Original Article / Özgün Makale

The role of potassium channels on vasorelaxant effects of elabela in rat thoracic aorta

Elabelanın sıçan torasik aortundaki vazorelaksan etkilerinde potasyum kanallarının rolü

Serdar Şahintürk ⬤, Naciye İşbil ⬤

Department of Physiology, Bursa Uludag University Faculty of Medicine, Bursa, Türkiye

ABSTRACT

Background: This study aims to investigate the roles of potassium channel subtypes in the vasorelaxant effect mechanism of elabela, which is a recently discovered endogenous apelin receptor ligand.

Methods: The vascular rings (4-mm) obtained from the thoracic aortas of 20 male Wistar Albino rats were placed into the isolated tissue bath system. The resting tension was set to 1 g. The aortic rings were contracted with 10^{-5} molar phenylephrine after the equilibration period (90 min). Elabela was applied cumulatively (10^{-10}-10^{-6} molar) to the aortic rings in the plateau phase. The experimental protocol was repeated in the presence of specific potassium channel subtype inhibitors to determine the role of potassium channels in the vasorelaxant effect mechanism of elabela.

Results: Elabela induced a concentration-dependent vasorelaxation ($p<0.001$). The maximum relaxation level was approximately 51% according to phenylephrine-induced contraction. Vasorelaxant effect level of elabela statistically significantly decreased after removal of the endothelium ($p<0.05$). Tetraethylammonium (1 milimolar), 4-Aminopyridine (1 milimolar), glyburide (10 micromolar), and barium chloride (30 micromolar) statistically significantly decreased the vasorelaxant effect level of elabela ($p<0.001$, $p<0.001$, $p<0.01$, and $p<0.05$ respectively). However, anandamide (10 micromolar) and apamin (100 nanomolar) did not statistically significantly change the vasorelaxant effect level of elabela.

Conclusion: Our results suggest that large-conductance calcium-activated, voltage-gated, adenosine triphosphate-sensitive, and inward-rectifier potassium channels are involved in the vasorelaxant effect mechanism of elabela in the rat thoracic aorta.

Keywords: Elabela; potassium channels; thoracic aorta; tissue bath; vasorelaxation.

ÖZ

Amaç: Bu çalışmada, yeni keşfedilen bir endojen apelin reseptör ligandı olan elabelanın vazorelaksan etki mekanizmasında potasyum kanal alt tiplerinin rolü araştırıldı.

Çalışma planı: Yirmi erkek Wistar Albino sıçanın torasik aortlarından elde edilen vasküler halkalar (4 mm) izole doku banyosu sistemine yerleştirildi. Dinlenim gerimi 1 gr olarak ayarlandı. Dengeleme periyodu (90 dak.) sonrasında aort halkaları 10^{-5} molar fenilefrin ile kasıldı. Plato fazında aort halkalarına kümülatif olarak (10^{-10}-10^{-6} molar) Elabela uygulandı. Potasyum kanallarının Elabelanın damar gevşetici etki mekanizmasındaki rolünü belirlemek için spesifik potasyum kanalı alt tip inhibitörlerinin varlığında deneysel protokol tekrarlandı.

Bulgular: Elabela konsantrasyon bağımlı olarak vazorelaksasyona neden oldu ($p<0.001$). Fenilefrin kaynaklı kasılmaya göre maksimum gevşeme seviyesi yaklaşık %51'di. Elabelanın damar gevşetici etki düzeyi, endotelin çıkarılmasından sonra istatistiksel olarak anlamlı düzeyde azaldı ($p<0.05$). Tetraetilamonyum (1 milimolar), 4-Aminopiridin (1 milimolar), gliburid (10 micromolar) ve baryum klorür (30 micromolar) Elabelanın vazorelaksan etki düzeyini istatistiksel olarak anlamlı düzeyde (sırasıyla; $p<0.001$, $p<0.001$, $p<0.01$ ve $p<0.05$) azalttı. Bununla birlikte, anandamid (10 micromolar) ve apamin (100 nanomolar) Elabelanın vazorelaksan etki seviyesini istatistiksel olarak anlamlı bir şekilde değiştirmedi.

Sonuç: Bulgularımız büyük iletkenlikli kalsiyum ile aktive olan voltaj kapılı, adenozin trifosfata duyarlı ve içeri doğrultucu potasyum kanallarının Elabelanın sıçan torasik aortundaki damar gevşetici etki mekanizmasında rol oynadığını göstermektedir.

