ORIGINAL ARTICLE

Effects of magnesium sulfate on airway smooth muscle contraction in rats

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ABSTRACT

Aim To investigate the effect of magnesium sulfate ($MgSO_4$) at different doses on isolated tracheal smooth muscle contraction in rats induced by different mechanisms.

Methods Twelve rats' tracheas were placed into organ bath. Consecutively, acetylcholine (10^{-6},10^{-5},10^{-4} M), histamine (10^{-8},10^{-5},10^{-3} M) and KCl (30,60 mM) solutions was administered for contractions. $MgSO_4$ from 10^{-4} to 10^{-1} M concentrations were subsequently administered after each constrictive agent and relaxation degrees were recorded.

Results In the acetylcholine and KCl groups, dose dependent strong contractions were observed, but not in the histamine group and that group was excluded. Significant relaxation occurred with gradually increasing doses of $MgSO_4$. In the high dose KCl group, a slight increase in contractions after the administration of 10^{-4} and 10^{-3} M $MgSO_4$ was recorded.

Conclusion We suggest that $MgSO_4$ is effective in relaxing airway smooth muscle contractions caused by different factors; however, it must be considered that low doses of $MgSO_4$ may only lead to a slight increase in contractions.

Key words: acetylcholine, histamine, potassium chloride

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INTRODUCTION

Magnesium (Mg) is the fourth most commonly found cation in the human body and the second most important intracellular one (1). Magnesium plays an active role in many enzyme systems. It acts as a natural antagonist of calcium in the cell (2). Low magnesium concentrations induce contractions; however, hypocalcemia stimulates relaxation (3). Low magnesium concentrations cause rapid and passive calcium release from the sarcoplasmic reticulum. Magnesium competitively blocks calcium entry into presynaptic endings (4). High magnesium levels reduce acetylcholine (ACh) release from the presynaptic interval. In addition, magnesium reduces the ACh effects on postsynaptic muscle receptors. It has been shown to be effective on the axonal stimuli threshold (5). Hypomagnesaemia has been shown to cause neuromuscular hyperexcitability, whereas hypermagnesemia is reported to cause neuromuscular weakness and loss of deep tendon reflexes (6). Magnesium has effects on adrenal medulla and adrenergic postganglionic sympathetic fibers, excess serum magnesium concentrations produce progressive inhibition of catecholamine release (7,8).

The first study of magnesium effects on bronchial smooth muscle was published by Trendelenburg in 1912 (9) showing that magnesium could cause bovine bronchial smooth muscle relaxation. However, since then, despite known effects, it has not entered routine clinical use. In fact, magnesium sulfate ($MgSO_4$) is known to be used by asthmatic cases by intravenous (IV), inhaler and oral routes (10). In recent years, the clinical importance of magnesium has increased because of updated information about magnesium's action as a direct calcium agonist receptor; as a result, magnesium plays a role reducing ACh release and depolarization in nerve muscle junctions affects prostaglandin-dependent smooth muscle relaxation and is necessary for β-agonist receptor complex interactions. It has been reported to be beneficial in severe acute bronchospasm attacks, particularly those that do not respond to other treatments (11-13).

The aim of this study was to investigate–the bronchodilator effects of different doses of MgSO4 in bronchospasm induced by ACh, histamine, and potassium chloride (KCl), showing effects through different mechanisms in isolated rat trachea in the *in vitro* environment.

MATERIALS AND METHODS

The study was performed after an approval had been obtained from the Ethics Committee of Suleyman Demirel University, Isparta, Turkey, for Animal Studies.

Twelve adolescent, female, 250-350 g Wistar albino rats were used. After mild ether anesthesia, the animals were sacrificed, and chests were speedily explored soon after which tracheas were removed. Adjacent lipid and connective tissues were cleaned with maximum care to prevent any damage. Three millimeter ring cross-sections were prepared from the trachea and thereafter, were bound to little triangular shaped wires and to a transducer via tight strings. A micro positioner coupled with a transducer was placed in a four channel isolated organ bath, which contained 25 mL *Krebs* solution in each channel. Thus, the experiment could be carried within four tracheal rings concurrently. One gram of tension was applied to the hanging rings that were resting at 37°C continuous 95% O_2 and 5% CO_2 conditioned Krebs' solution for 1.5 h to allow adaptation to the medium, while the solutions were replaced every 15 min.

