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Mechanisms of magnesium-induced vasodilation in cerebral penetrating arterioles

Takahiro Murata[a,*], Hans H. Dietrich[b,c], Tetsuyoshi Horiuchi[a], Kazuhiro Hongo[a], Ralph G. Dacey Jr.[b]

[a] Department of Neurosurgery, Shinshu University School of Medicine, Matsumoto 390-8621, Japan
[b] Department of Neurological Surgery, Washington University School of Medicine, St. Louis, MO 63110, United States
[c] Hope Center for Neurological Disorders, Washington University School of Medicine, St. Louis, MO 63110, United States

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ABSTRACT

We investigated in cerebral penetrating arterioles the signaling mechanisms and dose-dependency of extracellular magnesium-induced vasodilation and also its vasodilatory effects in vessels preconstricted with agonists associated with delayed cerebral vasospasm following SAH. Male rat penetrating arterioles were cannulated. Their internal diameters were monitored. To investigate mechanisms of magnesium-induced vasodilation, inhibitors of endothelial function, potassium channels and endothelial impairment were tested. To simulate cerebral vasospasm we applied several spasmogenic agonists. Increased extracellular magnesium concentration produced concentration-dependent vasodilation, which was partially attenuated by non-specific calcium-sensitive potassium channel inhibitor tetraethylammonium, but not by other potassium channel inhibitors. Neither the nitric oxide synthase inhibitor L-NNA nor endothelial impairment induced by air embolism reduced the dilation. Although the magnesium-induced vasodilation was slightly attenuated by the spasmogen ET-1, neither application of $PF_{2\alpha}$ nor TXA_2 analog effect the vasodilation. Magnesium induced a concentration- and smooth muscle cell-dependent dilation in cerebral penetrating arterioles. Calcium-sensitive potassium channels of smooth muscle cells may play a key role in magnesium-induced vasodilation. Magnesium also dilated endothelium-impaired vessels as well as vessels preconstricted with spasmogenic agonists. These results provide a fundamental background for the clinical use of magnesium, especially in treatment against delayed cerebral ischemia or vasospasm following SAH.

1. Introduction

Development of delayed cerebral ischemia (DCI) following subarachnoid hemorrhage (SAH), which results in an unfavorable clinical outcome, is thought to be caused by the combined effects of delayed cerebral vasospasm, arteriolar constriction and microthrombosis, cortical spreading ischemia, and processes triggered by early brain injury (Macdonald, 2014). Delayed cerebral vasospasm is one of the major causes of DCI, however, its pathophysiological mechanisms still remain unresolved despite progress in experimental and human investigations, thus limiting the number of available effective therapies. Magnesium is a well-known neuroprotective as well as vasodilatory agent with various experimental and clinical profiles. Several clinical studies have demonstrated the safety and efficacy of intravenous magnesium therapy for aneurysmal SAH (Veyna et al., 2002; van den Bergh et al., 2003). Unfortunately, the "Magnesium in Aneurysmal Subarachnoid Hemorrhage" (MASH-2) study, a phase III randomized, clinical, international multicenter trial, recently indicated that intravenous magnesium sulfate infusion therapy failed to improve clinical outcome after aneurysmal SAH (Dorhout Mees et al., 2012). These trials suggested that magnesium, administered in addition to the oral calcium blocker nimodipine, may not improve neuroprotection in patients when given intravenously. In contrast, Mori et al. (2009a,b) demonstrated that intracisternal injection of magnesium sulfate solution has a vasodilatory effect on spastic cerebral arteries both in human aneurysmal SAH and canine SAH models. In addition, we recently reported that increased extracellular magnesium concentration ($[Mg^{2+}]_o$) significantly induced dilation in cerebral penetrating arterioles (Murata et al., 2011); however, little

* Corresponding author at: Department of Neurosurgery, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. Tel.: +81 263 37 2690; fax: +81 263 37 0480.
E-mail address: tmurata@shinshu-u.ac.jp (T. Murata).

