Endothelium-Dependent Influence of Small Changes in Extracellular Magnesium Concentration on the Tone of Feline Middle Cerebral Arteries

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The aim of this study was to investigate the effect of small alterations in the extracellular magnesium concentration on the tone of feline middle cerebral arteries and to examine the role of the endothelium in these responses. We measured the isometric tension of isolated arterial rings placed between two stainless steel wires in a tissue chamber containing Krebs-Henseleit solution aerated with a gas mixture containing 95% O2 and 5% CO2 at 37°C. After precontraction with noradrenaline, a decrease of the extracellular magnesium concentration from 1.2 mM to 1.0 and 0.8 mM resulted in sustained relaxations, whereas elevation of the extracellular magnesium concentration from 0.8 mM to 1.2 mM caused an increase in vascular tone when the endothelium was intact. The magnesium deficiency-related dilations were absent in endothelium-denuded vessels and were inhibited by 5×10^(-7) M oxyhemoglobin and 10^(-5) M methylene blue, suggesting the involvement of an endothelium-derived relaxing factor in this vascular response. However, 5×10^(-7) M nifedipine or 3×10^(-5) M dichlorobenzamil did not affect the magnesium deficiency-related relaxations. Therefore, nifedipine-sensitive calcium channels or the sodium/calcium antiport system are not involved in this vascular action of magnesium. We conclude that small alterations in the extracellular magnesium concentration, possibly within the physiological range, are able to modify the basal formation and release of endothelium-derived relaxing factor and thus alter arterial smooth muscle tone in this vascular bed. This endothelium- and magnesium-dependent system appears to be more sensitive than the direct smooth muscle actions of magnesium and might exert a protective effect against magnesium deficiency-induced direct cerebrovascular contraction. (Stroke 1991;22:785-789)

It is well known that extracellular magnesium acts as a calcium antagonist at the level of the vascular smooth muscle. Contractile responses are potentiated and basal tone is increased in magnesium-deficient solutions; it has been suggested that low plasma magnesium concentrations might facilitate the occurrence of cerebral and coronary vasospasm.1-8

In the last decade, the endothelium and endothelium-derived relaxing factor (EDRF) have been shown to play an important role in the regulation of vascular function.9,10 The endothelium exerts an inhibitory effect against vasospasm by releasing prostacyclin and EDRF, both of which inhibit platelet aggregation.11 Furthermore, most platelet-derived vasoconstrictor substances also release EDRF, which attenuates their direct contractile action.12,13 If the endothelium is damaged, however, vasospasm might occur.10-12

Sustained release of EDRF depends on extracellular calcium influx.13-16 Recent work from Moncada's laboratory clearly shows the capability of the endothelial nitric oxide-synthesizing pathway to respond to changes in the concentration of calcium around the physiological range, thus modulating vascular tone.16 Extracellular magnesium might also exert its inhibitory action on calcium influx into cells at the level of the endothelium.17-19

Thus, the effect of magnesium deficiency on vascular tone might consist of two components: 1) the well-known facilitation of smooth muscle contrac-
tion1-8 and 2) an enhancement of EDRF release, resulting in sustained17 or transient18 relaxation. There are no observations on involvement of the endothelium regarding the cerebrovascular actions of magnesium. Furthermore, previous reports focusing on smooth muscle1-8 or endothelium17,18 studied the effect of total magnesium deficiency. Such a condition is unlikely to occur in vivo.20,21 Therefore, we first examined the effect of small alterations in the extracellular magnesium concentration, probably occurring under physiological conditions,20,21 on the tone of feline middle cerebral artery and then clarified the involvement of EDRF in these responses.

Materials and Methods

We used nine cats of either sex weighing 1.9-2.8 kg, anesthetized with 30 mg/kg i.p. sodium pentobarbital, and exsanguinated via a femoral artery catheter. The brain was quickly removed from the skull and placed into cold Krebs-Henseleit solution. Microsurgical methods and a Zeiss surgical microscope (Jena, FRG) were used to remove and clean the artery. Care was taken to avoid stretching or injuring the vessel.