All tracheas were first pretreated with 10^{-6} M ACh. Ten-minute contractions were awaited to reach a plateau. Formed contractions were first released by means of 10^{-4} M $MgSO_4$ administration and afterwards were gradually increased to 10^{-3}, 3×10^{-3}, 5×10^{-3}, 10^{-2}, 3×10^{-2}, and finally 10^{-1} M at 10 min intervals. After the process the tissues were left to rest for 30 min along with Krebs solution refreshing every 15 min. All procedures were then followed for 10^{-5} and 10^{-4} M ACh.

Histamine was administered after the rest. A 10^{-8} M dose was first used before the 10-min wait for stabilization and then higher doses were used (10^{-5} and 10^{-3} M) following a rest period for each dose.

A dose of 30 mM KCl was administered first after the rest. However, a 20-min interval for stabilization was set apart from other groups. 10^{-4}, 10^{-3}, 3×10^{-3}, 5×10^{-3}, 10^{-2}, 3×10^{-2}, and 10^{-1} M $MgSO_4$ administrations followed within 10-min intervals. The procedure was repeated with 60 mM KCl treatment after 30-min rest (Figure 1).

In all groups the first values measured before drug application were recorded as control valu-

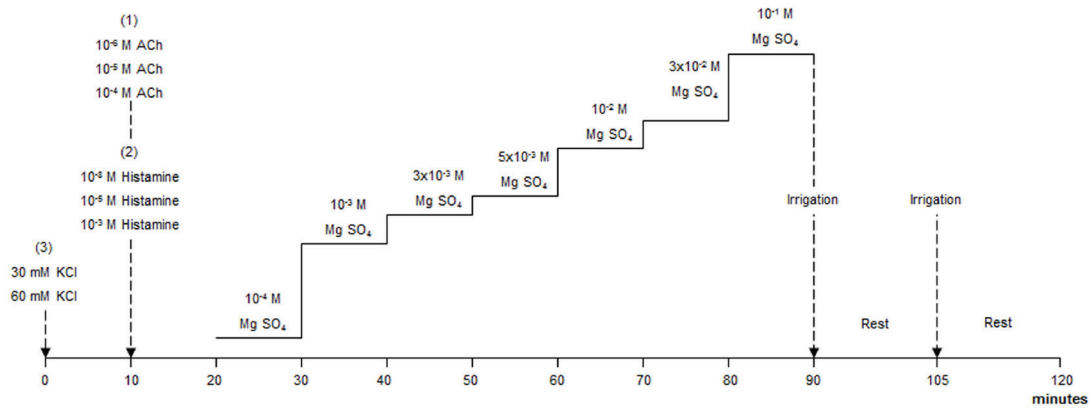

Figure 1. Procedure of the experiment applied to time course. (1) 10^{-6} M ACh was first administered to the tracheae, following a 10 minute interval. 10 minute relaxation periods were observed after 10-minute wait with increasing doses of $MgSO_4$, afterwards, cleansing twice with a 15-minute rest was given. Next, the same procedure was repeated with 10^{-5} and 10^{-4} M ACh; 10^{-8}, 10^{-5}, and 10^{-3} M histamine (2); and 30 and 60 mMKCl (3). The rest was adjusted to 20 minute for KCl group with exception ACh, acetylcholin; $MgSO_4$, magnesium sulfate; KCl, potassium chloride

es, and the measured values after the contracting agent was administered were recorded as contraction values. Results after $MgSO_4$ administration were calculated, and the relaxation was evaluated by a percentage value (contraction value: 0%; control value: 100%).

For statistical analyses, the significance of differences was evaluated by using Wilcoxon test; p value<0.05 was considered significantly different.

