Interactions between magnesium and drugs in cell membranes

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Zusammenfassung

^{28}MG-Fluxe wurden an der Media-Innenschicht von Kaninchenaorten gemessen. Aus einer niedrig konzentrierten Lösung (4 µM) akkumuliert ^{28}Mg in der glatten Gefäßmuskulatur mit der Zeit während einer 3stündigen Beobachtung. Dagegen fanden sich äquilibrierte ^{28}Mg-Aufnahme-Mengen innerhalb von 20 Minuten aus Lösungen mit 1,5 mM nicht-markiertem Mg. Die Rate des ^{28}Mg-Effluxes war in Gegenwart von 1,5 mM nicht-markiertem Mg nach einer Verzögerung von 5–10 Minuten erhöht; dagegen war der Efflux schnell und deutlich nach EDTA (0,5–1,5 mM) erhöht. Die zelluläre Akkumulation von ^{28}Mg wurde signifikant gehemmt durch 60 mM K^+ (80 %), 1,5 oder 15,0 mM La^{++} (93 %) bzw. 98 %), 1 mM Lidocain (36 %), 0,1 mM Oubain (47 %), 7 mM Neomycin (71 %), 4,08 mg/ml Gentamycin (52 %), 1 µg/ml Antimycin A (34 %). Diese Kationen bzw. Pharmaka beeinflußten den ^{28}Mg-Efflux nicht signifikant, wenn sie während der langsamen Auswaschphase zugegeben wurden. Hieraus folgt, daß akkumuliertes Mg^{2+} anscheinend gespeichert und intrazellulär ausgetauscht wird und nur sehr gering an oberflächlichen Austauschstellen vorhanden ist. EDTA könnte die ^{28}Mg-Efflux-Raten durch Erhöhung der Permeabilität der vaskulären Membran steigern, während Ionen und Pharmaka das zelluläre Mg^{2+} eher durch verminderte Aufnahme als durch erhöhte Verluste absenken.

Summary

Fluxes of ^{28}Mg were examined in the rabbit aortic media-intimal layer. Accumulation of ^{28}Mg in rabbit aortic smooth muscle from a low Mg^{++} (4µM) solution increased with time over a three-hour period. In contrast, equilibrated levels of ^{28}Mg uptake were obtained within 20 min from solutions containing 1.5 mM nonradioactive Mg^{++}. The rate coefficient of ^{28}Mg efflux was enhanced by 1.5 mM nonradioactive Mg^{++} following a delay of 5–10 minutes, whereas the efflux rate was increased rapidly and in a sustained manner by EDTA (0.5–1.5 mM). Cellular accumulation of ^{28}Mg was inhibited significantly by 60 mM K^+ (80 %), 1.5 or 15.0 mM La^{+++} (93, 98 %), 1 mM lidocaine (36 %), 0.1 mM ouabain (47 %), 7 mM neomycin (71 %), 4.08 mg/ml gentamicin (52 %), 1 µg/ml antimycin A (34 %), or 1 µg/ml oligomycin (32 %). These cations and drugs did not significantly alter ^{28}Mg efflux when added during the slow component phase of the washout. Thus, accumulated Mg^{++} appears to be stored and exchanged intracellularly with very little present at surface exchangeable sites. EDTA may enhance the ^{28}Mg efflux rate by increasing the permeability of the vascular membrane, whereas ions and drugs decrease cellular Mg^{++} content by reducing uptake rather than increasing loss.