Vessel segments 1–3 mm long were placed on two L-shaped stainless steel specimen holders 0.1 mm in diameter, one of which was attached to a Grass FT03 force transducer (Quincy, Mass.), for isometric tension measurements. The position of the other holder could be adjusted by a micromanipulator. The vessel segments were placed into a tissue chamber containing Krebs-Henseleit solution (millimolar composition: 119 NaCl, 4.6 KCl, 1.5 CaCl2, 1.2 MgCl2, 15 NaHCO3, 1.2 NaH2PO4, and 6 glucose) and bubbled with a gas mixture containing 95% O2 and 5% CO2. The temperature of the solution was kept at 37°C and the pH was 7.4.22 The vessels were incubated for 60–90 minutes at a tension of 400–600 mg. All measurements were carried out in the presence of 5×10⁻⁶ M indomethacin and 5×10⁻⁷ M propranolol to block the production of cyclooxygenase products and the activation of β-receptors, respectively. The magnesium concentration of the Krebs-Henseleit solution was adjusted to 1.0 and 0.8 mM by altering the NaCl concentration; thus, isotonicity of the solution was maintained at normal. First, cumulative dose–response curves for 10⁻⁸ to 10⁻⁵ M noradrenaline and 10⁻⁸ to 10⁻⁵ M acetylcholine were tested. Acetylcholine caused a dose-dependent relaxation, bringing the vascular tone to the level measured before application of the precontractile agent. (In some vessel preparations, acetylcholine caused dose-dependent, but only partial, relaxations, bringing the vascular tone maximally to approximately 50% of the level of precontraction. Since this response might reflect partial damage to the endothelium, these vessels were excluded from the present investigations.)

The vessels were precontracted with 5×10⁻⁶ M noradrenaline. After the tone reached a steady level, the incubation medium containing a given concentration of magnesium was replaced with a medium containing the same concentrations of noradrenaline, indomethacin, and propranolol but a slightly altered level of magnesium. After the new tone stabilized, the solution was replaced again, restoring the magnesium concentration in the medium to the original value. Finally, responses to acetylcholine were tested. After repeated washings with the normal solution containing 1.2 mM magnesium, vessel tone returned to baseline. Then, after precontraction, the magnesium concentration was changed again. Changes in the extracellular magnesium concentration from 1.2 to 1.0 and 0.8 mM and from 0.8 to 1.2 mM were tested (in the latter case vessels were incubated for 5 minutes in the solution containing 0.8 mM magnesium before the application of noradrenaline). Changes in the extracellular magnesium concentration from 1.2 to 0.8 mM were also tested in vessels pretreated with 5×10⁻⁶ M oxyhemoglobin, 10⁻⁵ M methylene blue, 5×10⁻⁷ M nifedipine, or 3×10⁻⁵ M dichlorobenzamil for 15 minutes followed by contraction with 5×10⁻⁶ M noradrenaline. The endothelium was removed by gently rubbing the intimal surface of the arterial segments with a stainless steel wire. The effectiveness of endothelium removal was verified in each ring by the absence of relaxation in response to acetylcholine.9,10

The following drugs were used: acetylcholine chloride (Sigma Chemical Co., St. Louis, Mo.), indomethacin (Sigma), noradrenaline (Gedeon Richter Chemical Works, Budapest, Hungary), propranolol hydrochloride (Sigma), nifedipine (Bayer, Germany), methylene blue (Sigma), dichlorobenzamil (Dr. E.J. Crago Jr., USA). All drugs were dissolved in saline, except indomethacin and nifedipine (dissolved in 50% ethanol), dichlorobenzamil (dissolved in dimethylsulfoxide), and noradrenaline (dissolved in distilled water containing 1 mM ascorbic acid). Oxyhemoglobin was prepared as previously described23 from the carefully oxygenated arterial blood of a cat. The blood was collected through a polyethylene cannula from the femoral artery into heparin-containing tubes. The blood was centrifuged at 2,000g and washed several times with Krebs-Henseleit solution. Red blood cells were then hemolyzed with distilled water and diluted with Krebs-Henseleit solution. The oxyhemoglobin concentration of the stock solution was measured spectrophotometrically.