RESULTS

10^{-6}M ACh administration achieved a weak contraction, whereas increasing concentrations provided better responses. A dose of 10^{-4} M $MgSO_4$, administered after 10^{-6} M ACh did not produce dilatation, however increasing concentrations succeeded. Doses of 10^{-3} M and 10^{-2}M, provided evident dilatation ($p<0.05$). Concentrations beginning from 3×10^{-2} M had much more dilated muscles than those in the controls. The highest dilatation value was 140% (Table 1, Figure 2A).

Table 1. Relaxation values after $MgSO_4$ applications

MgSO4 concentration	10-6 M ACh	10-5 M ACh	10-4 M ACh	30 mM KCl	60 mM KCl
10-4 M	0±0	-0,87±7,00	-2,32±6,63	5,05±13,95	-7,70±8,94†
10-3 M	27,65±23,67*	1,5±8,98	-0,35±9,03	39,75±21,17*	-8,01±12,88†
3x10-3 M	36,87±21,46*	10,05±10,27*	2,41±8,38	87,84±22,35*	1,82±15,33
5x10-3 M	48,07±25,03*	16,18±10,25*	6,32±9,82	103,63±21,22*	13,23±17,88*
10-2 M	74,50±21,49*	27,54±8,98*	11,6±11,32*	108,14±19,36*	52,97±18,51*
3x10-2 M	116,61±28,94*	57,35±19,30*	30,37±17,86*	109,97±19,27*	107,43±17,89*
10-1 M	140,04±16,59*	93,57±19,16*	71,69±15,20*	109,97±19,27*	107,43±17,89*

The values are described as percentage (contraction value: 0%; control value: 100%; mean±SD); *p < 0.05, when consisting relaxing response compared to basal muscle tonus; †p < 0.05, when consisting contracting response compared to basal muscle tonus; Ach, acetylcholine; KCl, potassium chloride;

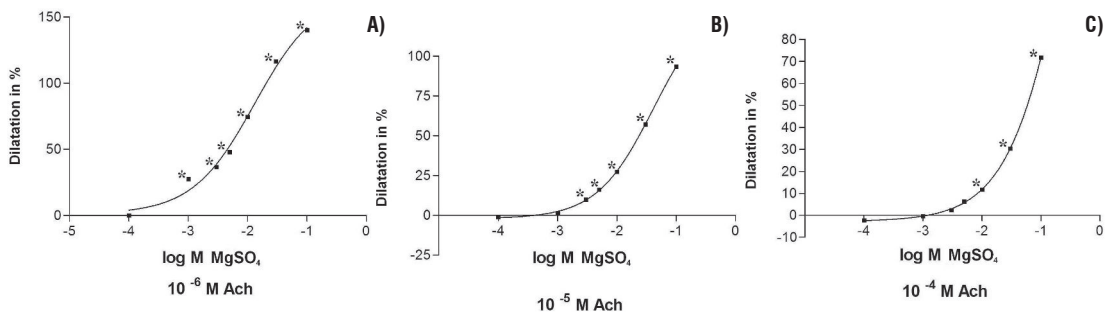

Figure 2. Logarithmical relaxation curve caused by increasing doses of magnesium sulfate ($MgSO_4$) over tracheal rings, which are contracted with A) 10^{-6} M, B) 10^{-5} M, and C) 10^{-4} M acetylcholine (ACh) *p<0.05

Muscles contracted with 10^{-5} M ACh were given 10^{-4} M $MgSO_4$, and a minor contraction was observed but the results were not significant ($p>0.05$). 10^{-3} M $MgSO_4$ did not achieve a dilatation ($p>0.05$), whereas evident dilatations were seen at higher doses ($p<0.05$). Concentrations $>3 \times 10^{-2}$ M provided fast dilatation. The most powerful dilatation was 93.6% (Table 1, Figure 2B).