Résumé

Nous avons étudié les flux de Mg^{28} dans la média et l'intima d'aorte de lapin. Sur une période de trois heures, nous avons constaté une accumulation croissante de Mg^{28} dans les muscles lisses d'aorte de lapin en présence d'une solution peu concentrée en Mg^{++} (4 µm). En revanche, lors des expériences menées avec des solutions contenant 1,5 mmole de Mg^{++} non radioactif, on a enregistré en l'espace de 20 minutes une captation équilibrée de Mg^{28}. Le coefficient d'efflux de Mg^{28} augmentait avec un temps de latence de 5 à 10 minutes sous l'effet de 1,5 mmole de magnésium non radioactif, alors que sous l'effet de l'EDTA (0,5–1,5 mmole) la vitesse d'efflux augmentait rapidement et de façon soutenue. L'accumulation cellulaire de Mg^{28} était significativement inhibée par 60 mmoles de K^+ (80 %), 1,5 ou 15 mmoles de La^{+++} (93 %, 98 %), 1 mmole de lidocaïne (36 %), 0,1 mmole de ouabaïne (47 %), 7 mmoles de néomycine (71 %), 4,08 mg/ml de gentamicine (52 %), 1 µg/ml d'antimycine A (34 %) ou 1 µg/ml d'oligomycine (32 %). Ces cations et médicaments ne modifiaient pas significativement l'efflux de Mg^{28} quand on les ajoutait pendant la phase lente du wash-out. Il semble donc que le Mg^{++} accumulé soit stocké et échangé dans les cellules et qu'il n'en existe que très peu au niveau des sites d'échange superficiels. Il est possible que l'EDTA augmente l'efflux de Mg^{28} en augmentant la perméabilité de la membrane vasculaire, tandis que les ions et médicaments diminuent la teneur cellulaire en Mg^{++} en réduisant la captation de Mg^{++} plutôt qu'en augmentant les pertes en Mg^{++}.

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Introduction

Though Mg^{++} is known to have critical roles in various cellular functions, few studies have been conducted to characterize transmembrane fluxes of Mg^{++} in isolated smooth muscle preparations [6]. Previous studies have used total tissue Mg^{++} content measurements to obtain indirect estimates of Mg^{++} fluxes. With these experimental approaches, the Mg^{++} contents of vascular smooth muscle have been shown to be very resistant to depletion, and only approximately half of

the original cation content was found to be exchangeable [12, 14, 16]. The studies to be discussed examine the binding and distribution of ^{28}Mg in the rabbit aorta as well as the effects of a number of cations and drugs on ^{28}Mg fluxes. The two ions studied were K^+ (the major monovalent intracellular cation) and La^{+++}, an ion known to affect transmembrane flux of Ca^{++} and Mg^{++} [4, 18] in various cellular systems. The seven drugs examined were the local anesthetic, lidocaine; the sodium pump inhibitor, ouabain, the divalent cation ionophore, A-23187; the aminoglycoside antibiotics, neomycin and gentamicin; and the mitochondrial poisons, antimycin A and oligomycin.

Materials and methods

New Zealand White rabbits (Marland Farms) were sacrificed by intravenous injection of sodium pentobarbital (50 mg/kg). The thoracic aorta was excised, cut into a spiral strip of 3 to 4 mm width, and the adventitia was separated from the media-intima as described previously [7].

The physiological salt solution contained (in mM): NaCl, 154; KCl, 5.4; $CaCl_2$, 1.5; glucose, 11.0; and tris (hydroxymethyl) aminomethane, 6. Solutions in which either Ca^{++} was omitted (0-Ca solution) or 1.5 mM Mg^{++} (as $MgCl_2$ $6H_2O$) was added, were also used. All solutions were adjusted to a pH of 7.4, maintained at 34.0 ± 0.5°C, and continuously aerated with 100 % oxygen.

Transmembrane fluxes of ^{28}Mg were studied with experimental approaches similar to those described previously for Ca^{++} movements [3,5,8-10]. For ^{28}Mg uptake measurements, muscles were preincubated for 30 min. in a nonradioactive solution similar to the incubation solution (unless specified otherwise), then transferred to the incubation solution containing ^{28}Mg (10 nM; specific activity, 1 – 2 μCi/ml) for 5 – 180 min. Excess superficial ^{28}Mg solution at the end of the incubation period was removed by rapidly rinsing and blotting the tissues, and radioactivity in the tissue was measured by liquid scintillation spectrometry as described elsewhere [13]. Tissue/medium ratios, representing the ratio (ml/g) of the amount of ^{28}Mg taken up per gram of wet tissue to the amount of ^{28}Mg per ml of the incubation solution, were calculated. Values for total Mg^{++} uptake (μmol/g) were calculated by multiplying the ^{28}Mg tissue/medium ratio by the concentration of Mg^{++} (μmol/ml) in the radioactive incubation solution. The calculations were based upon an extracellular (^{14}C-sucrose) space value of 0.41 ml/g [5].

For ^{28}Mg efflux experiments, muscles were incubated for 90 min with ^{28}Mg as in uptake experiments. After a similar removal of superficially-adhering ^{28}Mg, the muscles were placed into successive tubes containing 2 ml of washout solution. The bathing solution was changed after either 1 or 5 min periods for the duration of the washout (30 or 180 min). The amount of radioactivity in each washout sample was measured as before. Washout data were expressed either as desaturation curves (the percentage of initial radioactivity remaining after each time interval) or as rate coefficient plots (amount of radioisotope lost from the tissue per minute as a percentage of that present in the tissue during that minute of washout) as described by *Bianchi* [2] and *Weiss* [17].

