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[Pyr1]apelin-13 relaxes the rat thoracic aorta *via* APJ, NO, AMPK, and potassium channels

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Abstract. In this study, the effect and effect mechanisms of [Pyr1]apelin-13, the dominant apelin isoform in the human cardiovascular tissues and human plasma, on vascular contractility were investigated. The vascular rings obtained from the thoracic aortas of the male Wistar Albino rats were placed in the isolated tissue bath system. After the equilibration period, [Pyr1]apelin-13 (10^{-9} to 10^{-6} M) was applied cumulatively to the aortic rings pre-contracted with phenylephrine in the plateau phase. The protocol was repeated in the presence of specific signaling pathway inhibitors (F13A, L-NAME, dorsomorphin, TEA, U0126, or indomethacin) to determine the effect mechanisms of [Pyr1]apelin-13. [Pyr1]apelin-13 induced a dose-dependent relaxation in the pre-contracted aortic rings. APJ, eNOS, AMPK, and potassium channel inhibition statistically significantly decreased the vasodilator effect of [Pyr1]apelin-13. MAPK and COX inhibition didn't statistically significantly changed the vasodilator effect of [Pyr1]apelin-13. In conclusion, [Pyr1]apelin-13 relaxes the rat thoracic aorta *via* APJ, NO, AMPK, and potassium channels.

Key words: [Pyr1]apelin-13 — AMPK — APJ — NO — Potassium channels

Introduction

Apelin, which was isolated from bovine stomach extracts in 1998, is the first found endogenous apelin receptor (APJ) ligand (Tatemoto et al. 1998). Apelin has various isoforms such as apelin-12, apelin-13, apelin-17, apelin-36, and pyroglutamyl apelin-13 ([Pyr1]apelin-13). [Pyr1]apelin-13 is formed by posttranslational modification from apelin-13 and is a stable form that is highly resistant to enzymatic degradation. Tissue distribution and potency may differ between apelin isoforms. [Pyr1]apelin-13 is the predominant apelin isoform in plasma and cardiovascular tissues (Zhang et al. 2018).

Body-fluid regulation, energy metabolism, food intake, neuroendocrine-stress response, and immune responses are the major processes involved in apelin. Besides, the role of apelin in cardiovascular physiological processes is quite prominent. Apelin mediates angiogenic, cardioprotective, positive inotropic, and antihypertensive effects. The effects of apelin, which has an important role in the regulation of blood pressure, on vascular contractility, vary. It has been suggested that apelin, which generally acts as a vasodilator under physiological conditions, may have a vasoconstrictor effect in some pathological conditions (Mughal and O'Rourke 2018; Zhang et al. 2018; Read et al. 2019).

In the presented study, it was aimed to determine the effect and effect mechanisms of [Pyr1]apelin-13, which is the dominant apelin isoform in plasma and cardiovascular tissues, on the rat thoracic aortic contractility. APJ, endothelial nitric oxide synthase (eNOS)/nitric oxide (NO), mitogen-activated protein kinase (MAPK), cyclooxygenase (COX), and adenosine monophosphate-activated protein kinase (AMPK) pathways and the possible role of potassium channels have been questioned to identify the mechanisms mediating the effect of apelin on vascular contractility.

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Materials and Methods

Ethical approval and test animals

The ethical approval was obtained from Bursa Uludag University Animal Experiments Local Ethics Committee, dated 03.04.2019, and numbered 2019-04/11. In the study, 12 weeks old, 20 Wistar Albino male rats obtained from Bursa Uludag University Experimental Animal Breeding Administration and Research Center were used. The rats were kept 4–6 in each cage, at 22 ± 2°C, 12 h light/12 h dark cycle, and food/water intake was provided *ad libitum*.

Isolated tissue bath experiments

The rats were decapitated without anesthesia. The thoracic aortas were rapidly removed by excising the thoracoabdominal regions of the rats. The thoracic aortic tissues were placed in Petri dishes containing ice-cold Krebs solution (in mM: 2.5 $CaCl_2 \times 2\ H_2O$, 118 NaCl, 4.8 KCl, 1.2 KH_2PO_4, 11 $C_6H_{12}O_6 \times H_2O$, 25 $NaHCO_3$, 1.2 $MgSO_4 \times 7\ H_2O$). The vascular rings of 4 mm length were prepared from the vessels carefully cleared of perivascular tissues (4 vascular rings were obtained from the thoracic aorta of a rat). The vascular rings were placed in the glass chambers in the isolated tissue bath system (MAY IOBS99, Commat Ltd., Ankara) using the vessel hanging apparatus. The reservoirs were filled with Krebs solution. The temperature was kept constant at 37°C with hot distilled water circulating in the double jacketed system. Krebs solution was continuously gassed with a gas mixture of 95% O_2-5% CO_2, and the pH was adjusted to 7.4. After the first 30 min, the resting tension was set to 2 g (Barutcigil and Tasatargil 2018; Wani et al. 2020). And then one hour has been waited for the tissues to equilibrate. Krebs solution in the chambers is renewed every 15 min.

