Developmental change in magnesium sulfate-induced relaxation of rabbit pulmonary arteries

JEAN-FRANCOIS TOLSA, YUANSHENG GAO, AND J. USHA RAJ
Department of Pediatrics, Harbor-UCLA Medical Center, University of California, Los Angeles, School of Medicine, Torrance, California 90502

Tolsa, Jean-Francois, Yuansheng Gao, and J. Usha Raj. Developmental change in magnesium sulfate-induced relaxation of rabbit pulmonary arteries. J. Appl. Physiol. 87(5): 1589–1594, 1999.—Magnesium causes a variety of vascular smooth muscle to relax. The present study was designed to determine whether there is a developmental change in the magnesium-induced response of pulmonary vasculature. Isolated pulmonary arteries (PA) of newborn (1- to 3-day-old) and juvenile (4- to 6-wk-old) rabbits were suspended in organ chambers filled with modified Krebs-Ringer bicarbonate solution (95% O2-5% CO2, 37.0°C), and their isometric tension was recorded. In arteries preconstricted with endothelin-1 to a similar tension level, MgSO4 caused greater relaxation of juvenile rabbit PA than that of the newborn rabbit PA. Verapamil, a voltage-dependent Ca2+ channel blocker, attenuated magnesium-induced relaxation in juvenile rabbit PA but not in newborn PA. The uptake of Ca2+ of juvenile rabbit PA was inhibited by MgSO4, and the inhibition was attenuated by verapamil. The uptake of Ca2+ of newborn rabbit PA was smaller than that of the juvenile PA and was not significantly affected by MgSO4 and verapamil. These results demonstrate that there is a developmental increase in the dilator effect of MgSO4 in rabbit PA. In newborn rabbit PA, an incomplete maturation of the voltage-dependent Ca2+ channels may contribute to the smaller vasodilation induced by MgSO4.

perinatal pulmonary circulation; verapamil; voltage-dependent calcium channels; vasorelaxation

MAGNESIUM IS THE SECOND most plentiful cation of the intracellular fluid. It plays an important role in neurochemical transmission, muscular excitability, and regulation of vascular tone (3, 4, 6, 7, 19, 20). In a variety of blood vessels, an increase in extracellular magnesium concentration inhibits vascular contractile tension (2, 4, 5, 24, 32, 33). Magnesium has been shown to reduce acute hypoxia-induced pulmonary vasoconstriction (1, 9, 10) and to attenuate experimentally induced pulmonary hypertension (21). In clinical studies, intravenous magnesium has been proposed as effective in the treatment of persistent pulmonary hypertension in term (1, 30) and preterm (39) neonates.

Substantial evidence suggests that magnesium may modulate vasoactivity by affecting the influx of extracellular Ca2+ (3, 4, 6–8, 34). Agonists may stimulate the entry of extracellular Ca2+ through voltage-dependent Ca2+ channels and receptor-operated Ca2+ channels (26, 29). In cardiac, uterine, basilar arterial, and airway smooth muscle, extracellular magnesium has been shown to block voltage-dependent Ca2+ channels (12, 25, 28). In whole-cell patch-clamp studies done on capillary endothelial cells, high extracellular magnesium concentrations have been shown to reversibly depress the Ca2+ current (11). It is possible, therefore, that inhibition of voltage-dependent Ca2+ channels by magnesium may be one of the mechanisms by which the pulmonary vasculature relaxes during intravenous treatment with magnesium. However, the effect of magnesium on Ca2+ entry in newborn pulmonary vessels is not known and is likely to be different from that in the adult, as the structure and pharmacology of Ca2+ channels in newborn pulmonary vessels may be variable during the perinatal period as of the anatomic, functional, and pharmacological changes after the birth process in the first weeks of life (17, 18).

In the present study, we hypothesize that there is a developmental change in voltage-dependent Ca2+ channels. Such a difference affects relaxation of pulmonary vessels induced by magnesium. We tested this hypothesis in isolated pulmonary arteries of newborn and juvenile rabbits.