Anahtar sözcükler: Elabela; potasyum kanalları; torasik aort; doku banyosu; vazodilatasyon.

The apelinergic system consists of apelin receptor (APJ), apelin (firstly discovered endogenous APJ ligand), and elabela (ELA).[1-4] Elabela, a current endogenous APJ ligand, is widely expressed in cardiovascular tissues, and it is necessary for normal heart development and angiogenesis in the embryonic period. Furthermore, it exhibits positive inotropic, antihypertensive, and vasorelaxant effects.[4-7] However, the vasodilatory effect mechanism of ELA is unknown.

The efficiencies of ion channels in vascular smooth muscle cells play an important role in the regulation of vascular tone and the formation of contraction-relaxation responses. The main of these channels are cation channels such as potassium (K^+), calcium (Ca^{2+}), and sodium channels.[8] Potassium channels have a key role, particularly in changes in membrane potential. In studies on vascular smooth muscle, five types of potassium channel types have been defined. These are Ca^{2+}-activated (K_{Ca}), voltage-gated (Kv), ATP-sensitive (K_{ATP}), inward-rectifier (Kir), and two-pore domain (K_{2P}) potassium channels. These potassium channels, each with different subtypes, regulate membrane potential and ionic movements in vascular smooth muscle cells. Changes in membrane potential, on the other hand, determine the intracellular Ca^{2+} concentration by regulating the activity of L-type voltage-gated calcium channels. Changing Ca^{2+} concentration creates an effect in the direction of contraction or relaxation.[9] Therefore, potassium channels are of great importance in the regulation of vascular contractility.

It has been proposed that ELA may provide an effective treatment alternative in common cardiovascular diseases such as hypertension.[10] In addition, it is thought that the regulation of potassium channels is very important in the pathophysiological basis and treatment modalities of cardiovascular diseases.[11] Therefore, it is critical to reveal the effect of ELA on vascular contraction-relaxation responses and its effect mechanisms in detail. In the present study, we aimed to investigate the effect and the possible role of potassium channel subtypes in the effect mechanism of ELA on the rat thoracic aortic contractility. To the best of our knowledge, this is the first study to investigate in detail the possible roles of potassium channel subtypes in the vascular functional effects of ELA.

MATERIALS AND METHODS

This experimental study used 20 12-week-old male Wistar Albino rats weighing 250 to 300 g. The study protocol was approved by the Bursa Uludag University Animal Experiments Local Ethics Committee (Date: 15.06.2021, No: 2021-08/04). All experiments were conducted in accordance with the National Institutes of Health Guidelines on the Use of Laboratory Animals.

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 milimolar (mM): 2.5 $CaCl_2$ · $2H_2O$, 118 NaCl, 4.8 KCl, 1.2 KH_2PO_4, 11 $C_6H_{12}O_6$ · H_2O, 25 $NaHCO_3$, 1.2 $MgSO_4$ · $7H_2O$). The vascular rings of 4-mm length were prepared from the vessels carefully cleared of perivascular tissues. Four 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, Turkey) 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. The Krebs solution was continuously gassed with a gas mixture of 95% O2-5% CO_2, and the pH was adjusted to 7.4. After the first 30 min, the resting tension was set to 1 g.[12] The Krebs solution was renewed every 15 min during the 1-h equilibration period. After the equilibration period, phenylephrine (10^{-5} M) was used to stimulate vascular contraction. Elabela was applied, when vascular tension reached the plateau phase. An equal volume of distilled water was used instead of ELA in the control group. To determine the effect mechanisms of ELA, the potassium channel inhibitors (tetraethylammonium: TEA; glyburide, 4-Aminopyridine: 4-AP; apamin, anandamide, and barium chloride: $BaCl_2$) were administered 30 min before phenylephrine was. In cases where dimethyl sulfoxide (DMSO) was used as a solvent, an equal volume of DMSO was used in the vehicle group.

