 1.05 ± 0.00 g/kg dry weight in WKY rats versus 1.09 ± 0.00 g/kg dry weight in WKYs. Intracellular phosphate concentration in one month old SHRs was 18.71 ± 2.41 versus 21.36 ± 1.25 g/kg dry weight in WKYs (eight animals in each group). Aortic smooth muscle cells of SHRs are characterized by markedly lowered intracellular phosphate and magnesium concentrations, resulting in an altered ATP-metabolism, as described earlier. Possibly a membrane defect or a magnesium deficiency or disturbed magnesium channels are responsible for the early onset in the pathogenesis of primary hypertension.

Key words: phosphate, magnesium, SHR, ATPases

Changes in Mg²⁺ metabolism have been implicated in the pathogenesis of hypertension [1-4]. Whereas plasma Mg²⁺ concentrations have often been investigated, comparatively few data on intracellular Mg²⁺ concentrations are available [5-9]. In addition, a disturbed magnesium dependent phosphate metabolism is discussed in primary hypertension via intracellular ATP disorders [10-21].

The aims of the study presented here were to investigate intracellular magnesium and phosphate concentrations in intact vascular smooth muscle cells of spontaneously hypertensive and normotensive rats of different ages under in vivo conditions.

Methods

We used aortae from 10 SHRs and 10 WKY rats (systolic pressure 117.3 ± 6.1 mmHg, means ± SD) aged 9 months. The SHRs had reached a systolic blood pressure of 192.5 ± 10.55 mmHg at this age. Additionally
we investigated 8 SHRs (systolic blood pressure 175.2 ± 9.76 mm Hg) and WKYs (systolic blood pressure 115.7 ± 7.4 mmHg) aged 1 month.

For determination of intracellular magnesium and phosphate, smooth muscle cells from abdominal rat aorta were investigated under nearly *in vivo* conditions.

The aortae 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 µm from aortae were made and thereafter lyophilised. Then magnesium and phosphate measurements were performed in each sample by electronprobe microanalysis technique as described earlier [22, 23].

Briefly, for electronprobe microanalysis an electron microscope with an X-ray detector system was used (Camscan CS 24 apparatus, Cambridge, UK). 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 quantitation, the continuum method developed by Hall was used [24, 25].

Intracellular sites of measurements were identified by the morphology obtained by electron microscopy, and by simultaneous measurements of sulphur and phosphate, 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 different sites were calculated. All sites were within smooth muscle cells. The magnification was 5 x 10,000 so that the intracellular organelles could be identified (*figure 1*). For the magnesium and phosphate measurements only sites within the cytoplasm were chosen. Mg”” and phosphate contents were then expressed in g/kg dry weight of the tissue.

Additionally, ATP content was measured in red blood cells, expressed as µmol/L cells and the Na’/K’-ATPase activity was measured in erythrocyte membranes expressed as nmol inorganic phosphate/mg protein/min in the older animals, as described earlier [26, 27]. Briefly, rat red blood cells were separated from freshly drawn blood by centrifugation at 1,500 g for 15 min at room temperature. Cells were washed in 5 mL of 150 mmol/L NaCl, pH 7.4 and then lysed in hypotonical 10 mmol/L Tris/HCl buffer, pH 7.4, containing 0.05 mmol/L EGTA by mechanical shaking. After centrifugation at 20,000 g for 10 min at 4° C in Eppendorf tubes, the supernatant was discarded and the pelleted membranes were washed three times in Tris/HCl to remove the remaining hemoglobin. The membrane suspension obtained was diluted to contain 1.0-1.5 mg/mL protein. Protein measurements were performed in microtiter plates with bovine serum albumin as standard, according to established methods [28]. The Na’/K’-ATPase was calculated as the difference between the activity determined with and without ouabain. All measurements were corrected for unspecific phosphate release. The adenine nucleotide content was calculated from the integrated peak areas read from a calibration curve produced with nucleotide standards. All animal experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Data are means ± SD. Statistical analysis was performed for group comparison using unpaired Student’s *t*-test. P values below 0.05 (two-tailed test) were considered to be significant. Correlation analysis was performed by least square linear regression analysis.

Power analysis was performed based on pilot studies on intracellular magnesium and phosphate concentrations which showed standard deviations in the order of 14 per cent of the mean values. The sample size was chosen to detect a difference between groups of more than 20 per cent of the mean values, which we considered to be physiologically significant, with an alpha of 0.05 and a power of 0.8.
Results

Vascular smooth muscle Mg++ content was 0.90 ± 0.15 g/kg dry weight in SHRs versus 1.15 ± 0.10 g/kg dry weight in WKY rats aged 9 months (figure 2, p < 0.01).

In vascular smooth muscle cells phosphate concentrations were significantly decreased in 9 month old SHRs as compared with WKY rats (15.81 ± 1.22 versus 23.6 ± 0.79 g/kg dry weight) (figure 3, p < 0.01).

In aortic smooth muscle cells of one month old SHRs intracellular magnesium was measured 1.05 ± 0.08 versus 1.09 ± 0.09 g/kg dry weight in WKYs. Intracellular phosphate concentration in one month old SHRs was 18.71 ± 2.41 versus 21.36 ± 1.25 g/kg dry weight in WKY (eight animals in each group) (figures 2 and 3).

There was no correlation between intracellular magnesium or phosphate concentrations and blood pressure values in the normotensive or spontaneously hypertensive rats.

A positive correlation between intracellular phosphate and magnesium concentrations was observed in the SHRs group aged 9 months (r = 0.52, p < 0.02) (figure 4).