METHODS

Tissue preparation. Forty newborn rabbits (1–3 days old, either sex, 73.8 ± 2.2 g) and twenty-two juvenile rabbits (4–6 wk old, either sex, 1.75 ± 0.06 kg) were used. They were New Zealand White rabbits purchased from Irish Farms (Norco, CA). The newborn rabbits were killed by pentobarbital sodium (300 mg/kg ip) and by exsanguination. The juvenile rabbits were anesthetized with ketamine hydrochloride (30 mg/kg im) and killed by pentobarbital sodium (30 mg/kg) given by ear vein injection and by exsanguination.

The lungs were removed immediately and placed in a cold modified Krebs-Ringer bicarbonate solution of the following composition (mM): 118.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25.0 NaHCO3, and 11.1 glucose. As defined by Weibel and Taylor (38), who designated the left and right main branches of pulmonary arteries as the first, third, and fourth generation, pulmonary arteries were dissected from the lungs, cleaned of visible connective tissue, and cut into rings. The diameters of the rings were 0.62 ± 0.02 mm (n = 24) for the newborns and 1.54 ± 0.09 mm (n = 22) for the juveniles.

Organ chamber studies. Rings of pulmonary arteries were suspended in organ chambers filled with 10 ml of the modified Krebs-Ringer solution described in Tissue preparation, maintained at 37.0°C, and aerated with 95% O2-5% CO2 (pH 7.4). Each ring was suspended by two stirrups passed through the lumen. One stirrup was anchored to the bottom of the organ chamber, and the other was connected to a force displacement strain-gauge transducer (model FT03C, Grass Instruments, Quincy, MA) for the measurement of isometric force (15). A permanent record of the force developed by each ring was obtained by using a multichannel recorder. At the beginning of every experiment, pulmonary artery rings were brought to their optimal resting tension by stretching the tissues progressively until their contractile response to 100 mM KCl was maximal. The optimal resting tensions of the vessel rings were 0.50 ± 0.05 g/mm2 smooth muscle cross-sectional area

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(\text{CSA}_{\text{sm}}; n = 24) for the newborns and 0.27 ± 0.03 g/mm² for the juveniles; the method to determine the CSA_{sm} is described in Determination of CSA_{sm}. One hour of equilibration was allowed after tissues were brought to their optimal resting tension (15).

Experimental protocols. To determine the developmental change in voltage-dependent Ca²⁺ channels of pulmonary vessels, the response of these vessels to KCl (20–100 mM) was examined. It is known that potassium causes vasoconstriction predominantly by increasing the influx of Ca²⁺ into cells through voltage-dependent channels (26). The response of these vessels to endothelin-1 (10⁻¹⁰ M to 3 × 10⁻⁷ M) was also determined. Endothelin causes vasoconstriction not only by stimulating the influx of extracellular Ca²⁺ but also by mobilizing the intracellular Ca²⁺, sensitizing myofilaments to Ca²⁺, and by other mechanisms (27).

To determine the vasodilator effect of magnesium, pulmonary vessels of newborn and juvenile rabbits were preconstricted with different concentrations of endothelin-1 (3 × 10⁻⁹ to 10⁻⁸ M) to a similar tension level. After the contraction became stable, the effect of MgSO₄ (2–8 mM) was determined.

To evaluate the role of voltage-dependent Ca²⁺ channels in the vasorelaxant effect of magnesium, pulmonary vessels of newborn and juvenile rabbits were pretreated with verapamil (10⁻⁵ M; a voltage-dependent Ca²⁺ channel blocker (29)) or solvent (distilled water, 0.58% of organ chamber volume). These vessels were contracted with different concentrations of endothelin-1 (3 × 10⁻⁹ to 2 × 10⁻⁸ M) to a similar tension level, and then the effect of MgSO₄ was evaluated.

To eliminate a possible involvement of prostaglandins and endothelium-derived nitric oxide (14, 15), all the experiments mentioned above were performed in the presence of indomethacin (3 × 10⁻⁵ M) and nitro-L-arginine (10⁻⁵ M), inhibitors of cyclooxygenase (36) and nitric oxide synthase (22), respectively. These inhibitors had no significant effect on the resting tension and endothelin-induced contraction of pulmonary arteries of newborn and juvenile rabbits (data not shown).