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 ELA were calculated over this value. The records of the control groups were taken at the beginning of each experiment. Afterward, the washing and equilibration periods were 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 could not 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. The endothelium was mechanically removed from some aortic rings by gentle rubbing of the intimal surface with a wooden stick. Endothelium-denuded (E-) rings were considered to have less than 10% relaxation response of phenylephrine-induced contraction to 10^{-5} M acetylcholine.[12] All surgical operations and isolated tissue bath studies were performed as previously described.[13,14]

Drugs

Elabela (ELA-32) was purchased from Tocris. The other chemicals and drugs were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, Missouri, USA). The doses of ELA, phenylephrine, acetylcholine, and potassium channel inhibitors were determined by the literature and the drugs were prepared by the instructions for use. Elabela (10^{-10}-10^{-6} M), phenylephrine (10^{-5} M), acetylcholine (10^{-5} M), TEA (Large-conductance calcium-activated potassium (BKCa) channel inhibitor; 1 mM), 4-AP (Kv inhibitor; 1 mM), $BaCl_2$ (Kir inhibitor; 30 micromolar [μM]), and apamin (Small-conductance calcium-activated potassium (SKCa) channel inhibitor; 100 nanomolar [nM])

were dissolved in distilled water. Glyburide (K_{ATP} inhibitor; 10 μM), and anandamide (K_{2P} inhibitor; 10 μM) were dissolved in DMSO. The final concentration of DMSO in the Krebs solution did not exceed 0.1% and DMSO did not affect vascular smooth muscle contraction or relaxation.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard error of the mean (SEM) (n=8) as a percentage of the plateau tension obtained with phenylephrine. One-way analysis of variance (ANOVA) was used for comparisons between multiple groups, while the Bonferroni test was applied as a post-hoc test. A p value of <0.05 was considered statistically significant.

RESULTS

Effect of ELA on the rat thoracic aortic tension

The percentage tension values in the ELA group were found statistically significantly lower compared to the percentage tension values in the control group (p<0.001). Elabela showed a statistically significant vasorelaxant effect in the rat thoracic aorta. The
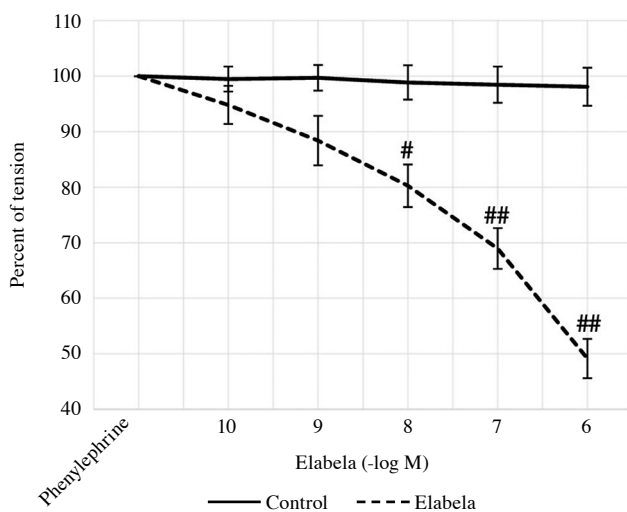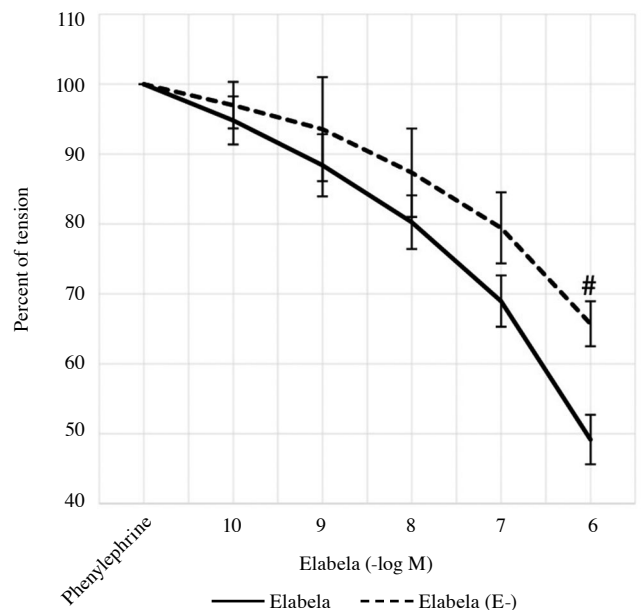

Figure 1. Vasorelaxant effect of elabela.

Data are expressed in mean ± SEM as a percentage of the plateau tension obtained with phenylephrine. Percentage tension values in the elabela group were found statistically significantly lower than in the control group. n=8 for each group. # p<0.05; ## p<0.001.

Figure 2. Effect of removal of the endothelium in the vasodilator effect of elabela.

Data are expressed in mean ± SEM as a percentage of the plateau tension obtained with phenylephrine. Percentage tension values in the elabela (E-) group were found statistically significantly higher than in the elabela group. n=8 for each group. # p<0.05; E-: Endothelium-denuded.