Student's t test for paired and unpaired samples was used to statistically analyze the data, and a difference was considered significant if p < 0.05. Data are shown as mean±standard error of the mean.

Results

Lowering of the extracellular magnesium concentration from 1.2 to 1.0 mM caused sustained relaxations of arteries precontracted with 5×10⁻⁶ M noradrenaline (Figures 1 and 2, left panels). Vessel tone stabilized at about 70% of the original tone induced by noradrenaline. If the magnesium concentration was lowered to 0.8 mM, tone decreased to 8% of original (Figure 1, right panel and Figure 2, middle
FIGURE 1. Typical tracings showing effect of rapid changes in extracellular magnesium concentration from 1.2 mM to 1.0 mM (left panel) and 0.8 mM (right panel) in feline middle cerebral artery precontracted with $5 \times 10^{-6}$ M noradrenaline (NA). In right panel effect of restoration of magnesium concentration to 1.2 mM and then response to $10^{-8}$ to $10^{-6}$ M acetylcholine (ACh) is also shown.

This relaxation represents the maximum magnesium deficiency response since reduction of the extracellular magnesium concentration to less than 0.8 mM caused a less pronounced relaxation, whereas at 0 mM magnesium the tone stabilized at about 50% of the original tone (not shown). The effect of variations in magnesium concentration on tone of the artery were reversible (Figure 1, right panel).

If the vessels were incubated first in a solution containing 0.8 mM magnesium and then the magnesium concentration was restored to 1.2 mM, we observed a marked increase in vascular tone (Figure 2, right panel).

Figure 3 shows that neither the calcium antagonist nifedipine at $5 \times 10^{-7}$ M nor the sodium/calcium antiport blocker dichlorobenzamil at $3 \times 10^{-5}$ M inhibited the relaxations induced by reduction of the extracellular magnesium concentration from 1.2 to 0.8 mM. On the other hand, $5 \times 10^{-6}$ M oxyhemoglobin, $10^{-5}$ M methylene blue, and endothelium removal each inhibited this relaxation. Dichlorobenzamil, oxyhemoglobin, methylene blue, and endothelium removal did not influence the absolute value of the noradrenaline-induced contraction. Nifedipine, however, significantly inhibited the contraction to $18.7 \pm 2.7\%$ of the control response ($p<0.001$). Dichlorobenzamil induced a significant $32.6 \pm 5.2\%$ relaxation of the noradrenaline-precontracted vessels and caused a $32.7 \pm 6.0\%$ relaxation of the little tone remaining after reduction of the extracellular magnesium concentration from 1.2 to 0.8 mM ($p<0.001$ in both cases). Dichlorobenzamil, however, did not change the tone of precontracted endothelium-denuded vessels.

Discussion

Two opposite effects of magnesium deficiency have been reported: increase of vascular tone and endothelium-dependent relaxation. We investigated the effect of small changes in the magnesium concentration on the tone of precontracted feline middle cerebral arteries. We believe that the effects and mechanisms of such small alterations have not been
previously investigated in vitro and that our data might help evaluate which effect of magnesium deficiency is dominating when the extracellular magnesium concentration is slightly altered. The plasma and cerebrospinal fluid magnesium levels are normally 0.8–1.2 mM under physiological conditions, but the magnesium concentration can be as low as 0.4 mM in pathophysiological states.