Determination of CSA_{sm}. To properly compare the constriction of pulmonary arteries of newborn and juvenile rabbits, vessel tensions have been standardized as previously described (14). First, the total tissue cross-sectional area (CSA_{tot}) was obtained by dividing the wet weight of the vessel (mg) by vessel density (mg/mm²) + optimal length of the vessel (mm). The vessel density was obtained by dividing the blotted wet weight of the vessel from its volume. The volume was determined by measuring the volume of Krebs-Ringer bicarbonate solution displaced by the vessel rings after the tissues were placed in a 1-ml graduated cylinder with an accuracy of 0.01 ml. The optimal length was determined, with the aid of a magnifying eyepiece and a micrometer with an accuracy of 0.01 mm, by measuring the distance between two crossbars passed through the lumen of the vessel under the optimal resting tension of the vessel.

After the CSA_{tot} was obtained, the CSA_{sm}/CSA_{tot} ratio was obtained by counting the total area and the area occupied by smooth muscle cells on a histological transverse section of the pulmonary arteries (5-µm thickness) viewed under a microscope. The histological sections were treated with either hematoxylin and eosin or Van Giesson stains to discriminate between smooth muscle cells and other components. The CSA_{sm}/CSA_{tot} ratios obtained from hematoxylin and eosin and from Van Giesson stains were averaged. The mean values were multiplied by the CSA_{tot} to obtain the CSA_{sm} of the vessel.

Ca²⁺ uptake. Ca²⁺ uptake was determined by using methods described by Godfraind (16) and by Turlapaty and Altura (32). Pulmonary artery rings were weighed and then placed in 10-ml vials containing a HEPES buffer containing the following composition (mM): 144.0 NaCl, 5.8 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 5.0 HEPES, 11.1 d-(+)-glucose, 10⁻⁵ M indomethacin, and 10⁻⁴ M nitro-L-arginine. The HEPES buffer was maintained at 37.0°C and aerated with 95% O₂-5% CO₂ (pH 7.4).

After a 1-h equilibration, vessel rings were exposed to 4⁵Ca²⁺ (3 µCi; specific activity: 10.82 mCi/mg; NEN Life Products, Boston, MA). Five minutes later, endothelin-1 (3 × 10⁻⁹ M and 10⁻⁸ M for vessels of juvenile rabbits and newborn rabbits, respectively) was added to the vials. Twenty minutes later, different concentrations of MgSO₄ or solvent were administered. In some experiments, the effect of magnesium on Ca²⁺ uptake was determined in the presence of verapamil (10⁻⁵ M). In these experiments, verapamil was added at least 45 min before incubation with Ca²⁺.

Twenty minutes after the administration of MgSO₄, vessel rings were taken out and placed individually into tubes containing 10 ml of an ice-cold Ca²⁺-free HEPES solution containing 50 mM LaCl₃ for 60 min. Then, the tissues were washed with the same ice-cold LaCl₃ solution (16). Afterward, pulmonary artery rings were blotted and transferred into tubes containing 5 ml EDTA (5 mM) and left overnight at room temperature. The next day, 5 ml of EDTA solution were mixed with 10 ml of scintillant (Ecolite+, ICN Biomedical, Irvine, CA) and the radioactivity was counted. The results of each determination have been converted to the apparent tissue content of 4⁵Ca²⁺ according to the following formula (16)

\[ \text{Calcium uptake (mmol/kg wet wt)} = \frac{\text{cpm in muscle}}{\text{muscle wet wt (kg)} \times \text{mmol Ca²⁺/l medium}} + \frac{\text{cpm in medium/l medium}} \]

where cpm is counts per minute.

Drugs. The following drugs were used: EDTA, HEPES, indomethacin, LaCl₃, verapamil (Sigma Chemical, St. Louis, MO); endothelin-1 (Peptides International, Louisville, KY); and N⁶-nitro-L-arginine (RBI, Natick, MA). Indomethacin was prepared with an equimolar amount of Na₂CO₃. This concentration of Na₂CO₃ did not significantly affect the pH of the solution in the organ chambers (15). All the other drugs were dissolved by using distilled water. Concentrations are expressed as final molar concentration in the organ chamber or in the incubation vial.