Table 1. Descriptive statistics and multiple comparisons

Groups	Concentration of elabela	Mean	SEM	$p*$
Control (n=8)	10^{-10} M	99.48	3.44	-
	10^{-9} M	99.71	4.45	-
	10^{-8} M	98.87	3.84	-
	10^{-7} M	98.47	3.67	-
	10^{-6} M	98.10	3.54	-
Elabela (n=8)	10^{-10} M	94.82	2.25	0.912
	10^{-9} M	88.40	2.31	0.379
	10^{-8} M	80.26	3.09	0.030
	10^{-7} M	68.97	3.26	0.000
	10^{-6} M	49.15	3.42	0.000
Elabela (E-) (n=8)	10^{-10} M	97.01	3.33	1.000
	10^{-9} M	93.58	7.44	1.000
	10^{-8} M	87.34	6.33	0.990
	10^{-7} M	79.45	5.08	0.725
	10^{-6} M	65.72	3.22	0.033
TEA (n=8)	10^{-10} M	99.88	3.47	0.918
	10^{-9} M	97.83	3.83	0.826
	10^{-8} M	94.81	4.63	0.387
	10^{-7} M	89.40	4.42	0.003
	10^{-6} M	81.20	5.21	0.000
4-AP (n=8)	10^{-10} M	98.70	5.56	0.988
	10^{-9} M	97.63	5.11	0.661
	10^{-8} M	93.30	3.71	0.157
	10^{-7} M	88.76	5.87	0.003
	10^{-6} M	80.50	7.61	0.000
$BaCl_2$ (n=8)	10^{-10} M	97.89	3.92	1.000
	10^{-9} M	95.62	4.82	0.977
	10^{-8} M	92.04	5.81	0.783
	10^{-7} M	84.35	7.65	0.680
	10^{-6} M	71.96	4.63	0.039
Apamin (n=8)	10^{-10} M	95.59	4.64	1.000
	10^{-9} M	89.68	6.49	1.000
	10^{-8} M	82.46	6.33	1.000
	10^{-7} M	71.70	4.60	1.000
	10^{-6} M	54.82	4.17	0.984
Vehicle (n=8)	10^{-10} M	96.21	5.24	-
	10^{-9} M	89.92	4.37	-
	10^{-8} M	80.84	4.06	-
	10^{-7} M	70.39	4.05	-
	10^{-6} M	52.65	3.64	-
Glyburide (n=8)	10^{-10} M	99.54	4.66	1.000
	10^{-9} M	97.08	7.53	0.996
	10^{-8} M	92.58	4.45	0.643
	10^{-7} M	86.23	3.26	0.008
	10^{-6} M	75.48	4.53	0.001
Anandamide (n=8)	10^{-10} M	97.65	4.52	1.000
	10^{-9} M	93.36	7.08	1.000
	10^{-8} M	84.46	7.47	1.000
	10^{-7} M	75.64	7.39	0.999
	10^{-6} M	56.43	3.71	0.996

SEM: Standard error of the mean; E-: Endothelium-denuded; TEA: Tetraethylammonium; 4-AP: 4-Aminopyridine; $BaCl_2$: Barium chloride; * p<0.05 indicates statistical significance.
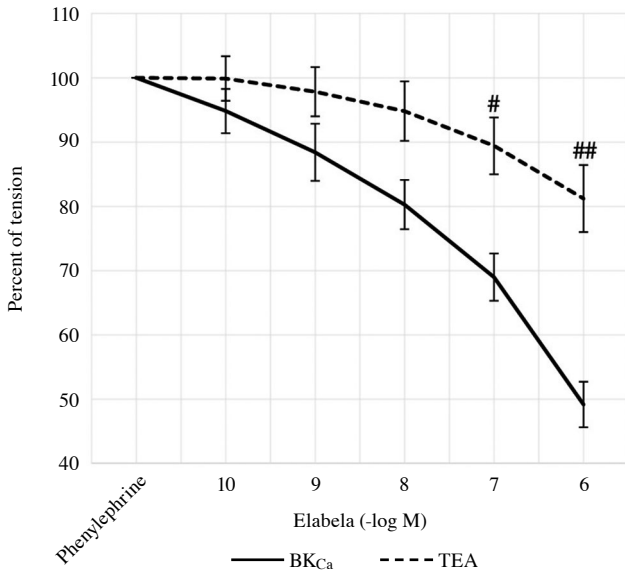
Figure 3. Effect of BKC$_a$ potassium channel inhibition on the vasorelaxant effect of elabela.