Agents employed in this study were EDTA (Fisher Scientific), gallium trinitrate (Pfaltz and Bauer), lidocaine hydrochloride (Astra), ouabain (ICN), A-23187 (Calbiochem), neomycin sulfate (Sigma), gentamicin sulfate (Sigma), antimycin A (Sigma), and oligomycin (Calbiochem). The pH of all solutions was adjusted to 7.4 except those containing the less soluble Ga^{+++} (adjusted to pH 7.0). A-23187, antimycin A, and oligomycin were dissolved in 95 % ethanol and diluted with bathing solution to a final ethanol concentration of less than 1 % (v/v). Values are expressed as the mean ± S.E., and the differences between means were compared by Student's t-test ($p < 0.05$).

Results

The initial rate of calculated Mg^{++} uptake in the rabbit aortic smooth muscle is much greater in solutions containing 1.5 mM added Mg^{++} than in solutions without added Mg^{++} (Fig. 1). Uptake of ^{28}Mg in a 1.5 mM Mg^{++} solution shows very rapid equilibration (within 20 min), whereas uptake in a solution without added Mg^{++} increases with the duration of exposure to the radioisotope with about 80 % of the total 180 min accumulation occurring during the first 90 min of the incubation period. Uptake of total Mg^{++} in muscles incubated with 1.5 mM added Mg^{++} were significantly greater than the corresponding uptakes obtained in the absence of added nonradioactive Mg^{++} at all incubation time intervals.

Washout experiments were performed in order to examine the release and exchangeability of ^{28}Mg from the media-intimal layer of rabbit aorta.The effects of added nonradioactive Mg^{++} and EDTA on the rate of efflux of ^{28}Mg from rabbit aorta preloaded with the radioisotope for 90 min are summarized in Fig. 2. Addition of 1.5 mM Mg^{++} to the washout solution for 10 min

after a 20-min washout in a 0-Mg solution resulted in little change in the efflux rate of ^{28}Mg from smooth muscle (Fig. 2, top). A sustained increase in the efflux rate of ^{28}Mg was obtained with added Mg^{++} during a longer (180 min) washout, but only after an initial delay of 5–10 min (Fig. 2, bottom). In contrast to this, addition of 1.5 mM EDTA caused an immediate and maintained increase in the rate of efflux of ^{28}Mg from the muscle (Fig. 2, bottom).

Experiments were designed to examine the effects of Ca^{++}, La^{+++}, or Ga^{+++} on ^{28}Mg efflux. Media-intimal strips of rabbit aorta were incubated for 30 min in either 0-Ca Tris or Tris solution followed by an additional 90 min in the same solution plus ^{28}Mg. The muscles were then washed out for 20 min with either 0-Ca Tris or Tris solution and for an additional 10 min with the same solutions with 1.5 mM Ca^{++} or La^{+++} added to the 0-Ca solution, or 0.36 mM Ga^{+++} added to the Tris solution. The rate of efflux of ^{28}Mg from the tissues was not altered by exposure to any of the three added cations during the slow component phase of the ^{28}Mg washout.

The effects of several cations and drugs on total ^{28}Mg uptake were also measured. The two cations examined were K^+ (60 mM) and La^{+++} (1.5 and 15.0 mM), and the seven drugs investigated were lidocaine (1 mM), ouabain (0.1 mM), A-23187 (1 μM), neomycin (7 mM), gentamicin (4.08 mg/ml), antimycin A (1 μg/ml), and oligomycin (1 μg/ml). Ethanol (1 %, v/v), the solvent used for A-23187, antimycin A, and oligomycin, was also examined for effects on total ^{28}Mg uptake. Muscles were incubated with the indicated cation or drug for 90 min in presence of ^{28}Mg. All muscles (excluding those incubated in K^+ or A-23187) were subjected to an additional 30 min preincubation with the same solution without added ^{28}Mg before incubation with the radioactive solution. As can be seen in Table 1, the accumulation of Mg^{++} in the media-inti-