In seven animals aged 9 months, erythrocytic ATP content was 180 ± 102 in SHRs versus 432 ± 72 μmol/L cells in WKY rats (p < 0.01).

In these animals the Na+/K+-ATPase activity was significantly decreased in SHRs as compared to controls (6.49 ± 2.3 versus 12.64 ± 2.9 nmol Pi/mg protein/min (p < 0.01)).

Furthermore there was a significant increase in heart muscle to body weight ratio in SHRs versus WKY rats (p < 0.01) in 9 month-old animals.

In addition no correlation between intracellular Mg++ or phosphate concentrations, blood pressure values or total heart muscle weight was found.

In one month-old animals phosphate content was significant lower in smooth muscle cells as compared to age matched WKY (p < 0.01). Intracellular Mg++ in 1 month old SHRs showed a reduced tendency as compared to age matched WKYs, without reaching statistical significance.
Discussion

To assess intracellular Mg$^{++}$ and phosphate stores appropriately still remains difficult [3, 5, 7, 16-18]. Red blood cells are not a generally accepted indicator of cellular Mg$^{++}$ or phosphate stores [5]. In human studies only blood cells can routinely be used to measure intracellular Mg$^{++}$ or phosphate concentrations [3, 4, 7, 16]. Measurements in lymphocytes and platelets are complicated by the fact that the volume of these cells is difficult to assess. A role for cellular Mg$^{++}$ concentration in vascular tone has been postulated in hypertension [1-5, 7-9]. In essential hypertensives Resnick et al. found decreased intracellular free Mg$^{++}$ concentrations in red blood cells as estimated by nuclear magnetic resonance spectroscopy [7].

Analogous findings were reported in the spontaneously hypertensive rat [5, 6, 29, 30]. 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 [5].

An intracellular Mg$^{++}$ deficiency and possibly a defect in intracellular Mg$^{++}$ transport could play a pathogenetic role in the development of primary hypertension [1, 2, 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 [31]. These possible mechanisms are supported by three lines of evidence. First, the extracellular Mg$^{++}$ concentration can influence Ca$^{++}$ metabolism of vascular smooth muscle by changing the Ca$^{++}$ influx through the plasma membrane. 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 [31-34]. Second, changes in the extracellular Mg$^{++}$ concentration induced inverse changes in the Ca$^{++}$ concentrations [35-37].

- interactions between Mg$^{++}$ and Ca$^{++}$ and K$^{+}$ channels. Indeed, Mg$^{++}$ interferes with voltage-gated Ca$^{++}$ channels and with various K$^{+}$ channels (delayed-gated K$^{+}$ (Ca$^{++}$) channels and particularly K$^{++}$-ATP channels) in vascular smooth muscle cells and endothelial cells [38-41].

In this context only sparse data exist concerning the intracellular phosphate and ATP content of vascular smooth muscle cells in essential hypertension [13-19]. These authors described a loss of cellular magnesium resulting in a loss of critically important phosphagens: Mg$^{++}$ ATP and creatine phosphate. Thus, under conditions where cellular Mg$^{++}$ is depleted, the Na$^{-}$K$^{+}$ pump and phosphagen stores will be compromised, leading to alterations in resting membrane potentials.

Other authors speculated that the regulation of an increased energy demand is performed by an increased capacity of intracellular ATP synthase. In hypertensive cells this ATPase synthase is fully activated even during low energy demands. In addition, SHR cells are unable to increase their capacity of their ATP synthase with increasing energy demand. This may result in a disturbed cell and membrane function in hypertension [12]. We speculate that this insufficient increase in ATP synthase corresponds well with low intracellular phosphate and magnesium levels in vascular smooth muscle cells. This intracellular loss can be explained either due to a disturbed transmembrane transport mechanism in essential hypertension or to the described fully activated ATP synthase even during low energy demand in hypertensive cells.

Previous investigations showed a dynamic regulation of ATPases in the regulation of vascular smooth muscle tone and endothelial function in addition to alterations in cations metabolism [36-41]. In this context the data presented here show significantly decreased intracellular Mg$^{++}$ and phosphate concentrations in vascular smooth muscle cells of the aorta, in addition to lowered ATP content and ATPases activity in hypertensive animals.

In this context the Na$^{-}$K$^{+}$ ATPase may be coupled in the intracellular magnesium and phosphate content indicating that the development of high blood pressure in SHR may be related to an alteration in the transport of magnesium and phosphate across the cell membranes.

Fischer et al. investigated the effects of dietary magnesium on the sodium-potassium pump action in the heart of Sprague-Dawley rats. In their study the authors concluded that a magnesium deficiency appears to have no effect on the number of sodium-potassium pump sites, but does decrease the activity of the pump. It is suggested that this leads to an increase in intracellular sodium, resulting in a change in the membrane potential, and may furthermore contribute to arrhythmias and disturbed endothelial function and development of atherosclerosis and increased intima media thickening associated with a magnesium deficiency [42-45].

The results obtained in the study presented are limited to smooth muscle cells of the aorta. However, what occurs in resistance microvessels in hyperten-
sion still remains an open question and demands further investigation [61].

There are clues that a magnesium and phosphate deficiency are pathogenetic factors for the development of hypertension, but further studies are still necessary in this context. The data presented here stress the pathogenetic role of a magnesium deficiency in hypertension.

Conclusion

Aortic smooth muscle cells from SHRs are characterized by markedly lowered intracellular phosphate and magnesium concentrations, resulting in an altered ATP-metabolism, as described earlier. Possibly, a membrane defect or a magnesium deficit or disturbed magnesium channels are responsible for the early onset in the pathogenesis of primary hypertension [46-61].

References


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