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RESEARCH

Rosuvastatin relaxes rat thoracic aorta, pulmonary artery, and trachea via nitric oxide, prostanoids, and potassium channels

Rosuvastatin, sıçan torasik aortunu, pulmoner arterini ve trakesini nitrik oksit, prostanoidler ve potasyum kanalları yoluyla gevşetir

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Abstract

Purpose: This study aimed to determine the functional effects and mechanisms of the action of rosuvastatin on vascular and tracheal smooth muscle tissues.

Materials and Methods: Vascular and tracheal rings (2-3 mm) isolated from the thoracic aortas, pulmonary arteries, and tracheas of Wistar Albino male rats (250-300 g) were placed in chambers in the isolated tissue bath system. As the resting tension, 1 g was selected. Vascular rings contracted with 10-6 M phenylephrine after a 90-minute equilibration period. Tracheal rings contracted with 10-5 M acetylcholine. After the contraction was steady, rosuvastatin (10-8-10-4 M) was cumulatively applied to the vascular and tracheal rings. The defined experimental methodology was repeated following the incubation of selective inhibitors of signaling pathways and K+ channel blockers to ascertain rosuvastatin's functional effect mechanisms.

Results: In the precontracted rat vascular and tracheal rings, rosuvastatin induced concentration-dependent relaxation. The maximal relaxation level in vessel samples was 96%. On the other hand, the maximal relaxation level in tracheal samples was found to be 75%. The vasorelaxant effects of rosuvastatin were dramatically attenuated by endothelium removal, L-NAME treatment, and indomethacin incubation (up to 27%). With the incubation of tetraethylammonium, glyburide, 4-Aminopyridine, and anandamide, rosuvastatin-mediated vascular smooth muscle relaxation levels were significantly decreased (up to 38%). Moreover, With the incubation of tetraethylammonium, glyburide, and 4-Aminopyridine rosuvastatin-mediated tracheal smooth muscle relaxation levels were significantly decreased (up to 30%).

Conclusion: Rosuvastatin has a noticeable relaxing effect on the vascular and tracheal smooth muscles. The vasorelaxant effect of rosuvastatin involves intact

Öz

Amaç: Bu çalışma rosuvastatin'in vasküler ve trakeal düz kas dokuları üzerindeki işlevsel etkilerini ve etki mekanizmalarını belirlemeyi amaçladı.

Gereç ve Yöntem: Wistar Albino erkek sıçanların (250-300 g) torasik aortları, pulmoner arterleri ve trakealarından izole edilen vasküler ve trakeal halkalar (2-3 mm), izole doku banyosu sistemindeki haznelere yerleştirildi. Dinlenme gerimi olarak 1 g seçildi. Vasküler halkalar, 90 dakikalık dengeleme periyodundan sonra 10-6 M fenilefrin ile kasıldı. Trakeal halkalar ise 10-5 M asetilkolin ile kasıldı. Kasılma stabil hale geldikten sonra, rosuvastatin (10-8-10-4 M) vasküler ve trakeal halkalara kümülatif olarak uygulandı. Rosuvastatin'in işlevsel etki mekanizmalarını belirlemek için seçici sinyal yolak inhibitörleri ve K+ kanal blokerlerinin inkübasyonu sonrasında belirlenen deneysel metodoloji tekrar edildi.

Bulgular: Rosuvastatin, ön kasılma uygulanmış sıçan vasküler ve trakeal halkalarında doz bağımlı bir gevşeme oluşturdu. Damar örneklerindeki maksimal gevşeme düzeyi % 96 idi. Öte yandan, trake örneklerindeki maksimal gevşeme düzeyi % 75 olarak bulundu. Rosuvastatin'in vazodilatör etkileri, endotelin çıkarılması, L-NAME tedavisi ve indometazin inkübasyonu ile anlamlı olarak azaldı (% 27'ye kadar). Tetraethylammonium, gliburid, ve 4-Aminopiridin inkübasyonu ile rosuvastatin kaynaklı vasküler düz kas gevşeme düzeyleri önemli ölçüde azaldı (% 38'e kadar). Dahası, tetraethylammonium, gliburid ve 4-Aminopiridin inkübasyonu ile rosuvastatin ile uyarılan trakeal düz kas gevşeme düzeyleri anlamlı olarak azaldı (% 30'a kadar).

Sonuç: Rosuvastatinin vasküler ve trakeal düz kaslarda belirgin bir gevşetici etkisi vardır. Rosuvastatin'in vazodilatör etkisi, sağlam endotel, nitrik oksit, prostanoidler ve K+ kanalları (BKCa, KV ve KATP kanalları) ile ilişkilidir. Ayrıca, nitrik oksit, prostanoidler,

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endothelium, nitric oxide, prostanoids, and K+ channels (BKCa, KV, and KATP channels). Furthermore, nitric oxide, prostanoids, BKCa channels, KV channels, and KATP channels play a role in rosuvastatin-induced tracheal smooth muscle relaxation.
Keywords: Aorta, nitric oxide, potassium, pulmonary artery, rosuvastatin, trachea.

BKCa kanalları, KV kanalları ve KATP kanalları, rosuvastatin tarafından indüklenen trakeal düz kas gevşemesinde rol oynamaktadır.

Anahtar kelimeler: Aort, nitrik oksit, potasyum, pulmoner arter, rosuvastatin, trake.

INTRODUCTION

The common medication for treating hypercholesterolemia, statins, are selective HMG-CoA reductase (HMG-CoA) reductase inhibitors[1]. The most efficient medications for decreasing cholesterol levels nowadays are statins, which were discovered in the late 1970s[2,3]. It is proposed that statins, which are frequently used in medical clinics to prevent coronary artery disease and stroke, have pleiotropic effects separate from their cholesterol-lowering benefits. Improved endothelial dysfunction, antioxidant effects, stabilization of atherosclerotic plaque, and suppression of inflammatory responses are the key outcomes of these interventions[4–7].