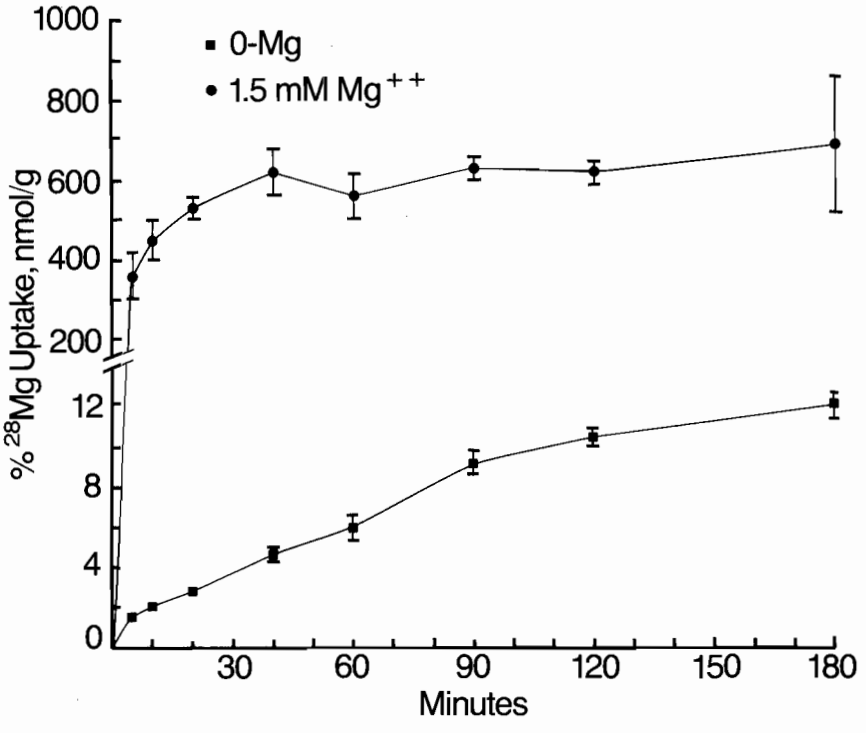

Fig. 1: Uptake of Mg^{++} in rabbit aortic smooth muscle. Values obtained represent averaged total Mg^{++} uptake in muscles incubated in either 1.5 mM Mg^{++} solution or in a solution with no added nonradioactive Mg^{++}. The 1.5 mM Mg^{++} was present during the ^{28}Mg incubation period as well as the preceding 30 min. Each point on the curves represents the average ± S.E. of three muscle strips

Tab. 1: Effects of cations and drugs on Mg^{++} uptake in rabbit aortic smooth muscle

^{28}Mg Incubation Conditions[1]	N	Mg^{++} Uptake[3]	Change in Mg^{++} Uptake
		nmol/g	%
Control	8	8.14 ± 0.40	
K^+ (60 mM)	4	1.64 ± 0.04	−79.9
La^{+++} (1.5 mM)	4	0.60 ± 0.08	−92.6
La^{+++} (15 mM)	4	0.20 ± 0.08	−97.5
Lidocaine (1 mM)	4	5.21 ± 0.72	−36.0
Ouabain (0.1 mM)	4	4.33 ± 0.28	−46.8
A-23187 (1 μM)	4	9.54 ± 1.04	+17.2
Neomycin (7 mM)	4	2.33 ± 0.12	−71.4
Gentamicin (4.08 mg/ml)	4	3.93 ± 0.24	−51.7
Antimycin A (1 μg/ml)	7	5.37 ± 0.60	−34.0
Oligomycin (1 μg/ml)	7	5.57 ± 0.76	−31.6
Ethanol (1 % v/v)[2]	3	7.30 ± 0.28	−10.3

[1] Muscles were incubated with the indicated cation or drug for 90 min in presence of ^{28}Mg. All muscles (excluding those incubated in K^+ or A-23187) were preincubated for an additional 30 min in the same solution without ^{28}Mg

[2] Ethanol (1 %, v/v) was the solvent used for A-23187, antimycin A and oligomycin

[3] Mg^{++} uptake was calculated as the product of the extracellular Mg^{++} concentration (approximately 0.004 mM, present as trace contaminants in the bathing solution) and the difference between the ^{28}Mg tissue/medium ratio and the extracellular (^{14}C-sucrose) space of 0.41 ml/g. Values are expressed as means ± S.E

mal layer of rabbit aortic strips is inhibited significantly by all agents examined except A-23187. The inhibition of Mg^{++} accumulation ranged from 32 % (oligomycin) to 98 % (La^{+++}, 15 mM).