Phenylephrine (10^{-5} M) was used to stimulate vascular contraction (Panthiyaa et al. 2019). [Pyr1]apelin-13 was applied when vascular tension reached the plateau phase. To determine the effect mechanisms of [Pyr1]apelin-13, the signaling pathway inhibitors (F13A, Nω-Nitro-L-arginine methyl ester (L-NAME), dorsomorphin, tetraethylammonium (TEA), U0126, or indomethacin) were administered 20 to 30 min before phenylephrine was and the protocol repeated in the same vascular rings. To evaluate the effect of perivascular adipose tissue (PVAT), PVAT+ and PVAT– groups were created and [Pyr1]apelin-13 was applied to these vascular rings. In a different set of experiment, phenylephrine challenge (10^{-9} to 10^{-4} M) was applied to aortic rings. The protocol repeated in the presence of 10^{-6} M [Pyr1]apelin-13 in the same vascular rings.

Tension changes in the vascular rings were detected by isometric force transducers (MAY FDT05) and recorded by computer software (BIOPAC MP36). The plateau tension created with phenylephrine was accepted as 100%. The tension values created with [Pyr1]apelin-13 were calculated over this value. The records of the control groups were taken at the beginning of each experiment. Afterward, the washing and equilibration period was repeated and the experimental protocols of the related study groups were performed in the same aortic vascular rings. The vessels for which a sufficient contractile response couldn't be obtained with phenylephrine were excluded from the study. The aortic rings were challenged with 10^{-5} M of acetylcholine, and if the vasorelaxant response was greater than 90% of the phenylephrine-induced contraction, endothelium of the aortic rings was considered intact (E+) (Panthiyaa et al. 2019). The endothelium was mechanically removed from some aortic rings by gentle rubbing of the intimal surface with a wooden stick (Wang et al. 2015). Endothelium-denuded (E–) rings were considered to have less than 10% relaxation response of phenylephrine-induced contraction to 10^{-5} M acetylcholine (Panthiyaa et al. 2019).

Drugs

The chemicals used in this study were obtained from Sigma-Aldrich. The doses of [Pyr1]apelin-13 and signaling pathway inhibitors have been determined by the literature and the drugs have been prepared by the instructions for use. Phenylephrine (10^{-5} M or 10^{-9} to 10^{-4} M), acetylcholine (10^{-5} M), [Pyr1]apelin-13 (10^{-9} to 10^{-6} M), F13A (10^{-7} M), L-NAME (10^{-3} M), and TEA (10 mM) were dissolved in distilled water. Dorsomorphin (10 μM), U0126 (1.5 μM), and indomethacin (5 μM) were dissolved in DMSO. The final concentration of DMSO in the Krebs solution didn't exceed 0.1% and DMSO didn't affect vascular smooth muscle contraction or relaxation.

Statistical analysis

The data obtained are expressed as mean ± standard deviation (SD) ($n = 8$) as a percentage of the plateau tension obtained with phenylephrine. Paired Samples T-Test was used for the comparison between the 2 groups. One-Way ANOVA test was used for comparisons between multiple groups, and then the Bonferroni test was applied as a post hoc. p values less than 0.05 were considered statistically significant.

Results

Effect of [Pyr1]apelin-13 on the rat thoracic aortic tension

Percentage tension values of the control (an equal volume of distilled water instead of [Pyr1]apelin-13) and the [Pyr1]apelin-13 groups were compared. Percentage tension values in the [Pyr1]apelin-13 group were found statistically significantly lower at all doses compared to the control