One of the most remarkable pleiotropic effects of statins is their cardiovascular protective benefits[4–7]. Statins, which are known to be effective, particularly in treating cardiovascular disorders including atherosclerosis and heart attacks, have been found to relax vascular smooth muscle (VSM)[8-10]. Sönmez Uydes-Doğan et al. reported that pravastatin, atorvastatin, and cerivastatin were effective as vasorelaxants in rat thoracic aorta. The researchers have shown that the NO pathway and prostanoids play a role in the vaso-relaxing mechanism of action of statins[9]. In a recent study, it was determined that simvastatin stimulated vascular relaxation in rat thoracic aorta. Besides NO pathway activation, mechanisms such as activation of potassium channels and inhibition of calcium channels have been shown to contribute to simvastatin-induced vascular relaxation[11].

Diseases such as essential hypertension, pulmonary arterial hypertension, heart failure, asthma, and chronic bronchitis are common and difficult to treat. The discovery of new smooth muscle relaxant agents is important to effectively treat these diseases associated with increased activity of vascular and airway smooth muscle tissue. Rosuvastatin is an important statin with widespread clinical use. The data obtained in previous studies suggest that rosuvastatin may exert a vasodilatory effect and stimulate relaxation in different vessels such as the pulmonary artery outside the aorta and smooth muscle tissues such as the trachea. However, its effects and mechanisms of action on the functioning of the vascular and tracheal smooth muscles have not yet been thoroughly investigated. This study aimed to determine the possible relaxant effect and mechanism of action of rosuvastatin on vascular and tracheal smooth muscle tissues. The thoracic aorta, pulmonary artery, and trachea of rats were used to examine the functional effects and mechanisms of action of rosuvastatin. It was questioned whether endothelium, nitric oxide (NO) pathway, prostaglandins, and potassium (K^+) channel subtypes affect rosuvastatin-related vascular relaxation functions.

MATERIALS AND METHODS

Experimental procedure

This work was given ethical permission for it by the Local Ethics Committee for Animal Experimentation at Bursa Uludag University on 18/10/2022 with the number 2022-14/02. Wistar Albino rats (250-300 g, male) were used in the present study. The experimental animals were cared for by national and international guidelines. Experiments of the study were carried out in the Cardiovascular Physiology Laboratory of Bursa Uludag University Physiology Department.

At the beginning of the experiments, rats were decapitated without anesthesia. The tracheal and vascular tissues were rapidly dissected and immersed in the Krebs solution. The thoracic aorta, pulmonary artery, and trachea were prepared from surrounding connective tissues to form vascular and tracheal rings that were 2-3 mm. The isolated organ bath system was continuously gassed with a 95% O_2-5% CO_2 gas combination while the rings were mounted in 20 ml organ bath chambers filled with Krebs solution (in mM: 118 NaCl, 1.2 KH_2PO_4, 25 $NaHCO_3$, 4.8 KCl, 1.2 $MgSO_4 \cdot 7H_2O$, 11 $C_6H_{12}O_6 \cdot H_2O$, and 2.5 $CaCl_2 \cdot 2H_2O$). The temperature of the system was

maintained at a constant 37 °C. The vascular rings' basal tension was set at 1 g. On the other hand, the tracheal rings' basal tension was set at 1.5 g. A force-displacement transducer measured the tension in the vascular and tracheal rings (SS12LA force transducer, BIOPAC Systems, Inc. Aero Camino, USA). Signal recording and analysis were carried out using computer software (BIOPAC MP36, Santa Barbara, CA, USA) coupled with a 4-channel isolated tissue bath system (MAY IOBS99, Commat Ltd., Ankara, Turkey).

Acetylcholine (10^{-6} M)-induced relaxation of greater than 80% in phenylephrine pre-contracted vessel rings in all experiments served as confirmation that the endothelium (10^{-6} M) was intact. In certain studies, the vascular rings' endothelium was mechanically removed. The inner surface of the vascular rings was lightly rubbed to complete this process. Less than 10% of acetylcholine (10^{-6} M)-induced relaxation in phenylephrine (10^{-6} M) pre-contracted vessel rings served as confirmation of the absence of endothelium. A 90-minute equilibration time was applied before each experiment. Every 15 minutes during this time, the Krebs solution in the bath chambers was changed.

Rosuvastatin (10^{-8}-10^{-4} M) was cumulatively administered to endothelium-intact and endothelium-denuded vascular rings that had been precontracted with phenylephrine (10^{-6} M) to produce concentration-response curves. The same experimental protocol was repeated in the presence of Nω-nitro-L-arginine methyl ester (L-NAME; endothelial nitric oxide synthase (eNOS) inhibitor), indomethacin (non-selective cyclooxygenase (COX 1/2 inhibitor), and K^+ channel blockers (TEA, glyburide, and 4-Aminopyridine) to determine the mechanisms of action of rosuvastatin. The K^+ channel blockers and signaling pathway inhibitors employed in this study were given 30 minutes before the application of cumulative concentrations of rosuvastatin. The percentage of phenylephrine-induced maximal contraction level, considered 100%, was used to express all results.

Tracheal rings were contracted with 10^{-5} M acetylcholine. The maximal contraction level was accepted as 100%. A similar protocol was repeated with vascular rings in drug applications. It was discovered how cumulatively taking rosuvastatin affected tracheal rings (10^{-8}-10^{-4} M). K^+ channel blockers, L-NAME, and indomethacin were given 30 minutes before rosuvastatin injection to identify the mechanisms of action of rosuvastatin in the rat trachea. All experiments were performed as indicated in previous studies[12,13].

Drugs

The following substances were bought from Sigma-Aldrich: phenylephrine, acetylcholine, TEA, L-NAME, indomethacin, glyburide, 4-Aminopyridine, and rosuvastatin (St. Louis, Missouri, USA). L-NAME (10^{-4} M), tetraethylammonium (TEA; 1 mM), acetylcholine (10^{-5} or 10^{-6} M), 4-Aminopyridine (1 mM), phenylephrine (10^{-6} M), and rosuvastatin (10^{-8}-10^{-4} M) were dissolved in distilled water. Dimethylsulfoxide (DMSO) was used to dissolve indomethacin (5 μM) and glyburide (10 μM). Results were not significantly changed by the DMSO concentration used in the experiment. A vehicle group was formed as a control for drugs using DMSO as a solvent.

