Electron-probe X-ray Microanalysis of Magnesium and Sodium Ion Content in Vascular Smooth Muscle Cells from Spontaneously Hypertensive and Normotensive Rats

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Abstract. Whereas 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 in vascular smooth muscle cells. Therefore, in aortic smooth muscle cells from seven spontaneously hypertensive rats (SHR) of the Münster strain and seven normotensive Wistar-Kyoto rats (WKY), the intracellular magnesium and sodium content were measured by electron-probe X-ray microanalysis. Measurements were performed in aortic cryosections 3 μm thick. The magnesium ion content was 0.93 ± 0.17 g/kg dry weight in SHR vs 1.14 ± 0.12 g/kg dry weight in WKY (p < 0.05). Vascular smooth muscle sodium ion content was measured at 6.85 ± 0.59 g/kg dry weight in WKY and 12.47 ± 1.62 g/kg dry weight in SHR (p < 0.05). In conclusion, aortic smooth muscle cells from SHR are characterized by a markedly lowered intracellular magnesium ion content and increased sodium ion concentrations compared with normotensive cells. The results may be due to genetically determined disturbances in transmembrane magnesium and sodium ion transport. Cellular magnesium and sodium handling may be disturbed in SHR aortic smooth muscle as it is in hypertensive blood cells.

Introduction

Changes in Mg2+ and Na+ metabolism have been implicated in the pathogenesis of hypertension [1–4]. Earlier studies mainly focused on disturbances in cellular Na+ transport using blood cells of essential hypertensive patients [3,4]. Although plasma Mg2+ concentrations have often been investigated, comparatively few data on intracellular Mg2+ concentrations are available. Furthermore, the role of intracellular Mg2+ content in essential hypertension is also controversially discussed. Decreased intracellular-free Mg2+ concentrations in erythrocytes of essential hypertensive patients have been described [5], but other authors were unable to confirm these findings [6,7]. These different results in intracellular Mg2+ content in essential hypertension may be due to various analytical techniques. Most of the results showing a decreased cellular magnesium ion content and increased cellular sodium concentrations were obtained from blood cells, but only sparse data exist concerning cellular magnesium and sodium ion concentrations in vascular smooth muscle cells. Therefore it was of interest to develop a method to determine total Mg2+ and Na+ content in vascular smooth muscle cells of spontaneously hypertensive and normotensive rats.

Methods

We used aortae from seven SHR and seven WKY rats (systolic pressure 116.4 ± 6.2 mmHg, mean ± SD) aged 3 months. The SHR had reached a systolic pressure of 190.4 ± 10.1 mmHg at this age. 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. Then 3-μm-thick cryosections were made and lyophilized. For the electron-probe microanalysis, an electron microscope with an X-ray detector system is used. 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 Hull [8] was used. Intracellular sites of measurement were identified by the morphology obtained by electron microscopy, and by simultaneous measurements of sulfur and phosphorus, the concentrations of which were markedly elevated in the intracellular compared with the extracellular space. In each aorta, mean values of the least five intracellular measurements at different sites were calculated. All sites were within smooth muscle cells. The magnification was 5 × 10,000, so that organelles could be identified. For the Mg2+ and Na+ measurements only sites within the...
Mg$^{2+}$ concentrations in red blood cells, as estimated by the fluorescent dye Mag-fura 2, have been postulated in hypertension [1,5,7]. In essential hypertensives, Resnick et al. [5] found decreased intracellular free Mg$^{2+}$ concentrations in aortic smooth muscle cells of one animal were 19.8% of the mean value in SHR and 13.2% in WKY.

Na$^+$ concentrations of vascular smooth muscle cells of WKY animals were 6.85 ± 0.59 g/kg dry weight. In the SHR, cellular Na$^+$ content in vascular smooth muscle cells was significantly increased (12.47 ± 1.62 g/kg dry weight, means ± SD, p < 0.05, Fig. 2). Variations in 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.

There was no correlation between cellular Mg$^{2+}$ and Na$^+$ concentrations in WKY and SHR vascular smooth muscle cells.

Discussion

To assess intracellular Mg$^{2+}$ and Na$^+$ stores appropriately still remains difficult. Red blood cells are not a generally accepted indicator of cellular Mg$^{2+}$ or Na$^+$ stores. In human studies, only blood cells can routinely be used to measure intracellular Mg$^{2+}$ or Na$^+$ concentrations. Mg$^{2+}$ or Na$^+$ 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$^{2+}$ by the fluorescent dye Mag-fura 2 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$^{2+}$ concentration in vascular tone has been postulated in hypertension [1,5,7]. In essential hypertensives, Resnick et al. [5] found decreased intracellular free Mg$^{2+}$ concentrations in red blood cells, as estimated by nuclear magnetic resonance spectroscopy. Analogous findings were reported in the spontaneously hypertensive rat [9].

Whereas from investigations in blood cells a magnesium deficiency in primary hypertension seems likely, comparatively few data exist on cellular electrolyte concentrations in vascular smooth muscle cells in hypertension. These data were obtained in animals [10,11].

An intracellular Mg$^{2+}$ deficiency and possibly a defect in cellular Mg$^{2+}$ transport could play a pathogenetic role in the development of primary hypertension [12,13]. On the basis of experimental data, the mechanisms underlying the Mg$^{2+}$-induced vasodilation may be a modification of the response to vasopressor hormones, and an interaction with cellular Ca$^{2+}$ handling [1]. These possible mechanisms are supported by three lines of recent evidence. First, the extracellular Mg$^{2+}$ concentration can influence Ca$^{2+}$ metabolism of vascular smooth muscle by changing the Ca$^{2+}$ influx through the plasma membrane. Recently, in single myocytes from frog ventricle, the site of interaction between Mg$^{2+}$ and Ca$^{2+}$ was identified as the Ca$^{2+}$ inward current that is dependent on phosphorylation by cyclic adenosine monophosphatase [14]. Second, changes in the extracellular Mg$^{2+}$ concentration induced inverse changes in the Ca$^{2+}$ content of vascular smooth muscle and in exchangeable Ca$^{2+}$ [15,16].

Third, a decrease in the intracellular free Mg$^{2+}$ concentration results in diminished membrane Na$^+$, K$^+$-adenosine triphosphatase and Ca$^{2+}$ ATPase activities [17], and, as a corollary, increased Na$^+$-Ca$^{2+}$ exchange and increased intracellular Na$^+$ and Ca$^{2+}$ concentrations [13].

The results obtained in our study show significantly lower total intracellular Mg$^{2+}$ concentrations in vascular smooth muscle cells of SHR rats compared with WKY rats (p < 0.05). The findings are similar to those in red blood cells of essential hypertensives or in spontaneously hypertensive rats.

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

There are only few data on intracellular Na$^+$ concentrations in arterial smooth muscle cells from hypertensive animals. Tobian and Binion [18] found that intracellular Na$^+$ was elevated in arterial smooth muscle in one-kidney, one-clip hypertensive rats [18]. In contrast Massingham and Shevde [19] described a principally unchanged total Na$^+$ content in aortae from genetically hypertensive rats, but did not distinguish between intracellular and extracellular Na$^+$ concentrations.

The inhibition of Na$^+$,K$^+$-ATPase has been postulated to cause hypertension as a consequence of elevated intracellular Na$^+$ concentrations [20]. Increased intracellular Na$^+$ con-