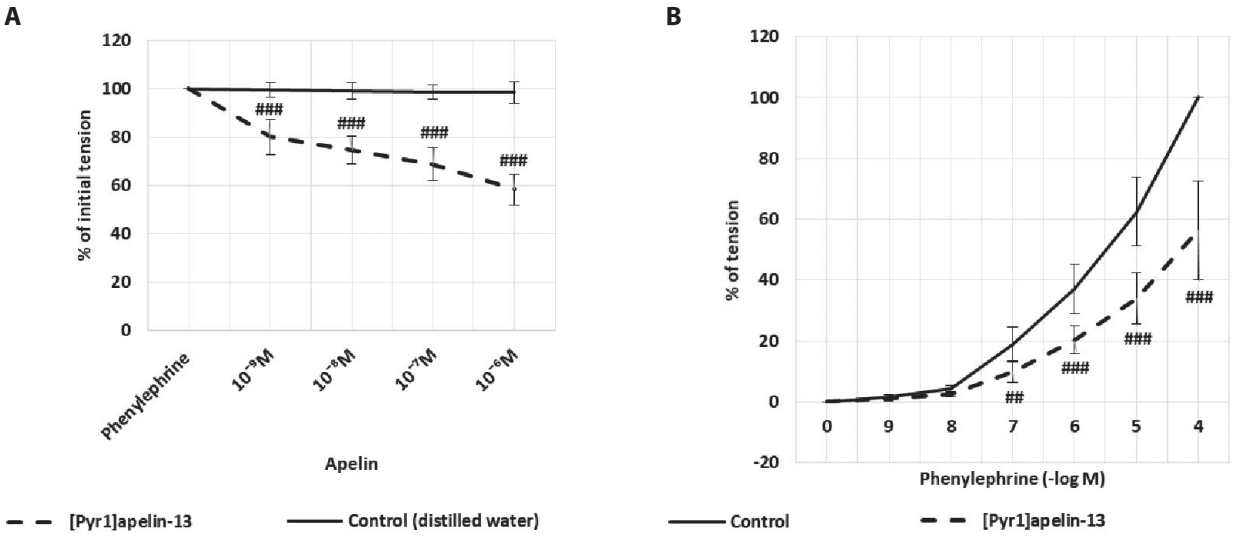

Figure 1. Vasodilator effect of [Pyr1]apelin-13 in the rat thoracic aorta pre-contracted with phenylephrine (**A**) and using Phenylephrine challenge protocol (**B**). Data are expressed as mean ± SD as a percentage of the plateau tension obtained with phenylephrine. Percentage tension values in the [Pyr1]apelin-13 group were found statistically significantly lower than in the Control group. $n = 8$ for each group. $^{\#\#}$ $p < 0.01$; $^{\#\#\#}$ $p < 0.001$.

group. [Pyr1]apelin-13 showed a statistically significant vasodilator effect in the rat thoracic aorta pre-contracted with phenylephrine. The maximum relaxation level was approximately 42% (Fig. 1A). Similar results were obtained with the phenylephrine challenge protocol. In the presence of [Pyr1]apelin-13, percentage tension values were statistically significantly lower compared to the control group. The maximum relaxation level was approximately 44% (Fig. 1B).

Percentage tension values in the [Pyr1]apelin-13 (E-) group were found statistically significantly higher at all doses compared to the [Pyr1]apelin-13 (E+) group. The