Statistical analysis

Power analysis was performed at the beginning of the study. While the power of the study was 0.80 and the alpha value was 0.05, the minimum sample size required was estimated at n=5 per group. Power analysis was done under GPower 3.1. SPSS v.23.0 program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The number of the vessel or tracheal rings used in each group was expressed as "n". The results were presented as mean±standard deviation. The maximal smooth muscle tone levels induced by phenylephrine and acetylcholine were accepted as 100%. The mean percentages of muscle tone values after drug administration were used in the statistical analysis. One-way ANOVA test was used for multiple comparisons. Bonferroni post hoc test was used to determine the differences between groups. P values less than .05 were regarded as statistically significant.

RESULTS

In the rat thoracic aorta that had been precontracted with phenylephrine, rosuvastatin treatment (10^{-8}-10^{-4} M) led to vasorelaxation in a concentration-dependent manner ($p<.001$). Around 88% was found to be the greatest relaxation level (Figure 1). In the rat pulmonary artery that had been precontracted with phenylephrine, rosuvastatin treatment (10^{-8}-10^{-4} M) led to vasorelaxation in a concentration-dependent manner ($p<.001$). Around 96% was found to be the

greatest relaxation level (Figure 2A). Rosuvastatin's ability to relax blood vessels was markedly reduced when the endothelium was removed. In endothelium-denuded vessel rings, the highest percentage of relaxation was 34% (Figure 2B).

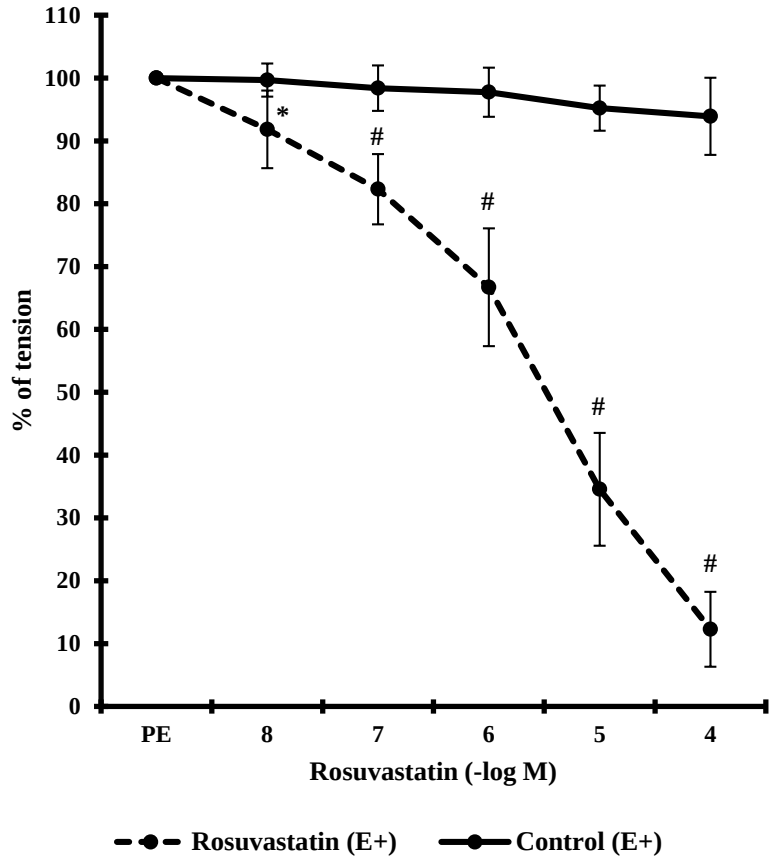

Figure 1. Concentration-dependent vasorelaxant effect of rosuvastatin in E+ rat thoracic aorta. n=8. *: p<.05. #: p<.001. E+: Endothelium-intact. PE: Phenylephrine.

Incubation with L-NAME significantly reduced the vasorelaxant effect level of rosuvastatin (p.<001) (Figure 2C). The degree of rosuvastatin's vasorelaxant effect was likewise significantly reduced after indomethacin application (p.<001) (Figure 2D).

In vascular rings that had been pre-contracted with KCl, the level of rosuvastatin's vasorelaxant action was significantly reduced. In these vessel rings, the highest relaxation level was around 24% (Figure 3A). TEA, a large-conductance calcium (Ca^{2+})-activated K^+ (BK_{Ca}) channel blocker, 4-Aminopyridine, a voltage-gated K^+ (K_V) channel blocker, and glyburide, an ATP-sensitive K^+ (K_{ATP}) channel blocker, significantly reduced the vasorelaxant effect level of rosuvastatin in rat pulmonary artery (p-values at maximal concentration <.001 in all groups) (Figure 3B-D).