Data are expressed in mean ± SEM as a percentage of the plateau tension obtained with phenylephrine. Percentage tension values in the TEA group were found statistically significantly higher than in the elabela group. n=8 for each group. TEA: Tetraethylammonium; SEM: Standard error of the mean; BKCa: Large-conductance calcium-activated potassium; # p<0.01; ## p<0.001.
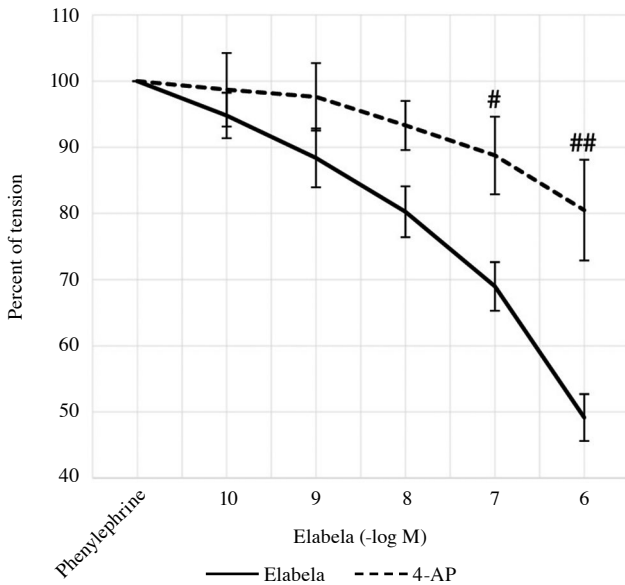

Figure 4. Effect of Kv potassium channel inhibition on the vasorelaxant effect of elabela.

Data are expressed in mean ± SEM as a percentage of the plateau tension obtained with phenylephrine. Percentage tension values in the 4-AP group were found statistically significantly higher than in the elabela group. n=8 for each group. SEM: Standard error of the mean; Kv: Voltage-gated potassium; 4-AP: 4-Aminopyridine; # p<0.01; ## p<0.001.

Figure 5. Effect of K$_{ATP}$ potassium channel inhibition on the vasorelaxant effect of elabela.

Data are expressed in mean ± SEM as a percentage of the plateau tension obtained with phenylephrine. Percentage tension values in the glyburide group were found statistically significantly higher than in the elabela group. n=8 for each group. K$_{ATP}$: ATP-sensitive potassium; SEM: Standard error of the mean; # p<0.01.

Figure 6. Effect of Kir potassium channel inhibition on the vasorelaxant effect of elabela.

Data are expressed in mean ± SEM as a percentage of the plateau tension obtained with phenylephrine. Percentage tension values in the BaCl$_2$ group were found statistically significantly higher than in the elabela group. n=8 for each group; Kir: Inward-rectifier potassium; SEM: Standard error of the mean; BaCl$_2$: Barium chloride; # p<0.05.

maximum relaxation level was approximately 51% (Figure 1). The percentage tension values in the ELA (E-) group were found statistically significantly higher
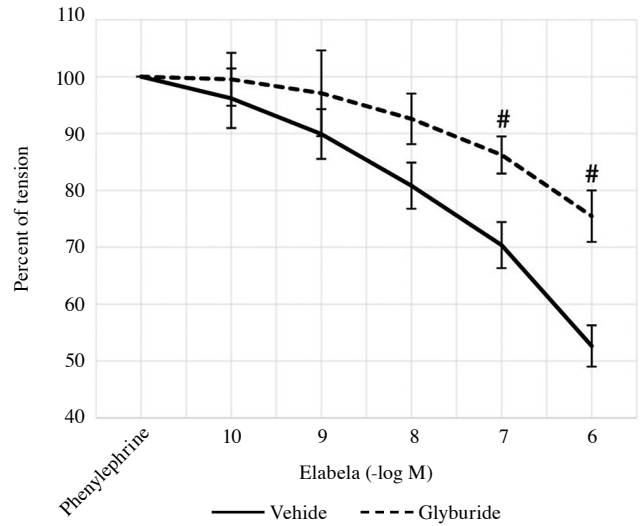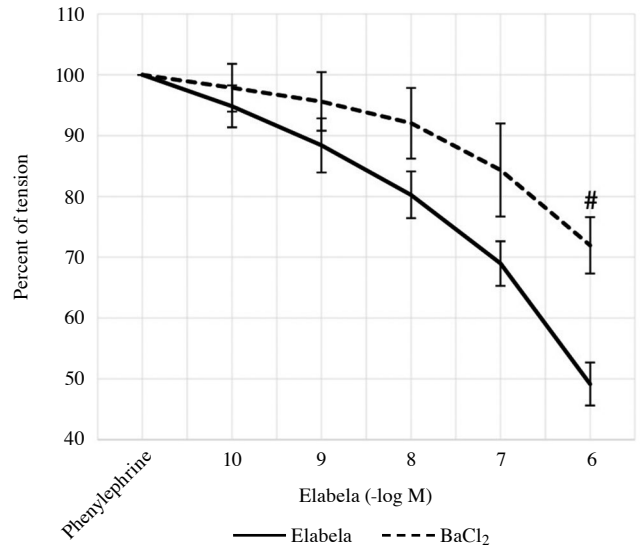
compared to the percentage tension values in the ELA group (p<0.05). Removal of the vascular endothelium statistically significantly decreased the vasorelaxant effect level of ELA up to approximately 34% (p<0.05) (Figure 2).