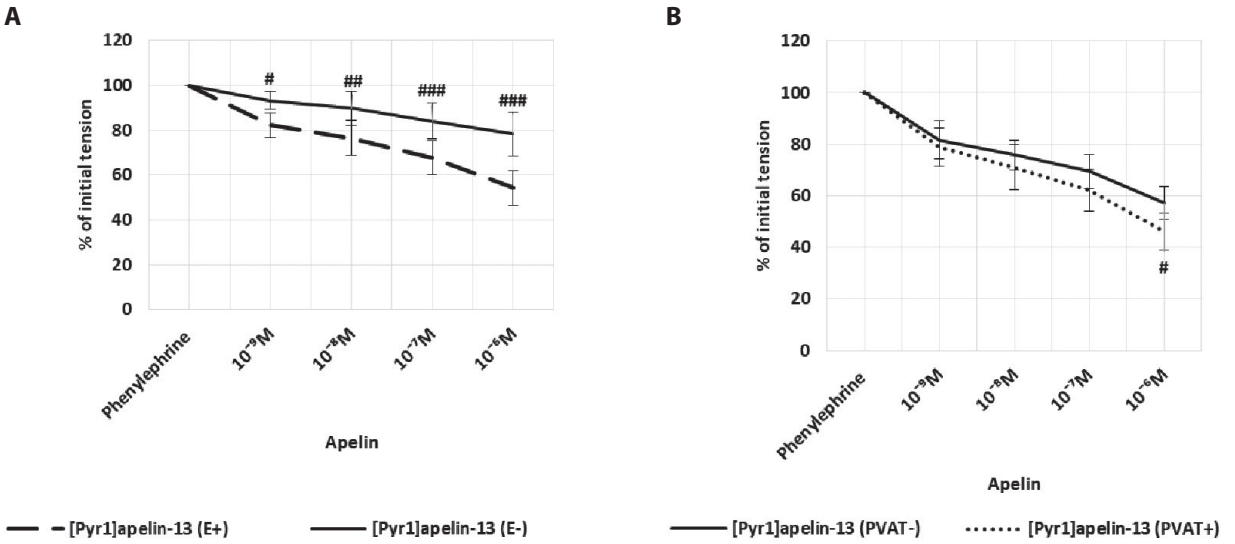

Figure 2. Effect of removal of the endothelium (**A**) and of preserving PVAT (**B**) in the vasodilator effect of [Pyr1]apelin-13. Data are expressed as mean ± SD as a percentage of the plateau tension obtained with phenylephrine. Percentage tension values in the [Pyr1]apelin-13 (E–) group were found statistically significantly higher than in the [Pyr1]apelin-13 (E+) group. Percentage tension values in the [Pyr1]apelin-13 (PVAT+) group were found lower than in the [Pyr1]apelin-13 (PVAT–) group. This reduction was statistically significant at the 10^{-6} M dose. $n = 8$ for each group. $^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$; $^{\#\#\#}$ $p < 0.001$. PVAT, perivascular adipose tissue.

vasodilator effect of [Pyr1]apelin-13 was statistically significantly decreased (from 46% to 22% at maximum dose) in the endothelium-denuded aortic rings (Fig. 2A).

Percentage tension values in the [Pyr1]apelin-13 (PVAT+) group were found lower compared to the [Pyr1]apelin-13 (PVAT−) group. This reduction was statistically significant at the 10^{-6} M dose. The vasodilator effect of [Pyr1]apelin-13 was statistically significantly increased (from 43% to 54% at maximum dose) in the presence of PVAT (Fig. 2B).

The role of APJ in the vasodilator effect of [Pyr1]apelin-13

Percentage tension values of the [Pyr1]apelin-13 and the F13A groups were compared. Percentage tension values in the F13A group were found statistically significantly higher compared to the [Pyr1]apelin-13 group. After APJ inhibition, the vasodilator effect of [Pyr1]apelin-13 in the rat thoracic aorta pre-contracted with phenylephrine was statistically significantly decreased (Fig. 3A).

The role of eNOS/NO pathway in the vasodilator effect of [Pyr1]apelin-13

Percentage tension values of the [Pyr1]apelin-13 and the L-NAME groups were compared. Percentage tension values in the L-NAME group were found statistically significantly higher than the [Pyr1]apelin-13 group. After eNOS inhibition, the vasodilator effect of [Pyr1]apelin-13 in the rat thoracic aorta pre-contracted with phenylephrine was statistically significantly decreased (Fig. 3B).

The role of MAPK pathway in the vasodilator effect of [Pyr1]apelin-13

Percentage tension values of the vehicle (an equal volume of DMSO instead of U0126) and the U0126 groups were compared. There was no statistically significant difference between the percent tension values in the U0126 group and the percent tension values in the vehicle group. After MAPK inhibition, there was no statistically significant change in the vasodilator effect of [Pyr1]apelin-13 on the rat thoracic aorta pre-contracted with phenylephrine (not shown).

The role of COX pathway in the vasodilator effect of [Pyr1]apelin-13

Percentage tension values of the vehicle (an equal volume of DMSO instead of indomethacin) and the indomethacin groups were compared. There was no statistically significant difference between the percent tension values in the indomethacin group and the percent tension values in the vehicle group. After COX inhibition, there was no statistically significant change in the vasodilator effect of [Pyr1]apelin-13 on the rat thoracic aorta pre-contracted with phenylephrine (not shown).

The role of AMPK pathway in the vasodilator effect of [Pyr1]apelin-13

Percentage tension values of the vehicle (an equal volume of DMSO instead of dorsomorphin) and the dorsomor-