10^{-4} M ACh provided clearly a stronger contraction initially. Though low doses (10^{-4} and 10^{-3} M) of $MgSO_4$ provided a mild contraction, the results were not significant ($p>0.05$). $MgSO_4$ gave significant results when the doses were elevated from 10^{-3} M to 10^{-2} M ($p<0.05$) with the knowledge that none of the doses gave a complete dilatation. The strongest dilatation was 71.7% (Table 1, Figure 2C).

No significant contraction response occurred after 10^{-8}, 10^{-5}, and 10^{-3} M histamine administrations. It was concluded that histamine had no contractile effects on the trachea, and thus, $MgSO_4$ was not administered to this group (Table 1).

A starting contraction response was produced from administration of KCl in a dose-dependent manner. The 30 mM KCl group gave no response of significant dilatation after 10^{-4} M $MgSO_4$ ($p>0.05$), nevertheless 10^{-3} M and more concentrated doses provided a faster dilatation ($p<0.05$). Doses beginning at 5×10^{-3} M provided stronger dilatations than those of starting values. The dilatation occurred when the dose of $MgSO_4$ was incrementally increased from 10^{-3} M up to 10^{-2} M was statistically significant ($p<0.05$). The strongest dilatation was 110% (Table 1, Figure 3A).

10^{-4} and 10^{-3} M MgSO4 administration to the 60 mM KCl group provided mild contractions ($p<0.05$). With increasing doses, dilatation responses were mounted. A dose of $\geq 3\times 10^{-2}$ M dilated the muscles more than the starting concentrations did. The differences in dilatation of 10^{-2} M were significant ($p<0.05$). The most intense dilatation was 107.4% (Table 1, Figure 3B).

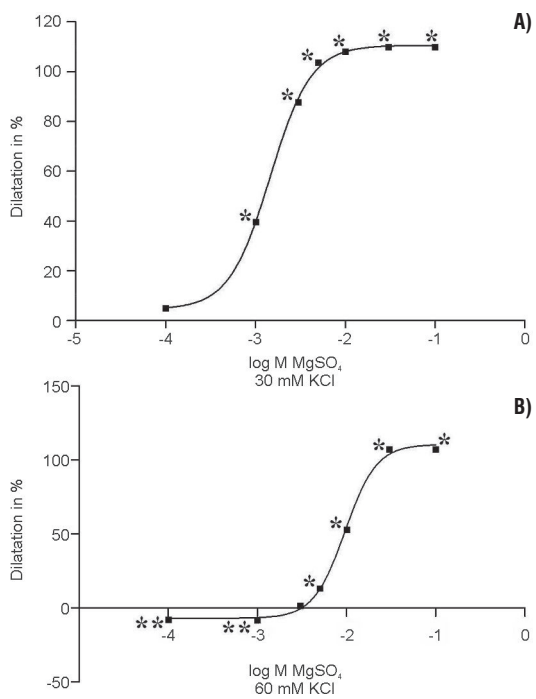

Figure 3. Logarithmical curve of relaxation caused by increasing doses of magnesium sulfate ($MgSO_4$) over tracheal rings, which are contracted with potassium chloride (KCl) of A) 30 mM (*$p<0.05$) **and B) 60 mM** (*$p<0.05$ when consisting relaxing response compared; **$p<0.05$ when consisting contracting response compared)

DISCUSSION

Airway smooth muscle contraction is the main component of acute asthma and other bronchospasms. Release of histamine, ACh and other mediators via inhaled allergenic or some other stimulants may cause bronchoconstriction. There are two major mechanisms in the pathophysiology of smooth muscle contraction. The first mechanism includes Ca^{++} influx into the cell via ligand-gated ion channels or Ca^{++} release from intracellular stores (14,15). ACh, histamine, hormones, and many drugs act via this pathway. The second mechanism is the influx of Ca^{++} via voltage-gated ion channels because of electrical depolarization of the membrane (16). KCl causes smooth muscle contractions by means of this mechanism (17). In this study, we performed these two different mechanisms for the airway smooth muscle contraction rat model.