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is known regarding the vasodilatory mechanisms of magnesium in the cerebral microcirculation. These vessels play an important role in the regulation of the cerebral circulation by maintaining normal blood flow and pressure to protect against cerebral ischemia and infarction (Faraci and Heistad, 1990; Nishimura et al., 2007). The failure of microvascular regulation after SAH suggests a pivotal role in the development and size of ischemia (Dietrich and Dacey, 2000). Therefore, the purpose of the present study was to clarify the signaling mechanisms of vasodilation induced by increased $[Mg^{2+}]_o$ in cerebral penetrating arterioles as it would occur after intracisternal magnesium injection in vivo and also to determine magnesium's vasodilatory potency in vessels preconstricted with spasmogen involved in delayed cerebral vasospasm following SAH.

2. Materials and methods

2.1. Isolation and cannulation of cerebral penetrating arterioles

All experimental procedures in this study were approved by the Washington University Advisory Committee for Animal Resources. The detailed techniques used in this study for the dissection and cannulation of intracerebral arterioles have also been described previously (Dacey and Duling, 1982). Briefly, 50 male Sprague-Dawley rats (300–450 g, 14–20 weeks olds, Harlan, Indianapolis, IN) were anesthetized (86.98 mg/kg ketamine; 13.40 mg/kg xylazine, intraperitoneally) and sacrificed. The brain was rapidly removed from the skull and placed in a cooled (4 °C) dissection chamber filled with physiological saline solution (PSS; see below) containing 1% dialyzed bovine serum albumin (BSA). Under a surgical microscope, a 2–3 mm slab of the cerebral cortex containing the middle cerebral artery (MCA) was dissected from the brain. The pia mater was gently reflected from the parenchyma to expose the intracerebral arterioles from MCA. The isolated arteriole was carefully transferred from the dissection chamber to a temperature-controlled vessel chamber (2.5-ml organ bath) mounted on the stage of an inverted video microscope (Zeiss TV 200, Thornton, NY). The holding and perfusion pipettes used for cannulation were fabricated by pulling and shaping glass tubes (Drummond Scientific Co.; 2.13 mm O.D., 1.63 mm I.D., and 1.19 mm O.D., 1.02 mm I.D., respectively; 15-cm length) using a microforge (Stoelting Co., Wood Dale, IL). The collecting pipettes were also fabricated from the same glass as the holding pipettes (2.13 mm O.D., 1.63 mm I.D.). The final holding pipette diameter was 80–90 μm and perfusion pipette diameter was 45–50 μm. The unbranched penetrating arteriole was cannulated at one end with the concentrically mounted perfusion (inner) and holding (outer) pipette system. The opposite end of the arteriole was occluded by the collecting pipette. All experiments were conducted without intraluminal flow. The transmural pressure (60 mmHg) was monitored continuously with a pressure transducer (model P23, Gould, Cleveland, OH) and recorded on a strip chart recorder (model 3200, Gould). The internal diameter of the vessel was observed with a high-resolution videocamera (CCD 72 with GenIIsis, Dage-MTI, Michigan City, IN) and displayed on a monitor. To measure the internal vessel diameter, we used both a calibrated video-dimensional analyzer (modified model 321, Colorado Video) and Diamtrak Edge-tracking software (T.O. Neild, Flinders University, Adelaide, Australia) as previously described (Neild, 1989). This method uses a video microscope to produce a digitized image of the blood vessel, the video signal was acquired on-line by a computerized diameter tracking system, allowing for a diameter measurement at a spatial resolution of 0.5 μm/pixel and a data acquisition rate of 10 Hz. The data were digitally stored as well as recorded on a strip-chart recorder for later evaluation. The vessel chamber temperature was increased to 37.5 °C, and the arterioles were allowed to develop spontaneous tone over approximately 45 min. The organ bath was continually replaced with a fresh PSS (pH 7.3) of the following composition (in mmol/L): 144 NaCl, 3.0 KCl, 2.5 $CaCl_2$, 1.4 $MgSO_4$, 2.0 pyruvate, 5.0 glucose, 0.02 ethylenediaminetetraacetic acid (EDTA), 1.21 NaH_2PO_4, and 2.0 3-(N-morpholino)propanesulfonic acid (MOPS) without BSA at a constant flow rate (0.5 ml/min) with a peristaltic pump (model 203, Scientific Industries, Bohemia, NY). After an equilibration period, the arterioles developed spontaneous tone, and we assessed their responsiveness and viability by changing the extraluminal pH from 7.3 to 6.8 and from 7.3 to 7.65. Arterioles with poor tone (less than 20% decrease from the maximal passive diameter) or poor response to pH (less than 15% change in diameter after pH change) were excluded. In some vessels, we compared vessel tone and pH-induced responses at the beginning and end of experiments to confirm the stability of the preparation.