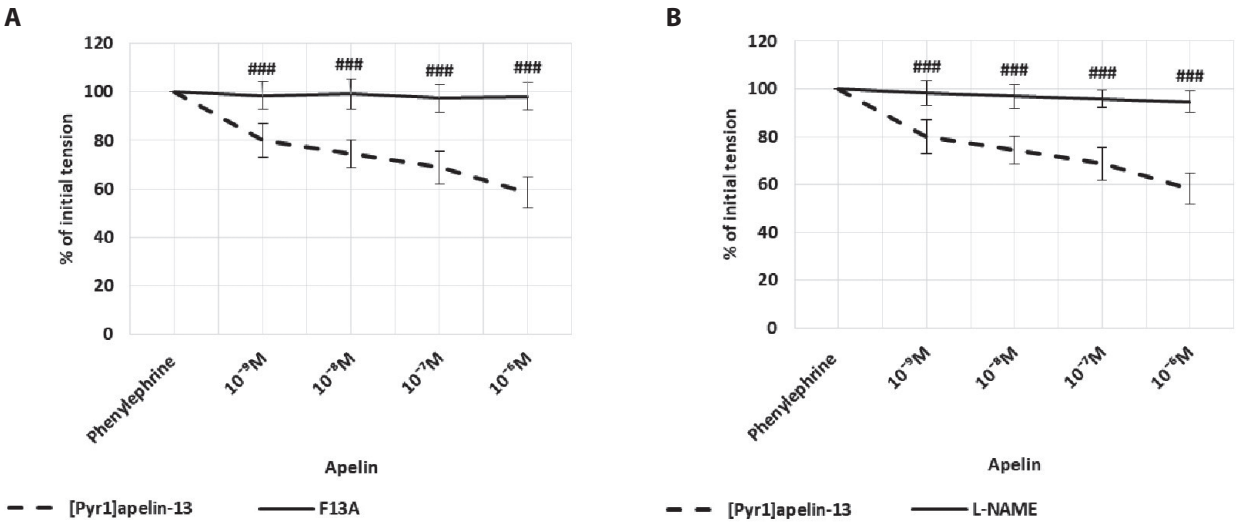

Figure 3. Effect of APJ inhibition (**A**) and eNOS inhibition (**B**) in the vasodilator effect of [Pyr1]apelin-13. Data are expressed as mean ± SD as a percentage of the plateau tension obtained with phenylephrine. Percentage tension values in the F13A group were found statistically significantly higher than the [Pyr1]apelin-13 group. Percentage tension values in the L-NAME group were found statistically significantly higher than in the [Pyr1]apelin-13 group $n = 8$ in each group. $^{###}$ $p < 0.001$. APJ, apelin receptor.

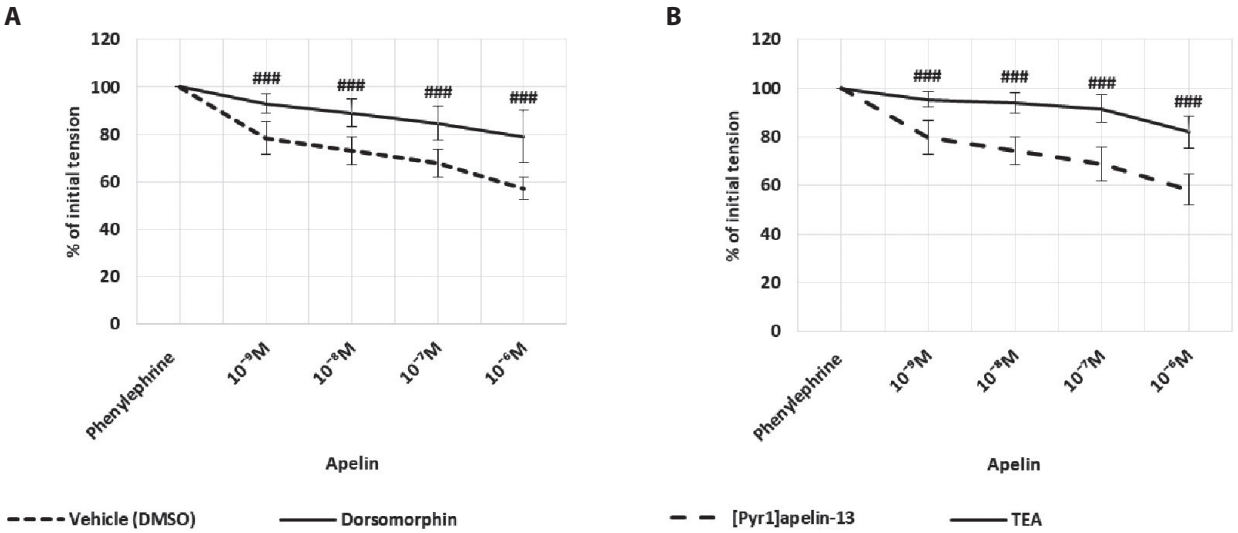

Figure 4. Effect of AMPK inhibition (**A**) and potassium channel inhibition (**B**) in the vasodilator effect of [Pyr1]apelin-13. Data are expressed as mean ± SD as a percentage of the plateau tension obtained with phenylephrine. The percent tension values in the dorsomorphin group were found statistically significantly higher than in the vehicle group. Percentage tension values in the TEA group were found statistically significantly higher than in the [Pyr1]apelin-13 group. $n = 8$ for each group. $^{###}$ $p < 0.001$. AMPK, adenosine monophosphate-activated protein kinase.