The role potassium channels in the vasorelaxant effect mechanism of ELA

The percentage tension values in the TEA group ($p<0.001$), 4-AP group ($p<0.001$), and $BaCl_2$ group ($p<0.05$) were found statistically significantly higher compared to the percentage tension values in the ELA group. The percentage tension values in the glyburide group were found statistically significantly higher compared to the percentage tension values in the vehicle group ($p<0.01$). After BK_{Ca}, Kv, K_{ATP}, or Kir potassium channel inhibition, the vasorelaxant effect level of ELA in the rat thoracic aorta was statistically significantly decreased (Table 1) (Figures 3-6).

On the other hand, there was no statistically significant difference between the percent tension values in the anandamide group and the percent tension values in the vehicle group. Also, there was no statistically significant difference between the percent tension values in the apamin group and the percent tension values in the ELA group. After K_{2P} or SK_{Ca} potassium channel inhibition, there was no statistically significant change in the vasorelaxant effect level of ELA in the rat thoracic aorta (not shown).

DISCUSSION

Our study demonstrates that ELA relaxes the precontracted rat thoracic aorta, and potassium channels are involved in the vasorelaxant effect mechanism of ELA. The main finding of this study is that ELA caused vasorelaxation in the endothelium intact rat thoracic aorta which was precontracted with phenylephrine. This finding is consistent with the data obtained in a few previous studies. Perjés et al.[15] reported that ELA exhibited a vasodilatory effect in the rat coronary artery. Wang et al.[16] showed that ELA caused vasodilation in a dose-dependent manner in the mouse aortic rings and determined that the maximum ELA-mediated vasorelaxant effect occurred at a level of approximately 74% at the 10^{-6} M dose. We concluded that the vasodilatory effect level of ELA in the rat thoracic aorta was approximately 51% at the dose of 10^{-6} M which was the maximum dose used in our study. This effect level is higher than the vasodilator effect level of other endogenous peptides that we have determined in our previous studies. In the first of two recent studies, we determined that the vasorelaxation level of 10^{-6} M pyroglutamyl apelin-13 in the rat thoracic aorta was approximately 42%.[13] In the second study, the vasorelaxation level of irisin at the same dose was approximately 40%.[17] Besides, in our study, it was observed that the vasorelaxant effect mediated by ELA in the rat thoracic aorta was

statistically significantly reduced up to approximately 34% as a result of the removal of the endothelium. The data we obtained indicate that the vasorelaxant effect of ELA is revealed by endothelium-dependent and endothelium-independent mechanisms. Besides, our result suggests that endothelium-independent mechanisms have a larger share in the vasodilatory effect of ELA. A similar result was obtained in the study of Wang et al.,[16] and it was observed that the vasorelaxant effect of ELA was largely preserved after the removal of the endothelium.