The inhibitory effects obtained with the two cations (K^+ and La^{+++}), an aminoglycoside antibiotic (neomycin), and a mitochondrial poison (antimycin A) were studied in washout experiments to ascertain whether the agents produced inhibitory effects on ^{28}Mg accumulation by increasing the loss of ^{28}Mg from the muscle or by blocking the uptake of ^{28}Mg. Washout experiments with ^{28}Mg indicated that the four cations and drugs did not affect the loss of radioisotope from the muscle when added to the bathing solution during the washout slow component phase (final 10 min of a 30 min washout). Washouts of ^{28}Mg into 0-Mg solution after incubation for 90 min with ^{28}Mg in similar solutions containing each of the four inhibitory agents were also performed. The muscles were preincubated for an additional 30 min in the presence of each of the agents before incubation with ^{28}Mg. Fig. 3 includes summarized desaturation curves for control muscles as well as for muscles incubated with neomycin or antimycin A. The slow components of the two latter washout curves are parallel to the control curve but their relative sizes are significantly smaller. Qualitatively similar results were obtained following incubations with K^+ or La^{+++}. Analysis of the amount of Mg^{++} present in each of the two washout components (Tab. 2) indicates that all of the cations and drugs decreased ^{28}Mg uptake into the slow component, whereas only La^{+++} inhibited ^{28}Mg uptake into the fast component.

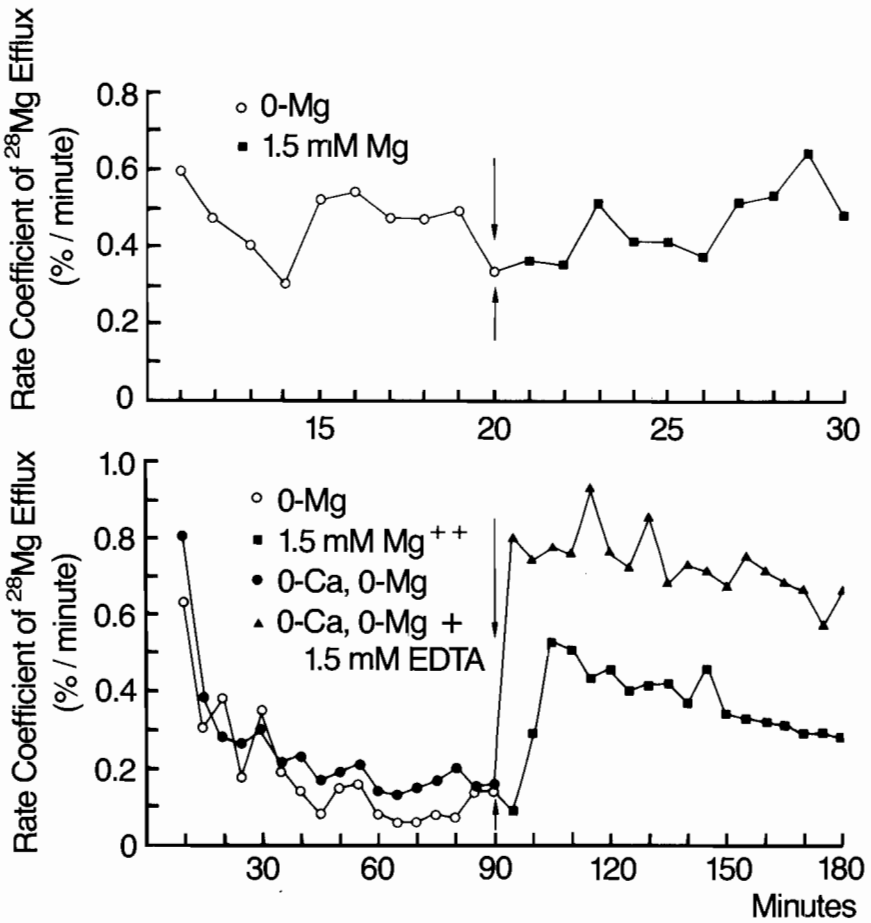

Fig. 2: Effect of 1.5 mM Mg^{++} or 1.5 mM EDTA on the rate at which ^{28}Mg is removed from the media-intimal layer of rabbit aorta. Muscles were incubated with ^{28}Mg for 90 min and then washed out either in 0-Ca, 0-Mg solution for 90 min or in 0-Mg solution for 20 min (top) or 90 min (bottom). Mg^{++} or EDTA was added to the bathing solution for the remainder of the washout as indicated by arrows. N = 3