phin groups were compared. It was determined that the percentage tension values in the dorsomorphin group were statistically significantly higher compared to the vehicle group. After AMPK inhibition, the vasodilator effect of [Pyr1]apelin-13 in the rat thoracic aorta pre-contracted with phenylephrine was statistically significantly decreased (Fig. 4A).

The role of potassium channels in the vasodilator effect of [Pyr1]apelin-13

Percentage tension values of the [Pyr1]apelin-13 and the TEA groups were compared. Percentage tension values in the TEA group were found to be statistically significantly higher compared to the [Pyr1]apelin-13 group. After potassium channel inhibition, the vasodilator effect of [Pyr1]apelin-13 in the rat thoracic aorta pre-contracted with phenylephrine was statistically significantly decreased (Fig. 4B).

The effects of the inhibitors to the phenylephrine-induced contraction

F13A, L-NAME, TEA, dorsomorphin, and U0126 didn't statistically significantly affect the phenylephrine-induced contraction. Indomethacin statistically significantly increased the phenylephrine-induced contraction (Fig. 5).

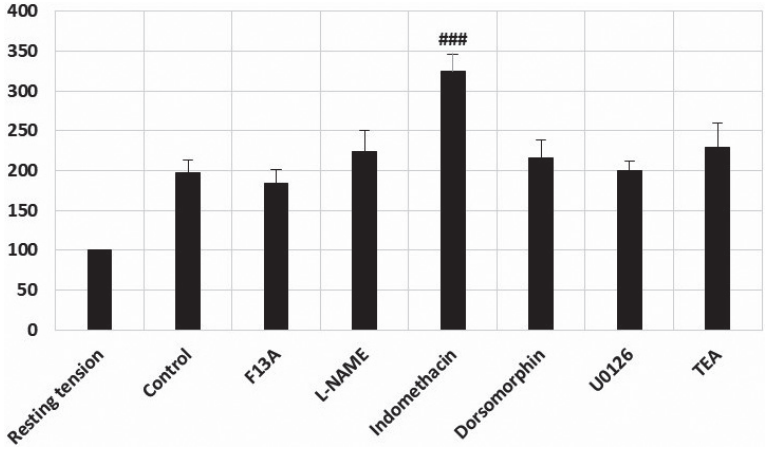

Figure 5. Effect of the inhibitors of phenylephrine-induced contraction. Data are expressed as mean ± SD as a percentage of the plateau tension obtained with phenylephrine. Percentage tension values in the indomethacin group were found statistically significantly higher than in the Control group (10^{-5} M phenylephrine). $n = 8$ for each group. There was no statistically significant difference in the other groups compared to the Control group. $^{###}$ $p < 0.001$.

Discussion

Apelin might provide an effective treatment alternative in common hypertensive diseases (Yang et al. 2015; Yamaleyeva et al. 2016; Wysocka et al. 2018). Therefore, it is critical to reveal the effect of apelin on vascular contractility and its mechanisms of action in detail. In this study, [Pyr1] apelin-13, which is the dominant apelin isoform in plasma and cardiovascular tissues, was determined to have a vasodilator effect. It was concluded that APJ, NO, AMPK, and potassium channels played a role in the vasodilator effect of [Pyr1]apelin-13.

In our study, it was determined that [Pyr1]apelin-13 showed a dose-dependent vasodilator effect in the endothelium-intact rat thoracic aorta. The maximum effect was approximately 42% of the phenylephrine-induced contraction. The vasodilator effect of [Pyr1]apelin-13 was greatly decreased after the removal of endothelium. These results are similar to previous studies. Vasodilator activity has been demonstrated by applying various apelin isoforms in some vascular beds (Gurzu et al. 2006; Salcedo et al. 2007; Andersen et al. 2009; Maguire et al. 2009; Wang et al. 2015; Mughal et al. 2018). Unlike previous studies, the rat thoracic aorta was used in our study. Mughal et al. (2018) reported that apelin-13 exerted a relaxing effect of 45% at a dose of 3×10^{-6} M in the coronary artery of rats pre-contracted with serotonin. Our study differs from this study because of the selection of [Pyr1] apelin-13 as apelin isoform, 10^{-6} M dose as the maximum drug dose, and the rat thoracic aorta as the vascular bed. On the other hand, these two studies are similar in terms of the maximum vasodilator effect level, which we detected at a level of approximately 42%. Wang et al. (2015), in their study using apelin-13 in mouse aorta, determined that 79% relaxation is at the maximum dose of 10^{-6} M. This level is quite high compared to the approximately 42% relaxation seen at the same dose in our study. We think that this difference may have resulted from the difference between the experimental animal species and the apelin isoforms in both studies.