The number of studies investigating potassium channels in the vascular functional effects of the apelinergic system is very limited. Modgil et al.[18] reported that apelin-13 inhibited BK_{Ca} potassium channels in cerebral artery smooth muscle cells via a PI3K-dependent mechanism. Mughal et al.[19] demonstrated that the vasorelaxant effect level of apelin-13 statistically significantly decreased due to BK_{Ca} channel inhibition in the rat coronary artery. In another study, Mughal et al.[20] found that apelin-13 reduced nitric oxide-induced relaxation of cerebral arteries by inhibiting activation of BK_{Ca} potassium channels. These data indicate that apelin may act as an activator or inhibitor on potassium channels according to the type of vascular bed. On the other hand, the role of potassium channels in the vascular functional effects of ELA has not been investigated, yet. In our study, for the first time, the roles of all major potassium channels subtypes in vasculature in ELA-induced vasorelaxation in rat thoracic aorta were evaluated comprehensively. Our results showed that BK_{Ca} inhibitor TEA, Kv inhibitor 4-AP, K_{ATP} inhibitor glyburide, and Kir inhibitor $BaCl_2$ statistically significantly diminished the vasorelaxant effect level of ELA. However, K_{2P} inhibitor anandamide and SK_{Ca} inhibitor apamin did not statistically significantly affect the vasorelaxant effect level of ELA. These results indicate that BK_{Ca}, Kv, K_{ATP}, and Kir potassium channels contribute to the vasorelaxant effect of ELA. Otherwise, there are differences between potassium channel subtypes in terms of their effect levels. The roles of BK_{Ca} and Kv potassium channels in the vasodilatory effect of ELA are higher than the other potassium channel subtypes. However, the role of Kir potassium channels in ELA-induced vasorelaxation is the least among all potassium channels with significant effect.

It has been reported that potassium channels play a role in the vasoactive effects of other endogenous peptides other than the apelinergic system. Demirel et al.[21] determined that irisin exerts a vasodilator effect through potassium channels in the rat thoracic

aorta. Similar to the study we presented, it was found that BK_{Ca}, Kv, Kir, and K_{ATP} potassium channels played a role in the vasodilator effect of irisin. However, in contrast to the present study, SK_{Ca} potassium channels were also found to be effective in irisin-induced vasodilation. The data we obtained in this study suggest that SK_{Ca} potassium channels do not contribute to the vasodilator effect of ELA. In the light of these findings, potassium channels likely to contribute significantly to vasodilation, but their role in the effect of different endogenous peptides may vary. On the other hand, in a study investigating the vascular functional effects of juglone in the porcine coronary artery, potassium channels did not mediate vasodilation, contrary to our study.[22]

The therapeutic potential of ELA in cardiovascular and hypertensive diseases such as heart failure, pulmonary arterial hypertension, myocardial infarction, and preeclampsia has been demonstrated in previous studies.[10,23] Also, the data we obtained in our study suggest that ELA may be beneficial in conditions such as hypertension and myocardial infarction, which are associated with increased vasoreactivity. In addition, ELA may also improve vascular congestion caused by widely used drugs such as fluoxetine.[24]

The main limitations of our study are that a muscular artery model such as a coronary artery or mesenteric artery was not used. Besides, direct measurement methods such as the patch-clamp technique were not used to demonstrate potassium channel activation. Furthermore, beneficial vascular functional effects of ELA could be supported using *in vivo* disease models, such as atherosclerosis and hypertension. Therefore, studies investigating the vascular functional effects and effect mechanisms of ELA in different vascular beds and *in vivo* disease models are needed.

In conclusion, elabela exhibits a prominent vasorelaxant effect in the precontracted endothelium intact rat thoracic aorta. The vasorelaxant effect level of elabela partially decreases after the removal of endothelium. The large-conductance calcium-activated, voltage-gated, ATP-sensitive, and inward-rectifier potassium channels are involved in the vasorelaxant effect mechanism of elabela. However, two-pore domain and small-conductance calcium-activated potassium channels do not play a role in the vasodilatory effect mechanism of elabela. The apelinergic system ligands are promising for the discovering of novel treatment agents in cardiovascular diseases owing to their potent vasodilatory effects. Based on these findings, elucidating the vasorelaxant effect mechanisms of elabela is of great importance,

and we believe that these findings would provide a contribution to the body of knowledge on this subject in the literature.

REFERENCES

1. Read C, Nyimanu D, Williams TL, Huggins DJ, Sulentic P, Macrae RGC, et al. International Union of Basic and Clinical Pharmacology. CVII. Structure and pharmacology of the Apelin receptor with a recommendation that Elabela/Toddler is a second endogenous peptide ligand. Pharmacol Rev 2019;71:467-502.

2. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 1998;251:471-6.

3. Zhang Y, Wang Y, Lou Y, Luo M, Lu Y, Li Z, et al. Elabela, a newly discovered APJ ligand: Similarities and differences with Apelin. Peptides 2018;109:23-32.