In the present study, the application of 30-60 mM KCl and 10^{-6} to 10^{-4} M ACh was observed to provide strong and dose-dependent contractions. Within these applications, the strongest contractions were obtained by 10^{-4} M ACh. Kumasaka et al. (18) studied the contraction responses of the trachea to 10^{-9} to 10^{-4} M carbachol and 10-140 mM KCl administration in an organ bath prepared with three different doses of $MgSO_4$ (1.2, 2.2, and 9.2 mM). Maximum contraction values were found to be similar in both carbachol and KCl conditions for each concentration of $MgSO_4$. Fifty percent of maximum contraction values (EC_{50})

differed in KCl group according to the dose of $MgSO_4$, whereas it was found similar for each carbachol doses. Gourgoulianis et al. (19) reported a strong contraction produced by 85 mM KCl and 10^{-4} M ACh. The results of these two latter studies were similar with the results of the present study. However, distinct results have been reported according to the location of the airway segment after the application of different substances in the literature (20). In a comprehensive study, bradykinin, histamine, neurokinin-A, serotonin, substance-P, tromboxane-A_2 (TxA_2) mimetic, carbachol and carbachol plus potassium were compared with regard to their bronchoconstrictive effects on the trachea, bronchi and bronchiole of Wistar and Fisher 344 rats (21). The most potent effect was exerted by carbachol on all airway segments; serotonin, bradykinin, and TxA_2 mimetics were found more effective on bronchioli, and carbachol plus potassium was more effective on trachea. On the basis of the given data, we considered that the use of the rat trachea would be an appropriate experimental model for evaluating the efficacy of ACh and KCl on tracheal contraction.

Although histamine is known to have a considerable bronchoconstrictor effect in both humans and animals (19), no significant contractions were determined in different doses of histamine in our study. In the mechanism of bronchoconstriction, histamine directly induces the smooth muscle cells and afferent endings leading to an increase in release of other bronchoconstrictor endogen mediators. Nowadays, histamine has been frequently used in provocation studies of asthmatic patients (22-24). This effect of histamine was also shown in animal studies. Hirota et al. (25) reported bronchoconstriction due to the use of 10 μM histamine in both *in vivo* and *in vitro* dog model. In contrast, there are also other studies reporting histamine as a weak bronchoconstrictor agent *in vitro*. Voorde et al. (21) described that the concentration of histamine used the same way we used it caused no effect in rat tracheae and main bronchi, and reported a faint bronchoconstriction in bronchiole in their model. A similar study demonstrated a feeble contraction in an isolated pig trachea model with the use of histamine compared to ACh, and this effect could have been augmented by tetraethylammonium and K^+ administration (26). Substantially, deprivation of bronchoconstrictor effect of histamine in isolated rat trachea may be because of many factors. Isolation of trachea blocks the pathway, which the afferent nerves and other mediators act on. In addition, it is known that airways of various species react in different degrees to histamine (27). Guinea pig is the most sensitive animal to the bronchoconstrictor effect of histamine. This condition may arise because of the number of histamine receptors placed in the airway of animal (28).

The results of the present study show that the strong airway smooth muscle contraction achieved via ACh and KCl could be managed by $MgSO_4$ in a dose-dependent manner. Moreover, high doses of $MgSO_4$ administrations were observed to produce a considerable dilatation as compared to control, particularly the KCl group. However, even high doses of $MgSO_4$ did not exert complete dilatation in the high dose ACh group. Similar results were also demonstrated in previous studies. Kumasaka et al. (18) reported that while $MgSO_4$ could revert the tracheal contraction, which occurred as a result of KCl and KCl + ACh administration, it was observed to be insufficient for reversing the tracheal contraction induced by only ACh itself. Gourgoulianis et al. (19) have revealed a complete dilatation of constructed tracheas induced by KCl after $MgSO_4$ administration, and reported residual contractions with $MgSO_4$ after giving ACh. This condition was interpreted in a way that $MgSO_4$ could block the voltage-gated ion channels stimulated by KCl, whereas it cannot affect the receptor-dependent channels and the intracellular Ca stores stimulated by ACh.