It has been reported that apelin can act as a vasorelaxant with endothelium-dependent and independent mechanisms and it was stated that endothelium has a major role in the vasodilator effect of apelin (Wang et al. 2015). In our study, it was observed that the vasorelaxant effect induced by [Pyr1] apelin-13 in the rat thoracic aorta was greatly reduced by the removal of endothelium. However, the vasodilatory effect of apelin didn't completely disappear. This result shows that endothelium-independent factors also play a role in the vasodilator mechanism of apelin. PVAT has been recognized to regulate vascular tone *via* releasing important adipokines. Apelin is one of these important adipokines (Kagota et al. 2019). Kagota and collegues reported that the vasorelaxant effect level of apelin was higher in the presence of PVAT. Our study determined that the vasodilator effect of [Pyr1] apelin-13 is higher in the vascular aortic rings where PVAT is preserved. These data reveal that endothelial integrity and PVAT play an important role in the vasodilator effect of apelin.

Apelin exhibits its physiological effects *via* APJ, a G protein-coupled receptor. It has been suggested that the APJ also plays a role in the effect of apelin on vascular contractility (Zhang et al. 2018; Read et al. 2019). Mughal et al. (2018) reported that the apelin-13-induced vasodilator effect in the rat coronary artery completely disappeared as a result of APJ inhibition with the APJ receptor antagonist F13A. In our study, F13A was applied at the same dose for APJ inhibition, similarly to the study of Mughal et al. (2018), and it was determined that the vasodilator effect of [Pyr1] apelin-13 in the rat thoracic aorta was eliminated after this inhibition. This finding suggests that apelin acts on the rat thoracic aortic tension *via* APJ.

The previous studies have suggested that the eNOS/NO pathway plays a very important role in the apelin-mediated vasodilator effect. Gurzu et al. (2006) determined that NO had a role in the apelin-13-mediated vasodilator effect in the rat portal vein pre-contracted with Ang II. Salcedo et al. (2007) reported that apelin-13 exerted a NO-mediated vasodilator effect in the human mesenteric artery pre-contracted with U46619. Mughal et al. (2018) suggested that apelin-13 exerted a NO-mediated vasodilator effect in the rat coronary artery pre-contracted with serotonin. In contrast to all those studies, it has been reported that the eNOS/NO pathway has no role in the vasodilator effect of apelin-13 in the human hepatic artery and [Pyr1]apelin-13 in the human internal thoracic artery (Salcedo et al. 2007; Maguire et al. 2009). Those data suggest that the effect mechanism of apelin may vary depending on the type of vascular bed. Until now, there has been no study investigating the role of the eNOS/NO pathway in the apelin-mediated vasodilator effect in the rat thoracic aorta. In our study, after the administration of eNOS inhibitor L-NAME the vasodilator effect of [Pyr1]apelin-13 in the rat thoracic aorta was abolished majorly. This finding shows that the eNOS/NO pathway plays a very important role in the vasodilator effect mechanism of apelin and it is also effective in the rat thoracic aorta.

It is considered that the MAPK pathway is associated with cardiovascular contractility and the apelinergic system (Perjés et al. 2016). It has been determined that ERK 1-2 activation has a role in the positive inotropic effect of Elabela, a newly discovered endogenous APJ ligand. Perjés et al. (2016) reported that Elabela showed positive inotropic and coronary vasodilator effects in a dose-dependent manner in the isolated rat heart. It was determined that the positive inotropic and coronary vasodilator effect of Elabela decreased due to ERK 1-2 inhibition. Those data suggest that the MAPK pathway may have a role in the vasodilator effect of the apelinergic system. Therefore, in our study, it was questioned whether the MAPK pathway contributed to

the vasodilator effect of [Pyr1]apelin-13 in the rat thoracic aorta. After the application of U0126, MAPK pathway inhibitor, no statistically significant change was observed in the vasodilator effect of [Pyr1]apelin-13, and this result shows that the MAPK pathway doesn't play a role in the vasodilator effect of apelin.