Data analysis. Contractions are expressed in grams per millimeter CSA_{sm}. Relaxations were expressed as percentage of tension elicited by pretreatment with endothelin-1. Data are shown as means ± SE. When mean values of two groups were compared, Student’s t-test for unpaired observations was used. When the mean values of the same group before and after stimulation were compared, Student’s t-test for paired observations was used. Comparison of mean values of more than two groups was made with one-way ANOVA test, with Student-Newman-Keuls test for post hoc testing of multiple comparison. Statistical significance was accepted when the P value (2 tailed) was <0.05. In all experiments, n represents the number of rabbits studied.

RESULTS

Organ chamber studies. The wet weights, optimal length, and CSA_{sm} of vessel rings used in the study were significantly different between pulmonary arter-
cies of newborn rabbits and those of juvenile rabbits. There is no significant difference in the tissue densities and in the CSA_{sm}/CSA_{tot} ratio between these two vessel types (Table 1).

KCl (20–100 mM) and endothelin-1 (10^{-10} M to 3 \times 10^{-7} M) caused a greater increase in tension in pulmonary arteries of juvenile rabbits than in those of newborn rabbits. In pulmonary arteries of the newborn rabbits, the maximal contraction induced by KCl was \sim 30\% of that induced by endothelin-1. For the vessels of juvenile rabbits, there is no significant difference in the maximal contraction between that induced by KCl and that by endothelin-1 (Fig. 1).

The effect of MgSO_{4} was examined in arteries preconstricted with different endothelin-1 concentrations (3 \times 10^{-9} M to 10^{-8} M) to a similar tension level (1.14 \pm 0.18 g/mm^{2} CSA_{sm} and 1.28 \pm 0.22 g/mm^{2} CSA_{sm} for the vessels from newborn and juvenile rabbits, respectively; n = 6–7, P < 0.05). After the contraction became stable, the administration of MgSO_{4} induced a concentration-dependent relaxation. The relaxation was significantly greater in arteries of juvenile rabbits than in those of newborn rabbits (Fig. 2).

Verapamil [10^{-5} M; a voltage-dependent Ca^{2+} channel blocker (29)] had no significant effect on the basal tension of pulmonary arteries of newborn and juvenile rabbits. After a 45-min exposure to verapamil, the vessels were contracted with endothelin-1 (3 \times 10^{-9} to 3 \times 10^{-8} M) to a tension similar to vessels that were not treated with verapamil (data not shown, n = 6 for each group; P < 0.05). In pulmonary arteries of juvenile rabbits, pretreatment with verapamil significantly reduced the vasodilator effect of MgSO_{4} (8 mM), whereas verapamil had no significant effect on the relaxation induced by MgSO_{4} in pulmonary arteries of the newborns (Fig. 3).

Ca^{2+} uptake. Under control conditions (1.2 mM MgSO_{4}), the Ca^{2+} uptake of pulmonary arteries of newborn and juvenile rabbits for 45 min was 0.23 \pm 0.02 mmol/kg wet wt tissue (n = 7) and 0.34 \pm 0.03 mmol/kg wet weight tissue (n = 8), respectively. These values are significantly different (P < 0.05). MgSO_{4} induced a concentration-dependent inhibition in the Ca^{2+} uptake of pulmonary arteries of newborn and juvenile rabbits.

Table 1. Morphological data for pulmonary arteries of newborn and juvenile rabbits