4. Xu J, Chen L, Jiang Z, Li L. Biological functions of Elabela, a novel endogenous ligand of APJ receptor. J Cell Physiol 2018;233:6472-82.

5. Chng SC, Ho L, Tian J, Reversade B. ELABELA: A hormone essential for heart development signals via the apelin receptor. Dev Cell 2013;27:672-80.

6. Pauli A, Norris ML, Valen E, Chew GL, Gagnon JA, Zimmerman S, et al. Toddler: An embryonic signal that promotes cell movement via Apelin receptors. Science 2014;343:1248636.

7. İşbil N, Şahintürk S, Demirel S. Vascular functional effects of the apelinergic system. J Lit Pharm Sci 2021;10:12-20.

8. Tykocki NR, Boerman EM, Jackson WF. Smooth muscle ion channels and regulation of vascular tone in resistance arteries and arterioles. Compr Physiol 2017;7:485-581.

9. Jackson WF. Potassium channels in regulation of vascular smooth muscle contraction and growth. Adv Pharmacol 2017;78:89-144.

10. Xu C. The Elabela in hypertension, cardiovascular disease, renal disease, and preeclampsia: An update. J Hypertens 2021;39:12-22.

11. Cheng J, Wen J, Wang N, Wang C, Xu Q, Yang Y. Ion channels and vascular diseases. Arterioscler Thromb Vasc Biol 2019;39:e146-e156.

12. Panthiya L, Pantan R, Tocharus J, Nakaew A, Suksamrarn A, Tocharus C. Endothelium-dependent and endothelium-independent vasorelaxant effects of tiliacorinine 12'-O-acetate and mechanisms on isolated rat aorta. Biomed Pharmacother 2019;109:2090-9.

13. Sahinturk S, Demirel S, Ozyener F, Isbil N. [Pyr1]apelin-13 relaxes the rat thoracic aorta via APJ, NO, AMPK, and potassium channels. Gen Physiol Biophys 2021;40:427-34.

14. Yılmaz A, Elbey B, Yazgan ÜC, Dönder A, Arslan N, Arslan S, et al. Protective effects of caffeic acid phenethyl ester on fluoxetine-induced hepatotoxicity: An experimental study. Biomed Res Int 2016;2016:1247191.

15. Perjés Á, Kilpiö T, Ulvila J, Magga J, Alakoski T, Szabó Z, et al. Characterization of apela, a novel endogenous ligand of apelin receptor, in the adult heart. Basic Res Cardiol 2016;111:2.

16. Wang Z, Yu D, Wang M, Wang Q, Kouznetsova J, Yang R, et al. Elabela-apelin receptor signaling pathway is functional in mammalian systems. Sci Rep 2015;5:8170.

17. Demirel S, Şahintürk S, Işbil N, Özyener F. Irisin relaxes rat thoracic aorta through activating signaling pathways implicating protein kinase C. Turk J Med Sci 2021.

18. Modgil A, Guo L, O'Rourke ST, Sun C. Apelin-13 inhibits large-conductance Ca2+-activated K+ channels in cerebral artery smooth muscle cells via a PI3-kinase dependent mechanism. PLoS One 2013;8:e83051.

19. Mughal A, Sun C, O'Rourke ST. Activation of large conductance, calcium-activated potassium channels by nitric oxide mediates apelin-induced relaxation of isolated rat coronary arteries. J Pharmacol Exp Ther 2018;366:265-73.

20. Mughal A, Sun C, O'Rourke ST. Apelin reduces nitric oxide-induced relaxation of cerebral arteries by inhibiting activation of large-conductance, calcium-activated K channels. J Cardiovasc Pharmacol 2018;71:223-32.

21. Demirel S, Sahinturk S, Isbil N, Ozyener F. Physiological role of K+ channels in irisin-induced vasodilation in rat thoracic aorta. Peptides 2021;147:170685.

22. Ahmad T, Shah AJ, Roberts R. Mechanisms mediating the vasodilatory effects of juglone in porcine isolated coronary artery. Eur J Pharmacol 2020;866:172815.

23. Zhou M, Wu Y. Effects and signaling pathways of Elabela in the cardiovascular system. Peptides 2021;147:170674.

24. Arslan S, Zeytun H, Basuguy E, İbiloğlu İ, Uygun İ, Yılmaz A, et al. Cordycepin prevents postoperative formation of intra-abdominal adhesion in a rat model: An experimental study. Ulus Travma Acil Cerrahi Derg 2017;23:273-8.