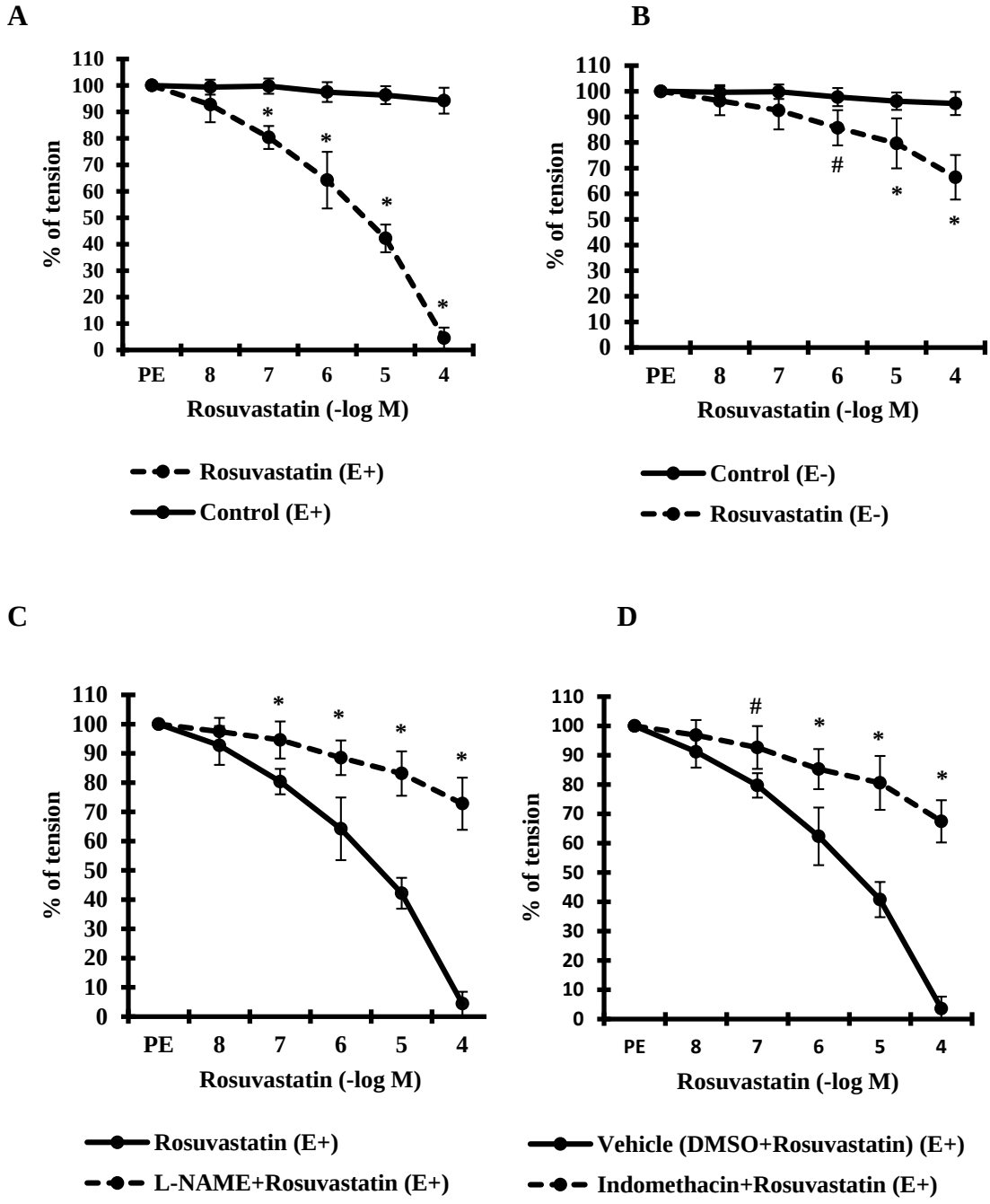

Figure 2. Rosuvastatin-induced vascular relaxation mechanisms. A. Concentration-dependent vasorelaxant effect of rosuvastatin in E+ rat pulmonary artery. B. The vasorelaxant effect of rosuvastatin in the E- rat pulmonary artery. C. Effect of L-NAME incubation on rosuvastatin-mediated vasorelaxation. D. Effect of indomethacin incubation on rosuvastatin-mediated vasorelaxation. n=8. #: p<.01. *: p<.001. E+: Endothelium-intact. E-: Endothelium-denuded. L-NAME: Nω-nitro-L-arginine methyl ester. PE: Phenylephrine.

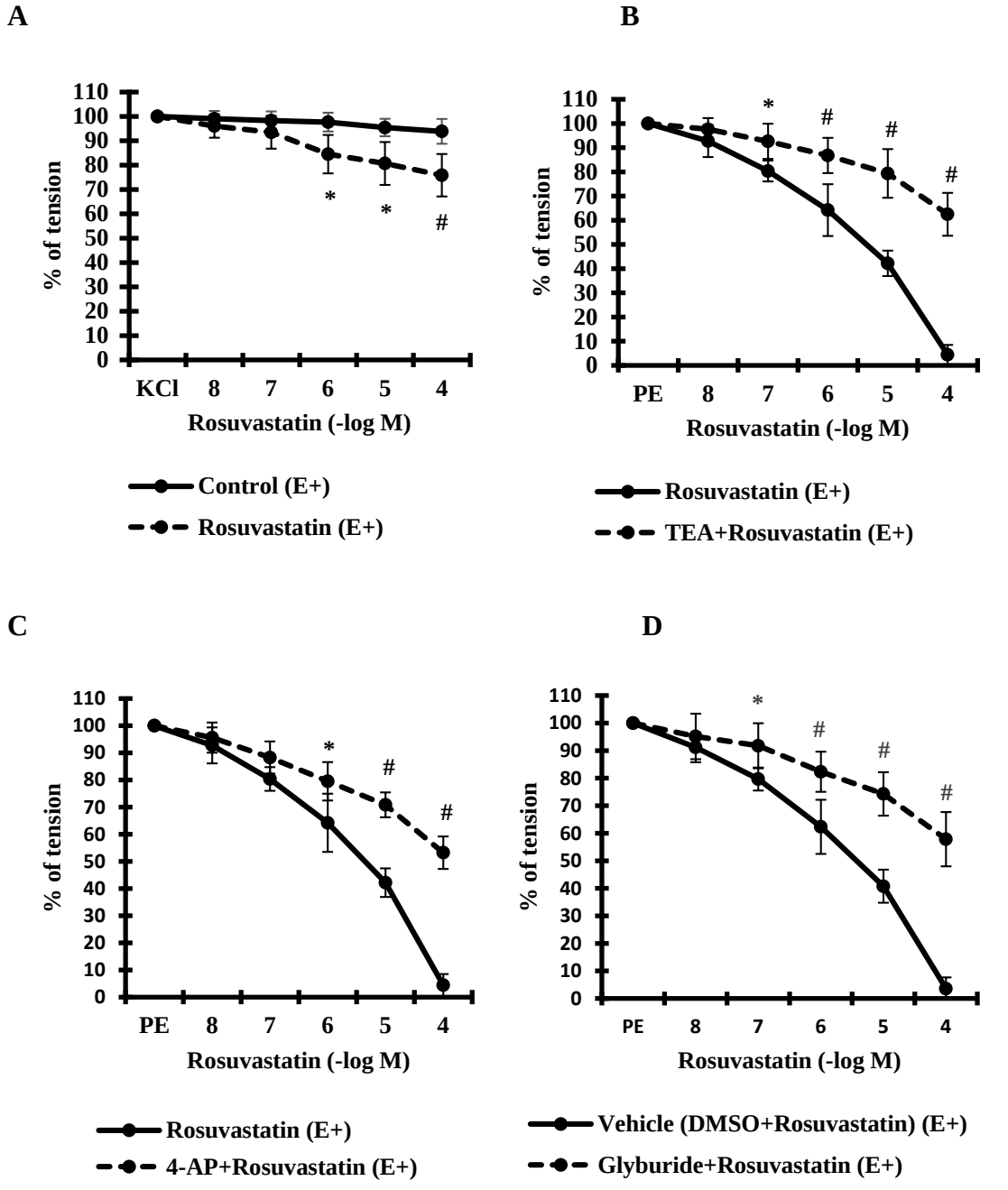

Figure 3. Rosuvastatin-induced vascular relaxation mechanisms. A. Effect of high K^+ concentration (60 mM KCl) on rosuvastatin-mediated vasorelaxation. B. Effect of TEA incubation on rosuvastatin-mediated vasorelaxation. C. Effect of 4-AP incubation on rosuvastatin-mediated vasorelaxation. D. Effect of glyburide incubation on rosuvastatin-mediated vasorelaxation. n=8. *: p<.01. #: p<.001. DMSO: Dimethylsulfoxide. E+: Endothelium-intact. PE: Phenylephrine. TEA: Tetraethylammonium. 4-AP: 4-Aminopyridine.