<table>
<thead>
<tr>
<th></th>
<th>Newborn Rabbit Pulmonary Artery</th>
<th>Juvenile Rabbit Pulmonary Artery</th>
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<tbody>
<tr>
<td>Vessel wet wt, mg</td>
<td>1.69 \pm 0.09 (24)</td>
<td>4.95 \pm 0.34 (22)*</td>
</tr>
<tr>
<td>Density, mg/mm^{3}</td>
<td>1.02 \pm 0.02 (6)</td>
<td>1.03 \pm 0.02 (6)</td>
</tr>
<tr>
<td>L_{o}, mm</td>
<td>0.97 \pm 0.03 (24)</td>
<td>2.42 \pm 0.15 (22)*</td>
</tr>
<tr>
<td>CSA_{sm}, mm</td>
<td>0.68 \pm 0.04 (24)</td>
<td>0.95 \pm 0.05 (22)*</td>
</tr>
<tr>
<td>CSA_{sm}/CSA_{tot}</td>
<td>H&amp;E stain</td>
<td>0.38 \pm 0.03 (6)</td>
</tr>
<tr>
<td></td>
<td>Van Giesson stain</td>
<td>0.39 \pm 0.02 (6)</td>
</tr>
</tbody>
</table>

Values are means \pm SE. Nos. in parentheses are no. of animals. L_{o}, optimal length of vessel ring; CSA_{sm}, cross-sectional area occupied by smooth muscle. CSA_{sm}/CSA_{tot}, ratio of CSA_{sm} to total cross-sectional area determined by using transverse histological section treated with hematoxylin and eosin (H&E) or Van Giesson stain. *Significantly different from pulmonary arteries of the newborn rabbits; P < 0.05.
of the juvenile pulmonary arteries but had no significant effect on the Ca\textsuperscript{2+} uptake of the newborn pulmonary arteries (Fig. 4).

In vessels pretreated with verapamil (10\textsuperscript{-5} M), the Ca\textsuperscript{2+} uptake of pulmonary arteries of newborn and juvenile rabbits was similar [0.21 ± 0.01 mmol/kg wet weight tissue (n = 7) and 0.24 ± 0.02 mmol/kg wet wt tissue (n = 8), respectively]. In the presence of verapamil, the reduction in the Ca\textsuperscript{2+} uptake of pulmonary arteries of juvenile rabbits caused by MgSO\textsubscript{4} (8 mM) was significantly attenuated. Verapamil had no significant effect on the Ca\textsuperscript{2+} uptake of pulmonary arteries of newborn rabbits (Fig. 5).

**DISCUSSION**

Magnesium as a vasodilator has been reported in a variety of vessel types (6). However, few studies have been done in isolated pulmonary vessels. Villamor et al. (37) found that, in 10- to 17-old-day piglets, magnesium is a weak dilator of isolated pulmonary arteries. The maximal reduction in tension of preconstricted pulmonary arteries was <20%. Such an observation is in line with our present finding in pulmonary arteries of newborn rabbits. In contrast, magnesium caused marked relaxation of pulmonary arteries of juvenile rabbits. These results demonstrate that there is a developmental increase in the vasorelaxant effect of magnesium in the rabbit lungs.

A rise in intracellular Ca\textsuperscript{2+} in smooth muscle cells is thought to be one of the key events for the initiation and the maintenance of contraction, with the inverse being true for relaxation (35). When stimulated with a variety of vasoconstrictors, extracellular Ca\textsuperscript{2+} may enter into the cell through voltage-dependent Ca\textsuperscript{2+} channels and receptor-operated Ca\textsuperscript{2+} channels (26, 29). In airway smooth muscle, electrophysiological studies have shown that magnesium inhibits voltage-dependent Ca\textsuperscript{2+}

![Graph](image)

**Fig. 4.** Effect of MgSO\textsubscript{4} on Ca\textsuperscript{2+} uptake of pulmonary arteries of newborn and juvenile rabbits. Values are means ± SE; n = 7–8 for each group. *Significantly different from newborn, P < 0.05. †Significantly different from control (1.2 mM MgSO\textsubscript{4}), P < 0.05. ‡Significantly different from vessels treated with 4.0 mM MgSO\textsubscript{4}, P < 0.05.