2.2. Experimental procedures

After cannulation and development of spontaneous tone we tested the vessel response to pH 6.8 and pH 7.65 to confirm vessel viability. Then we measured the vessel diameter dose–response to increasing extraluminal magnesium concentrations (2.1, 2.8, 3.5, 4.2 mmol/L, respectively) with a control $[Mg^{2+}]_o$ of 1.4 mmol/L after an equilibration period of 10–15 min for each concentration. The chosen increases in magnesium concentrations reflect physiologically and clinically relevant increases in $[Mg^{2+}]_o$ avoiding hypermagnesemia. Recovery back to control was also measured. To study the mechanisms involved in magnesium-induced vasodilation, we repeated the magnesium dose–response either after endothelial impairment or in the presence of inhibitors or spasmogens after equilibration (~60 min). To study the contribution of the endothelium on magnesium-induced vasodilation, the endothelium was impaired by passage of air through the arteriole at 60 mmHg intraluminal pressure. Air embolization does not necessarily remove the endothelium but has been shown to disrupt the endothelium in microvessels (Saito et al., 1994), and we previously confirmed to use sodium nitroprusside to ensure that vascular smooth muscle dilation was not changed before and after air embolization (Horiuchi et al., 2001). To assess endothelial damage, we applied adenosine tri-phosphate (ATP) extraluminally before and after air embolism. Complete endothelial impairment was confirmed by lack of dilation of ATP (Horiuchi et al., 2001). L-NNA (Nω-Nitro-L-arginine, 10 μmol/L) was used to inhibit endothelial nitric oxide (NO) production. Four potassium channel inhibitors were used as followed: (1) 30 μmol/L $BaCl_2$ (inward rectifier potassium (K_{IR}) channel inhibitor); (2) 1 mmol/L tetraethylammonium (TEA, non-specific calcium-sensitive potassium (K_{Ca}) channel inhibitor); (3) 100 μmol/L 4-aminopyridine (4-AP, voltage-dependent potassium (K_V) channel inhibitor); (4) 3 μmol/L glibenclamide (ATP-sensitive potassium (K_{ATP}) channel inhibitor). To simulate delayed cerebral vasospasm we applied several vasospasm-related vasoconstrictors including Endothelin-1 (ET-1, 1–21 peptides), Prostaglandin $F_{2\alpha}$ ($PF_{2\alpha}$) and Thromboxane A_2 (TXA_2) analog (U46619). All chemicals were applied extraluminally and obtained from Sigma–Aldrich, St. Louis, MO.

2.3. Statistical analysis

All data are presented as mean ± SEM, with n representing the number of observations. Statistics were conducted on absolute vessel diameters. All data analysis was done using a statistical software package (InStat, GraphPad Software, San Diego, CA). Differences were considered significant at $P < 0.05$ and determined by analysis of paired and Student's t-test. The data are presented as percent maximum diameter to correct for changed control diameters induced by treatments and was calculated

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