The debates about the use of magnesium for clinical treatment of bronchospasm continue. It is noteworthy that magnesium is successful in the treatment of severe acute asthma attacks. Skobeloff et al. (29) showed that for patients with acute severe asthma attacks IV applying magnesium treatment positively affected peak expiratory flow (PEF) values and duration of hospital stay. On the other hand, some serious clinical researchers have reported that MgSO4 infusion is not effective (30-31). In some randomized controlled studies of children the use of magnesium was recommended, while other studies reported negative results (32-34). These different interpretations in the literature may be related to the infusion duration and dose of MgSO4. In our study, all high doses of MgSO4 administered for bronchoconstriction caused by different mechanisms were shown to cause dilatation (even more than initial values). However, we observed that the use of low dose MgSO4 resulted in insufficient dilatation. There appear to be similar conclusions in clinical studies in the literature. While all studies

using 2 g MgSO4 with infusion durations longer than 20 min were ineffective (34,35), in studies with high concentrations and rapid administration like 10-20 g MgSO4 over 1 hour (36) and 2 g MgSO4 in less than 2 min (37) effective treatment was reported. The results of our study lead to the consideration that the use of clinically higher doses of MgSO4 may be beneficial. Additionally, care should be taken in terms of toxic dose.

We obtained an unexpected finding from the present study that low doses of $MgSO_4$ administration provided a mild increase in tracheal contraction in the high-dose KCl group. A similar contraction was also observed with 10^{-4} M $MgSO_4$ administration in the high-dose ACh group. This effect may be because of K^+ channel inhibition or a direct electrical stimulant causing contraction, although the design of this study did not include such these applications. This condition was not reported in literature. Nevertheless, some clinical studies showed that low dose and slow infusion of $MgSO_4$ is ineffective in treating bronchospasm (38,39). If the finding we have experienced in rat trachea *in vitro* is the same in humans, low doses of $MgSO_4$ used in the treatment of bronchospasm may produce a more severe reaction. Further studies are required to resolve this issue.

Nitric oxide (NO) related smooth muscle dilatation is another issue. Nitric oxide is a bronchodilator, plays a significant role in the physiological regulation of airway function, but the effect is not completely clear (40). Nitric oxide synthase (NOS) is an enzyme for the production of NO, which exists in severa1 isoforms. Constitutive NOS (cNOS) isoforms are inducible by intracellular calcium ions. Also, magnesium acts as a natural calcium antagonist. The other form of NOS, iNOS, is activated by proinflammatory cytokines. Nitric oxide is produced by a variety of cells in the airways. The cNOS is involved in the regulation of bronchial blood flow and in neutrally mediated bronchodilation. However, NO may also have deleterious effects as it may increase bronchial blood flow and plasma exudation in the airways. It is also possible that the production of endogenous NO results in a long-term deleterious effect, and may be involved in the orchestration of eosinophilic inflammation that characterizes asthma. The iNOS may be expressed in macrophages and in epithelial cells. Respiratory tract infections may induce iNOS expression and NO production is increased in epithelial cells in case of infection (41). These mechanisms can be *in vivo* although we used isolated tracheas in our study. Therefore, we think that these factors are not effective in our experiment.

The limitations of our study include studying isolated rat trachea to avoid effects on afferent nerves and via other mediators. Another limiting factor is that while we studied the trachea it is known that both muscle constrictor and dilatator medications have different effects on different localizations in the airway. It will be beneficial to complete future studies with these targets in mind.

In conclusion, we obtained evident dilatations with increasing doses of $MgSO_4$ in the contracted tracheal smooth muscle produced by both ACh and KCl in various doses. Besides that, histamine appears to be an inappropriate agent to produce smooth muscle contraction in trachea isolated from rats. We suggest that low doses of $MgSO_4$ could cause a slight increase in airway smooth muscle contraction. However, further studies are needed to confirm our proposals.

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TRANSPARENCY DECLARATIONS

Competing interest; none to declare.

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