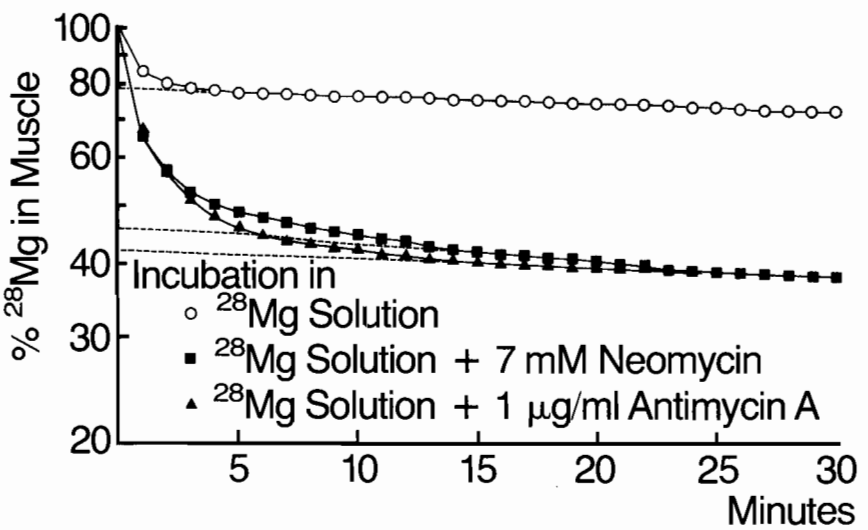

Fig. 3: Effects of exposure to 7 mM neomycin or 1 µg/ml antimycin A on subsequent slow washout ^{28}Mg component in rabbit aortic media intimal layer. Desaturation curves represent averaged washouts for three muscles. Dashed lines indicate extrapolation of the slow washout components to the beginning of the washout. Muscles were preincubated for 30 min with the indicated drug and for an additional 90 min in solutions containing both drug and ^{28}Mg before washout in 0-Mg solution

Discussion

In accordance with earlier observations in guinea-pig taenia coli [15] and rat uterus [11], uptake of Mg^{++} in the media-intimal strip of rabbit aorta was found to be a slow process. Uptake of ^{28}Mg in rabbit aorta occurred in two phases; an initial rapid phase observed within the first 5 min of incubation followed by a slower phase with a timecourse dependent on the extracellular concentration of Mg^{++}. The major portion of the Mg^{++} uptake during the rapid phase probably represents equilibration of Mg^{++} into the extracellular space. The second slow phase may reflect accumulation of Mg^{++} at previously depleted Mg^{++} binding sites and/or exchange with Mg^{++} at intracellular sites or stores. The accumulation of Mg^{++} in the rabbit aorta increased with the duration of incubation in the ^{28}Mg solution containig no added nonradioactive Mg^{++}, whereas muscles in 1.5 mM Mg^{++} solution showed equilibrated levels of Mg^{++} uptake within about 20 min of incubation in the radioactive solution. The exclusion of nonradioactive MG^{++} from the ^{28}Mg solution incerased the specific activity of the radioactive solution and, in this manner, enhanced the uptake of ^{28}Mg at Mg^{++} binding sites.

Previous studies have indicated that only a minor portion of vascular smooth muscle Mg^{++} is readily released after the tissue is transferred to a 0-Mg solution [12, 14]. These findings have been confirmed in this study of ^{28}Mg accumulation in rabbit aorta. As indicated in Fig. 3, the amount of ^{28}Mg retained by rabbit aorta at the end of a 30 min washout in 0-Mg solution is greater than 70 % of the amount present initially in the muscle. Thus, a major portion of the ^{28}Mg taken up by the vascular smooth muscle behaves as if it were bound and not readily depleted in the absence of extracellular Mg^{++}.