In rat tracheal rings, the potential functional effects and mechanism of action of rosuvastatin were examined. It was agreed that 10^{-5} M acetylcholine produced 100% of the maximum tension. In rat trachea that had been pre-contracted with acetylcholine, rosuvastatin treatment (10^{-8}-10^{-4} M) caused a relaxing effect in a concentration-dependent manner ($p<.001$). The maximal level of relaxation was determined to be around 75% (Figure 4A). In tracheal rings that had been pre-contracted with KCl (60 mM), the level of rosuvastatin's relaxant action was significantly reduced. In these tracheal rings, the highest relaxation level was around 28%. Rosuvastatin's relaxing effect on the rat tracheal rings was significantly reduced by L-NAME, indomethacin, TEA, 4-Aminopyridine, and glyburide (p-values at maximal concentration <.001 in all groups) (Figure 4B, 4C, 5A-D).

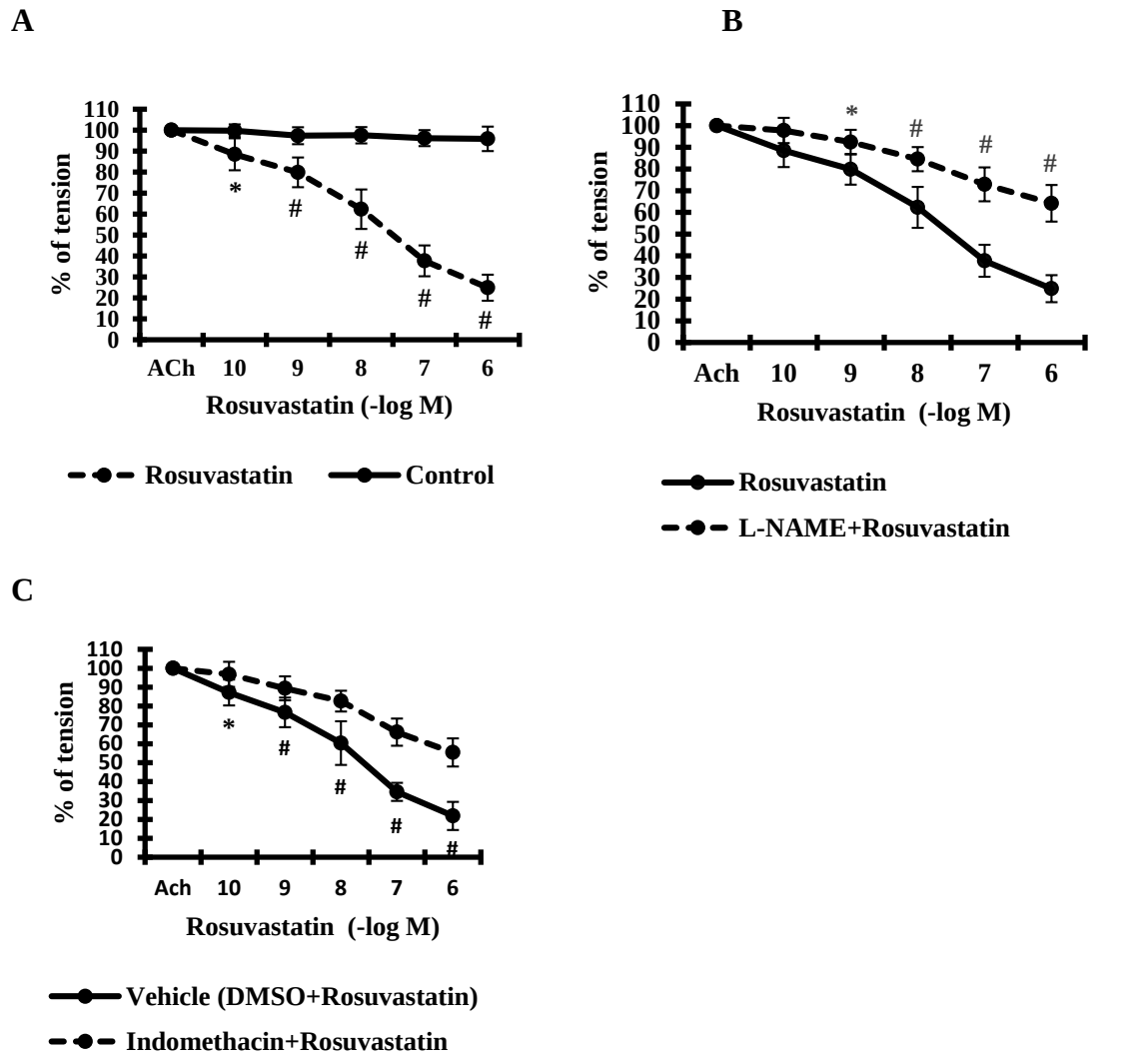

Figure 4. Rosuvastatin-induced tracheal relaxation. A. Concentration-dependent relaxant effect of rosuvastatin in rat trachea. B. Effect of L-NAME incubation on rosuvastatin-mediated tracheal relaxation. C. Effect of indomethacin incubation on rosuvastatin-mediated tracheal relaxation. n=8. *: p<.01. #: p<.001. ACh: Acetylcholine. DMSO: Dimethylsulfoxide. L-NAME: Nω-nitro-L-arginine methyl ester.