![Graph](image)

**Fig. 5.** Effect of MgSO\textsubscript{4} (8 mM) on Ca\textsuperscript{2+} uptake of pulmonary arteries of newborn and juvenile rabbits under control conditions or in the presence of verapamil (10\textsuperscript{-5} M). Values are means ± SE; n = 7–8 for each group. †Significantly different from control, P < 0.05.

channel current. The inhibition is quantitatively similar to MgSO\textsubscript{4}-induced relaxation of trachea smooth muscle strips (28). In the present study, MgSO\textsubscript{4}-induced relaxation of pulmonary arteries of juvenile rabbits was attenuated by verapamil, a voltage-dependent Ca\textsuperscript{2+} channel blocker (29). Furthermore, the inhibition of Ca\textsuperscript{2+} uptake caused by MgSO\textsubscript{4} in the juvenile pulmonary arteries was attenuated by verapamil. Hence, inhibition of Ca\textsuperscript{2+} entry through voltage-dependent Ca\textsuperscript{2+} channels may contribute to vasodilation of pulmonary arteries of juvenile rabbits caused by magnesium.

The voltage-dependent Ca\textsuperscript{2+} channels seem to be less well developed in pulmonary arteries of newborn rabbits in comparison to those of the juveniles. A similar suggestion was advanced earlier for newborn piglet arteries (17). It is well known that contraction of smooth muscle evoked by potassium results predominantly from extracellular Ca\textsuperscript{2+} entry via the voltage-dependent channels (26). In our study, the maximal contraction of pulmonary arteries of the newborn rabbits to KCl was only 15% of that of pulmonary arteries of the juveniles. Furthermore, verapamil reduced the Ca\textsuperscript{2+} uptake of the vessels from juvenile rabbits but had no significant effect on the Ca\textsuperscript{2+} uptake of the vessels from newborn rabbits. In addition, verapamil attenuated MgSO\textsubscript{4}-induced relaxation of the arteries from juvenile rabbits but had no significant effect on MgSO\textsubscript{4}-induced relaxation of the arteries from newborn rabbits. These observations indicate that the difference in the vasodilation effect of magnesium between the pulmonary arteries of the newborn rabbits and those of the juveniles is likely due to a difference related to the voltage-dependent Ca\textsuperscript{2+} channels.

Magnesium modulates the influx of extracellular Ca\textsuperscript{2+} into the cell not only via the voltage-dependent channels but also via the other pathways (5, 23, 31). For instance, in rat cultured aortic smooth muscle, magnesium inhibits receptor-mediated Ca\textsuperscript{2+}-permeable nonselective cation channels (23). It is interesting to note...
that the relative role of the voltage-dependent and receptor-operated Ca\(^{2+}\) channels in the effect of magnesium differs in vascular smooth muscle of Wistar-Kyoto rats and spontaneously hypertensive rats. In Wistar-Kyoto rats, extracellular magnesium modulates cytosolic Ca\(^{2+}\) concentration primarily through the voltage-dependent Ca\(^{2+}\) channels. In spontaneously hypertensive rats, extracellular magnesium affects cytosolic Ca\(^{2+}\) concentration through voltage-dependent Ca\(^{2+}\) channels, non-voltage-dependent Ca\(^{2+}\) channels, and the intracellular Ca\(^{2+}\) stores (3, 7, 31). The roles of the later two mechanisms in magnesium-induced vasodilation in the lungs are not clear.

Clinical studies have shown that MgSO\(_4\) infusion, to achieve a magnesium blood concentration between 3.5 and 5.5 mmol/l, can be an effective therapy for persistent pulmonary hypertension in preterm and term neonates (1, 30, 39). However, and under similar magnesium concentrations, results obtained from isolated animal newborn pulmonary arteries of our present study and those of others show that magnesium has only a moderate vasodilator effect (37). In in vivo studies, the observed effects of magnesium reflect the actions of magnesium on the whole pulmonary vascular tree. In contrast, our present results and those of others show that magnesium has more pronounced in small pulmonary arteries and arterioles and venules to constrictor agents: an in situ study on magnesium-induced vasodilation in the lungs.

We thank J. van Morris for technical assistance. This study was supported by National Heart, Lung, and Blood Institute Grants HL-38438 and HL-59435. J.-F. Tolsa was sponsored by Swiss grants (les Fonds du Département de Pédiatrique et de Perfectionnement du Centre Hospitalier Universitaire Vaudois, la Société Académique Vaudoise, and la Fondation Emma Muschop, Lausanne, Switzerland).

Received 12 March 1997; accepted in final form 30 June 1999.

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