Tab. 2: Effect of incubation with cations or drugs on ^{28}Mg washout components in the media-intimal layer of rabbit aorta

Incubation Conditions[1]	Total Mg^{++} Uptake[2]	Fast Component		Slow Component	
		Uptake	Relative Size	Uptake	Relative Size
	nmol/g	nmol/g	%	nmol/g	%
Control	13.07	2.81	21.5	10.26	78.5
60 mM K^{+}	2.73	1.80	66.0	0.93	34.0
1.5 mM La^{+++}	0.84	0.60	72.0	0.24	28.0
7 mM Neomycin	3.33	1.82	54.7	1.51	45.3
1 µg/ml Antimycin A	4.85	2.81	58.0	2.04	42.0

[1] Muscles were incubated with the indicated cation or drug for 90 min in presence of ^{28}Mg. All muscles (except those incubated in K^{+}) were preincubated for an additional 30 min in the same solution without ^{28}Mg

[2] Mg^{++} uptake was calculated as the product of the extracellular Mg^{++} concentration (approximately 0.004 mM, present as trace contaminants in the bathing solution) and the difference between the ^{28}Mg tissue/medium ratio and the extracellular (^{14}C-sucrose) space of 0.41 ml/g. N = 3

In order to study the exchangeability of bound Mg^{++}, washout experiments were performed in which 1.5 mM nonradioactive Mg^{++} was added to the bathing soluting during the slow component phase of the washout. Exposure to nonradioactive Mg^{++} (1.5 mM) for periods of 10 min caused no significant change in the rate of efflux of ^{28}Mg. This indicates that very little ^{28}Mg is available at superficial membrane sites for exchange with nonradioactive Mg^{++} and, also, that the slow component of ^{28}Mg efflux represents loss of intracellular Mg^{++}. The increases in rate of ^{28}Mg efflux following addition of 1.5 mM Mg^{++} to the bathing solution for periods longer than 5 – 10 min demonstrate the presence of an exchangeable intracellular ^{28}Mg pool. The delay in increased rate of ^{28}Mg efflux following addition of nonradioactive Mg^{++} indicates that nonradioactive Mg^{++} must first traverse the muscle membrane and, subsequently, displace intracellularly located and exchangeable ^{28}Mg.

In contrast to the delayed effect on ^{28}Mg loss produced by added nonradioactive Mg^{++}, EDTA (0.5 or 1.5 mM) elicited an immediate and sustained increase in the rate of ^{28}Mg efflux when added during washout in a 0-Ca, 0-Mg solution. Since EDTA is known to be confined to the extracellular space [2,19], the effect of this chelator of divalent cations on the rate of efflux of ^{28}Mg could be attributed to a nonspecific increase in muscle membrane permeability caused by removal by EDTA of superficial (stabilizing) divalent cations. Deficiency of external Ca^{++} and Mg^{++} is known to increase membrane permeability in rabbit aortic smooth muscle [1]. The lack of similar immediate effects (within 10 min) of added Ca^{++} (1.5 mM), La^{+++} (1.5 mM), or Ga^{+++} (0.36 mM) on ^{28}Mg efflux rate indicates that these cations do not modify membrane permeability to Mg^{++}.

The total uptake of Mg^{++} was found to be significantly inhibited by a variety of cations (K^{+};

La^{+++}) and drugs (lidocaine, ouabain, neomycin, gentamicin, antimycin A, and oligomycin). A more detailed analysis of the mechanism of action of four of these substances (K^+, La^{+++}, neomycin, and antimycin A) indicates that none of these agents altered the rate of efflux of ^{28}Mg when added during the slow component phase of a ^{28}Mg washout into a 0-Mg solution. Thus, these substances do not displace intracellularly located Mg^{++}. Information about the particular fraction of ^{28}Mg uptake inhibited by each of the above four agents was obtained by comparison of ^{28}Mg washout components in a 0-Mg solution following incubation with ^{28}Mg and different inhibitory agents in 0-Mg solutions. Analysis of the size of the two washout components showed that all 4 agents reduced the slower component of ^{28}Mg efflux but only La^{+++} reduced the fast efflux component to values that approximated the extracellular space [5]. The reduction in ^{28}Mg washout slow component magnitude in the presence of various inhibitory agents supports the view that all of these agents decrease Mg^{++} accumulation by reducing intracellular uptake of ^{28}Mg.

Thus, Mg^{++} accumulates slowly in the rabbit aortic smooth muscle and also exchanges slowly with added nonradioactive Mg^{++}. The accumulated Mg^{++} is stored and exchanged intracellularly with very little present at surface-exchangeable sites. The increased rate of ^{28}Mg efflux elicited with EDTA may result from nonspecific membrane permeability increases, but ions and drugs that decrease cellular Mg^{++} accumulation appear to act by reducing uptake rather than by increasing loss.

Acknowledgement

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