A **B**

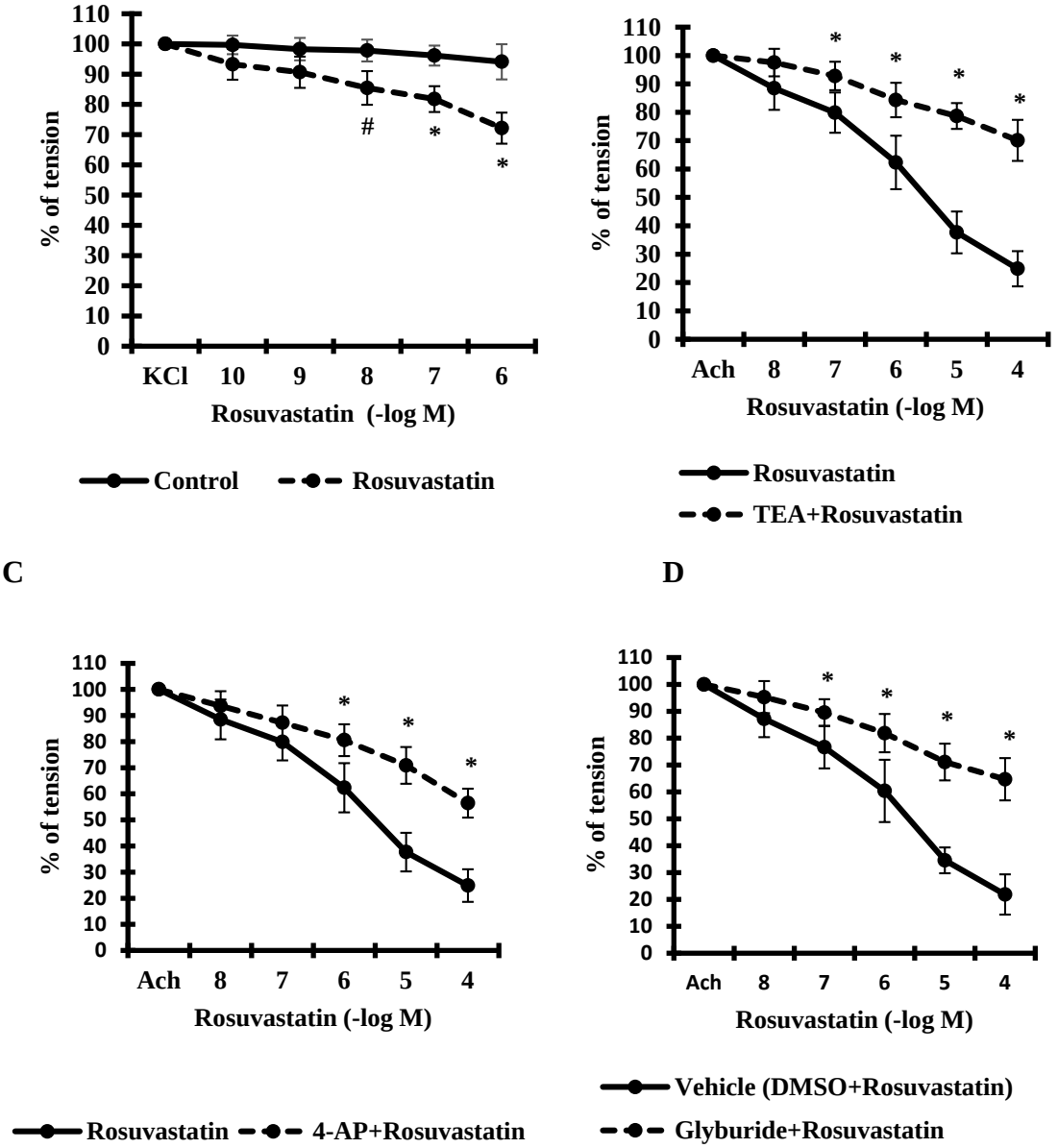

Figure 5. Rosuvastatin-induced tracheal relaxation mechanisms. A. Effect of high K^+ concentration (60 mM KCl) on rosuvastatin-mediated tracheal relaxation. B. Effect of TEA incubation on rosuvastatin-induced tracheal relaxation. C. Effect of 4-AP incubation rosuvastatin-induced tracheal relaxation. D. Effect of glyburide incubation rosuvastatin-induced tracheal relaxation. n=8. #: p<.05. *: p<.001. ACh: Acetylcholine. DMSO: Dimethylsulfoxide. TEA: Tetraethylammonium. 4-AP: 4-Aminopyridine.

DISCUSSION

Rosuvastatin's effects on vascular functions were thoroughly examined in this study, as well as the processes behind those effects. Rosuvastatin was found to elicit vasorelaxation in the rat thoracic aorta and pulmonary artery in a concentration-dependent manner, with a substantial endothelium-dependent component. The current study also demonstrated that prostaglandins, NO generation, and several K^+ channel subtypes, including BK_{Ca}, K_{ATP}, and K_V channels, are involved in rosuvastatin-mediated vasorelaxation in rat pulmonary arteries. In addition, this study revealed for the first time that rosuvastatin-mediated relaxation of tracheal smooth muscle and BK_{Ca}, $K_{ATP,}$ and K_V channels play a role in this effect.

In the first step of the present study, the effect of rosuvastatin on rat thoracic aortic contractility was determined. Rosuvastatin administered cumulatively in the rat thoracic aorta pre-contracted with phenylephrine caused vasorelaxation in a concentration-dependent manner. The relaxation level calculated over the maximum contraction stimulated with phenylephrine was found to be approximately 88%. In the present study, the rat thoracic aortic model was used because it is the gold standard for the development of antihypertensive drugs and for determining the functional effects of vasoactive agents[14]. In addition, examples of the thoracic aorta have also been used in previous studies[8,9]. Therefore, in the present study, the effect of rosuvastatin on pulmonary artery samples was investigated. Similar to the thoracic aorta, it was demonstrated that rosuvastatin also caused a concentration-dependent vasorelaxation effect in rat pulmonary arteries. The maximal level of relaxation in the pulmonary artery samples was 96%. It is known that factors such as endothelium-derived NO and prostanoids (especially prostacyclin (prostaglandin I_2)) have a critical role in the mechanism of action of vasoactive substances[15]. Therefore, to determine the contribution of endothelium to rosuvastatin-mediated vasorelaxation, rosuvastatin was applied cumulatively to the endothelium-denuded pulmonary artery rings and its effect level was found to be greatly reduced (up to 34%). These results indicate that rosuvastatin-induced vascular relaxation is largely endothelium-mediated and consistent with previous findings on the vasoactive effects of statins. There are few studies in the literature associated with the vasorelaxant effects of some other statins. In those studies, it has been shown that vasorelaxation occurs in various vascular beds by using pravastatin, atorvastatin, rosuvastatin, and cerivastatin[8,9,16]. In those studies, it was observed that statins have a vasorelaxant effect and this effect is largely dependent on the endothelium. In this study, unlike previous studies, the vasodilatory effect of rosuvastatin on the pulmonary artery was demonstrated.