Our results show that the slight decrease in the magnesium level from 1.2 to 1.0 or 0.8 mM causes very pronounced relaxations in the cerebral vessel studied. In contrast to previous works focusing only on the direct smooth muscle contractile effect of magnesium deficiency, our results also suggest that small decreases in the extracellular magnesium concentration, also possible in vivo, cause a relaxation rather than direct smooth muscle contraction.

This relaxation was not present in endothelium-denuded vessels and was blocked by melatonin blue and oxyhemoglobin, suggesting the involvement of endothelium and EDRF. Interestingly, reduction of the magnesium level to less than 0.8 mM caused a much smaller relaxation than reduction to 0.8 mM. Since, in vessels without endothelium, reduction of the magnesium concentration induces a dose-dependent increase in vascular tone that is only approximately 20% at 0.8 mM (Figure 3) but becomes very pronounced at 0 mM (unpublished observations, see also References 1–6), probably the direct smooth muscle contractile action of magnesium deficiency counteracting the relaxant effect of EDRF abolishes the relaxation at total magnesium deficiency. Recently, Gold et al studied the reactions of pulmonary vessels to changes in the extracellular calcium and magnesium concentrations and found that the algebraic sum of the smooth muscle and endothelial actions of the magnesium concentration gives the actual vascular change. These investigators reported transient endothelium-dependent relaxations followed by sustained endothelium-independent contractions of bovine intrapulmonary vessels in response to total magnesium deficiency.

The depressed contractile responses to noradrenaline in magnesium-deficient medium and the increase in vascular tone after restoration of the magnesium concentration to normal (Figure 2) clearly show that the enhanced basal release of EDRF caused by low extracellular magnesium concentrations cannot only relax precontracted vessels, but also sufficiently inhibit agonist-induced contractions.

Our findings confirm and extend the results of Ku and Ann and Gold et al, showing endothelium-dependent relaxations in response to total magnesium deficiency in canine coronary artery and bovine intrapulmonary artery and vein. Those authors showed that the relaxant effect of magnesium deficiency depends on extracellular calcium and is probably due to enhanced calcium influx into endothelial cells. Since in the present vessel neither nifedipine nor dichlorobenzamil inhibited the relaxations, the proposed magnesium deficiency–induced calcium influx into the endothelium must occur via some pathway other than the nifedipine-sensitive voltage-operated calcium channel or the sodium/calcium antiport system.

Our results also confirm the endothelium-dependent relaxant effect of dichlorobenzamil, which was first reported in canine coronary arteries, suggesting the presence of a functioning sodium/calcium antiport system in the membrane of the cerebrovascular endothelium.

Recent studies from our laboratory showed that cerebrovascular endothelium-dependent relaxations caused by acetylcholine are also very sensitive to changes in the extracellular magnesium concentration in a similar range as studied presently. Thus, extracellular magnesium appears to be an important and sensitive modulator in the basal and agonist-induced endothelium-dependent regulation of cerebrovascular tone.

Recent experiments showed the importance of EDRF in the inhibition of platelet aggregation and the maintenance of blood pressure. Although the present and previous data also suggest that circulating magnesium plays a role in the regulation of these processes via adjustment of EDRF release, further studies are needed to clarify this possibility.

Endothelium was suggested to play a protective role against platelet-induced vasospasm; many platelet-derived constrictors also cause relaxation via EDRF. At sites of endothelial damage, however, these substances might cause contraction. Our results indicate that endothelium might exert its protective role not only against the vasoconstrictor effect of platelet aggregation products, but also against magnesium deficiency–induced direct cerebrovascular smooth muscle contraction.

Acknowledgments

The authors express their gratitude to G. Csubák for the technical assistance and to E. Molnár for the drawings.

References


KEY WORDS: endothelium • magnesium • muscle, smooth • cats
Endothelium-dependent influence of small changes in extracellular magnesium concentration on the tone of feline middle cerebral arteries.
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doi: 10.1161/01.STR.22.6.785

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/22/6/785

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