It is thought that some prostanoids produced in the COX pathway such as PGD_2, PGE_2, and PGI_2 might mediate the vasodilator effect (Félétou et al. 2011). Especially, PGI_2, whose synthesis is highly reduced by the indomethacin application, is known to be a powerful vasodilator, and it is stated that it may play a role in apelin-mediated vasodilation (Read et al. 2019; Rikitake 2020). Inconsistent data have been obtained from previous studies investigating the role of prostanoids in the apelin-mediated vasodilator effect. In an *in vivo* study, Japp et al. (2008) determined that the [Pyr1]apelin-13 or apelin-36 infusion caused vasodilation in the forearm resistance arteries, but didn't alter the venous tone. In that study, it was reported that NO had a role in the vasodilator effect of [Pyr1] apelin-13, but not prostanoids. Similarly, Mughal et al. (2018) suggested that the vasodilator effect of apelin-13 in the rat coronary artery was NO-mediated, but prostanoids didn't contribute to this effect. On the other hand, Maguire et al. (2009) reported that the eNOS/NO pathway wasn't involved in the vasodilation caused by [Pyr1]apelin-13 in the human internal thoracic artery, but prostanoids were. The contradictory results obtained in those studies may have resulted from different experimental animal species and different vascular beds used. In our study, it was determined that the vasodilator effect of [Pyr1]apelin-13 in the rat thoracic aorta was mildly decreased but didn't statistically significantly changed after the administration of COX inhibitor indomethacin. The finding we obtained shows that prostanoids aren't involved in the vasodilator effect of apelin. The slight decrease in the vasodilator effect of apelin seen here may be due to the increased level of phenylephrine-induced contraction with indomethacin administration.

AMPK pathway is closely related to vascular contractile function and mediates the anticontractile effect. It has been reported that AMPK increases eNOS activity in vascular endothelial cells and causes vasodilation NO-mediated vasodilation (Salt and Hardie 2017). Ford et al. (2012) suggested that NO-mediated vasodilation occurred after AMPK activation in spontaneously hypertensive rats. No study has been found to associate the effect of the apelinergic system on vascular contractility with the AMPK pathway. In our study, after the AMPK inhibitor dorsomorphin administration, the vasodilator effect of [Pyr1]apelin-13 in the rat thoracic aorta was statistically significantly decreased. This result shows that AMPK activation has an important role in the vasodilator effect of apelin.

Potassium channels play a key role in determining vascular tone through regulation of membrane potential, opening or closing of voltage-gated calcium channels, calcium release from intracellular sources, and calcium sensitivity. An important subtype of potassium channels is Ca^{2+}-activated K^+ channels found in most cells, and these channels are largely inhibited with the administration of TEA. In the previous studies, potassium channels, especially large-conductance Ca^{2+}-activated K^+ channels, have been associated with apelin-mediated vascular contractile effects. Modgil et al. (2013) showed that apelin-13 inhibited large-conductance Ca^{2+}-activated K^+ channels in the rat cerebral artery smooth muscle cells by a phosphatidylinositol 3-kinase-dependent mechanism. Mughal et al. (2018) determined that there was a statistically significant decrease in the vasodilator effect of apelin-13 due to large-conductance Ca^{2+}-activated K^+ channel inhibition in the rat coronary artery. In our study, it was shown that the administration of Ca^{2+}-activated K^+ channel inhibitor TEA statistically significantly decreased the vasodilator effect of [Pyr1]apelin-13 in the rat thoracic aorta. This finding shows that Ca^{2+}-activated K^+ channel activation has an important role in the vasodilator effect of apelin.

In conclusion, apelin exhibits a vasodilator effect in the rat thoracic aorta. APJ, eNOS/NO pathway, AMPK, and Ca^{2+}-activated K^+ channels play a role in the vasodilator effect of apelin. MAPK pathway and prostanoids don't contribute to the vasodilator effect of apelin. Endothelial integrity and preserved PVAT contribute significantly to the vasodilator effect of apelin. According to our knowledge, this is the first study to demonstrate that apelin has a vasodilator effect in the rat thoracic aorta and the AMPK pathway plays a role in the vasodilator effect of apelin. Also, this is the first study to report that the MAPK pathway doesn't contribute to the vasodilator effect of apelin. We think that apelin is promising for the development of alternative treatment agents in hypertensive diseases due to its prominent vasodilator effect. Therefore, elucidating the vasodilator effect mechanisms of apelin is critical and the new data obtained in our study will make an important contribution to the literature.

Conflict of interest. All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version. The authors declare that there is no conflict of interest regarding the publication of this paper.

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