One of the important pleiotropic effects of statins is vasorelaxation[8,9]. However, data on the vascular functional effects of statins in studies so far are very limited. The mechanism of action of rosuvastatin-mediated vasorelaxation has not been demonstrated until now. Therefore, in the next step of the present study, it was aimed to determine the mechanisms of the vasorelaxant effect of rosuvastatin in the rat pulmonary artery. Endothelial-derived factors are known to have a critical role in vascular relaxation. The mains of these factors are NO and prostacyclin[15]. The present study determined that the vasorelaxant effect level of rosuvastatin was greatly reduced after 30 minutes of endothelial NO synthase inhibitor L-NAME incubation. Similarly, indomethacin incubation, which inhibits prostanoid synthesis, has been shown to reduce rosuvastatin-mediated vascular relaxation less than L-NAME, but at a statistically significant level. These findings showed that the eNOS/NO pathway and prostanoids play a role in rosuvastatin-induced endothelial-dependent vascular relaxation. In the study of Sonmez Uydes-Dogan et al., which is one of the first studies on the vasoactive effects of statins, pravastatin, atorvastatin, and cerivastatin were reported to be effective as vasorelaxants in the rat thoracic aorta[9]. In that study, it was shown that NO pathway and prostanoids are involved in the mechanism of action of all 3 statin group drugs. In a more recent study, rosuvastatin was administered to the thoracic aorta of cafeteria-style diet-applied rats. In that study, the researchers determined that rosuvastatin causes vasorelaxation and, the NO pathway and prostanoids play a role in its mechanism of action[8]. Nurullahoglu-Atalik et al. showed that rosuvastatin caused endothelium-dependent relaxation in the calf cardiac vein[17]. Guresir and Nurullahoglu determined that the NO pathway contributes to rosuvastatin-mediated cardiac vein relaxation[18]. In another study, it was reported that atorvastatin caused vasorelaxation in the rat thoracic aorta and the NO pathway played a role in this effect[16]. In a different study, Castro et al. showed that atorvastatin increased sildenafil-mediated vasodilation in the rat thoracic aorta[19]. Almukhtar et

al. reported that simvastatin caused endothelium-independent and mitochondria-related relaxation in the porcine coronary artery[20]. On the other hand, the findings from another study are in the opposite direction. In that study, it was suggested that simvastatin administration caused a contraction response in the thoracic aorta of the rat. It has been reported that the contraction response increases with the removal of endothelium and inhibition of NO synthase but decreases with the inhibition of COX[21]. Those findings suggest that statins generally cause vasorelaxation in arteries and veins in vitro conditions, but they can also cause vascular contraction like simvastatin. As a result, considering all the data obtained, endothelium-related factors such as NO and prostaglandins appear to be important mechanisms in the vasoactive effects of statins.

Another important finding in the present study is that the vasorelaxant effect level of rosuvastatin in rat pulmonary arteries is greatly reduced (up to 24%) at high K^+ concentrations. This data demonstrates that K^+ channel activation is involved in the vasorelaxant mechanism of action of rosuvastatin. Therefore, we questioned their possible role in rosuvastatin-induced vasorelaxation by administering specific K^+ channel blockers. It has been reported that 5 different types of K^+ channels are found in VSM cells (VSMCs). These channels are Ca^{2+}-activated K^+ channels (BK_{Ca}, IK_{Ca}, and SK_{Ca} channels), K_V channels, K_{ATP} channels, Kir channels, and K_{2p} channels. As a result of the activation of these channels, hyperpolarization occurs in the VSMCs, the influx of Ca^{2+} into the cell is reduced, and the VSM relaxes[22,23]. Many vasoactive substances cause vasorelaxation by causing K^+ channel activation[14,24]. In this study, the possible roles of main K^+ channel subtypes expressed in VSMCs in rosuvastatin-mediated vasoactive effects were investigated using selective K^+ channel blockers, and the effective ones were determined. The data of the present study showed that TEA, glyburide, and 4-Aminopyridine applications cause a statistically significant decrease in rosuvastatin-mediated vasorelaxant effect levels. These findings suggested that K^+ channel activation is an important mechanism in rosuvastatin-induced vasorelaxation. BK_{Ca}, K_V, and K_{ATP} channels are involved in the vasorelaxant effect of rosuvastatin. As far as it is known, the findings of the current study are the first data on K^+ channel activation in the vasoactive effects of rosuvastatin in rat pulmonary arteries. On the other hand, the study of López-Canales et al. showed that BK_{Ca} channel activation contributes to the vascular functional effects of rosuvastatin in the rat thoracic aorta[8]. Unlike that study, in the present study, rats have not applied a cafeteria diet, and the pulmonary arteries of healthy rats were used. In addition, in the present study, several other K^+ channel subtypes (K_V and K_{ATP}) were also determined to be involved in the vasorelaxant effect of rosuvastatin. In the study of Sonmez Uydes-Dogan et al., it was reported that activation of K_{ATP} channels has a role in cerivastatin-mediated vascular relaxation, but not in vasorelaxation induced by pravastatin and atorvastatin[9]. The present study's findings showed that both K^+ channels (K_V and K_{ATP}) play a role in the vasorelaxant effect of rosuvastatin. A recent study reported that simvastatin was effective as a vasorelaxant in rat thoracic aorta. Mechanisms of simvastatin-mediated relaxation have been demonstrated in detail. Unlike rosuvastatin, prostanoids do not contribute to the vasoactive effects of simvastatin. In contrast, Kir channels, as well as K_{ATP} and K_V channels, were also found to be involved in simvastatin-mediated vascular relaxation. In addition, the inactivation of Ca^{2+} channels and angiotensin II type 2 receptors, which were not investigated in the current study, were observed to be involved in the mechanism of action of simvastatin[11]. Considering all data, different subtypes of K^+ channels are thought to contribute to the vasorelaxant effect of statins. Different mechanisms can be activated depending on the type of vascular bed. On the other hand, recent studies show that inhibition of Ca^{2+} influx from intracellular and extracellular Ca^{2+} sources may also mediate statin-mediated smooth muscle relaxation.

The airway smooth muscle (ASM) layer lines the lower respiratory tract, which extends from the trachea to the terminal bronchioles. The smooth muscle layer in this layer enables the airways to expand and contract. The main element that ensures ventilation-perfusion balance and mechanical stability is the ability of the airways[25]. Many extracellular messengers that operate on certain membrane receptors regulate the contraction-relaxation reactions of tracheal smooth muscle cells[26]. Asthma and bronchitis, which influence the respiratory tract and induce bronchoconstriction, have become more common diseases[27]. In this context, exogenous substances and associated mechanisms that affect tracheal smooth muscle activity may help to improve the condition of the trachea. Statins can induce functional effects in

different smooth muscle tissues as well as VSM. Therefore, we investigated the effects of rosuvastatin on tracheal smooth muscle functions due to its relaxant effects on other smooth muscle tissues.

One of the important findings in the current study is the relaxant effect of rosuvastatin in rat tracheal smooth muscle and the contribution of K^+ channels in this mechanism. Diseases associated with airway hyperreactivity, such as asthma, are common in the population. These diseases are difficult to treat, and alternative treatment agents and bronchodilators are under investigation[28]. No direct effect of statins on tracheal smooth muscle has been demonstrated in studies to date. Therefore, the present study questioned whether rosuvastatin administration has any effect on tracheal rings. It was concluded that tracheal rings, which were pre-contracted with acetylcholine, were relaxed with rosuvastatin administration. In the next step, the possible role of K^+ channels was questioned because of their critical importance in smooth muscle relaxation. It was shown that BK_{Ca}, K_V, and K_{ATP} channels play a role in tracheal relaxation. These data indicated that rosuvastatin has relaxing effects on tracheal and VSM, and K^+ channels play a role in these effects. In addition, the present study determined that the NO pathway and prostanoids concerning the epithelium were involved in the tracheal smooth muscle relaxant effect of rosuvastatin.

It is well known that voltage-dependent Ca^{2+} entry routes, which aid in ASM contraction, are inhibited by K^+ channel modulation of membrane potential. K^+ channel activators can partially relax ASM by K^+ efflux-induced hyperpolarization, which makes them effective as bronchodilators, especially in asthma. K_V, K_{ATP}, and BK_{Ca} channels are among the K^+ channels expressed in ASM[29]. As a result, we investigated the function of K^+ channels expressed in ASM in rosuvastatin's broncho-relaxant processes in the rat trachea.

An increase in intracellular free Ca^{2+} concentration that encourages the production of actin-myosin cross-bridges causes the ASM to contract. The key factor controlling the potential of the ASM membrane is the activation of K^+ channels, especially K_V channels. By adjusting membrane potential and modifying the opening of L-type Ca^{2+} channels, K_V channels have a considerable effect on intracellular Ca^{2+} ($[Ca^{2+}]$ i). ASM cells are physiologically hyperpolarized when K_V channels are activated. This decreases the activity of voltage-gated Ca^{2+} channels (VGCCs), which relaxes ASMs by reducing intracellular Ca^{2+} influx. On the other hand, contraction results from blocking K_V pathways[30]. As a result, we began by examining the part played by K_V channels in the concentration-dependent relaxation brought on by rosuvastatin. This study found that pretreatment with the K_V channel blocker 4-AP dramatically decreased the broncho-relaxant action of rosuvastatin.

The functional link between membrane excitability and cellular metabolism includes K_{ATP} channels. Depolarization and bronchoconstriction are brought on by blocking K_{ATP} channels[30]. In this study, we discovered that rosuvastatin-induced bronchodilation in the rat trachea is mediated in part by K_{ATP} channels. It has been demonstrated that Ca^{2+}-activated K^+ (K_{Ca}) channels, particularly BK_{Ca}, are crucial for controlling bronchial tone. The passage of K^+ ions, hyperpolarization of the plasma membrane, and inhibition of VGCC opening result from the activation of K_{Ca} channels on the plasma membranes of ASM cells. These circumstances lead to a relative decrease in $[Ca^{2+}]i$, inhibition of Ca^{2+}-dependent contraction, and the development of bronchodilation[30]. The present study's findings also indicated that BK_{Ca} channels work with rosuvastatin to cause bronchodilation.

The main limitation of this study is that K^+ channel current was not directly measured with the patch clamp technique. In addition, molecular methods were not used to support the data. In addition, the contribution of mechanisms associated with beta-adrenergic receptors, angiotensin receptors, and Ca^{2+} channels to rosuvastatin-mediated smooth muscle relaxation was not investigated in the current study.

In conclusion, the findings of the present investigation demonstrate that rosuvastatin produces vasorelaxation in the rat thoracic aorta and pulmonary artery. Endothelium-dependent processes play a significant role in this effect. The relaxing effect of rosuvastatin on the vascular smooth muscle in the rat pulmonary artery is due to the activation of the prostanoids and NO signaling pathways, as well as large-conductance Ca^{2+}-activated K^+ channels, voltage-gated K^+ channels, and ATP-sensitive K^+ channels. Because of their potent vasorelaxant effects, rosuvastatin and other members of the statin group of medications, which are used to prevent and treat a variety of cardiovascular diseases like atherosclerosis, coronary artery disease, and ischemic stroke, may also benefit the treatment of comorbid

hypertensive diseases by lowering cholesterol levels. Moreover, rosuvastatin relaxes tracheal smooth muscle by opening voltage-gated K^+ channels, ATP-sensitive K^+ channels, and large-conductance Ca^{2+}-activated K^+ channels. These findings imply that statins may be therapeutically effective for conditions such as asthma and bronchitis linked to airway smooth muscle hyperreactivity.

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