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

Elabelanın sıçan torasik aortundaki vazorelaksan etkilerinde potasyum kanallarının rolü

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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 ($10^{-10}$-$10^{-4}$ 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 rolünü araştırıldı.

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

Bulgular: Elabela konsantrasyon başlı olarak vazorelaksasyonun neden olduğu ($p<0.001$). Fenilefrin kaynaklı kasılmaya göre maksimum gevşeme seviyesi yaklaşık %51’di. Elabelanın damar gevşetici etki düzeyi, endotelin çıkarılması sonrasında istatistiksel olarak anlamlı ölçüde 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 azalttı ($p<0.001$, $p<0.001$, $p<0.01$, ve $p<0.05$) azaltı. Bununla birlikte, anandamid (10 micromolar) ve apamin (100 nanomolar) Elabelanın vazorelaksan etki seviyesini istatistiksel olarak anlamlı bir şekilde değiştirdi.

Sonuç: Bulgularımız büyük iletezlik kalsiyum ilavesi ile aktive olan voltaj kaplı, adenosin trifosfata duyarlı ve içeri doğruktulu potasyum kanallarının Elabelanın sıçan torasik aortındaki damar gevşetici etki mekanizmasında rol oynadığını göstermektedir.

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

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The apelinergic system consists of apelin receptor (AP), apelin (firstly discovered endogenous AP ligand), and elabela (ELA). Elabela, a current endogenous AP 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. However, the vasodilatory effect mechanism of ELA is unknown.

Based on these considerations, we aimed to investigate the effect and the possible role of potassium channel subtypes in the vascular functional potential. In studies on vascular smooth muscle, five types of potassium channel types have been defined. These are Ca2+-activated (KCa), voltage-gated (Kv), ATP-sensitive (KA TP), inward-rectifier (KIR), and two-pore domain (K2P) 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 Ca2+ concentration by regulating the activity of L-type voltage-gated calcium channels. Changing Ca2+ concentration creates an effect in the direction of contraction or relaxation. 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. In addition, it is thought that the regulation of potassium channels is very important in the pathophysiological basis and treatment modalities of cardiovascular diseases. 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 millimolar (mM): 2.5 CaCl2 · 2H2O, 118 NaCl, 4.8 KCl, 1.2 KH2PO4, 11 C6H12O6 · H2O, 25 NaHCO3, 1.2 MgSO4 · 7H2O). 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% CO2, and the pH was adjusted to 7.4. After the first 30 min, the resting tension was set to 1 g. 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: BaCl2) 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 ($10^{-4}$ 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\(_{\text{ATP}}\) inhibitor; 10 µM), and anandamide (K\(_{\text{P2}}\) 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

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**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.
| 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         | 94.82                    | 2.25  | 0.918|      |
| 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         | 97.01                    | 3.33  |     | 1.000|
| 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|      |
| **BaCl2 (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; BaCl2: Barium chloride; * p<0.05 indicates statistical significance.
The 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 BaCl2 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 BKCa, Kv, KATP, 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 K2P or SKCa 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. reported that ELA exhibited a vasodilatory effect in the rat coronary artery. Wang et al. 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%. In the second study, the vasorelaxation level of irisin at the same dose was approximately 40%. 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., 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. reported that apelin-13 inhibited BKCa potassium channels in cerebral artery smooth muscle cells via a PI3K-dependent mechanism. Mughal et al. demonstrated that the vasorelaxant effect level of apelin-13 statistically significantly decreased due to BKCa channel inhibition in the rat coronary artery. In another study, Mughal et al. found that apelin-13 reduced nitric oxide-induced relaxation of cerebral arteries by inhibiting activation of BKCa 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 BKCa inhibitor TEA, Kv inhibitor 4-AP, KATP inhibitor glyburide, and Kir inhibitor BaCl2 statistically significantly diminished the vasorelaxant effect level of ELA. However, K2P inhibitor anandamide and SKCa inhibitor apamin did not statistically significantly affect the vasorelaxant effect level of ELA. These results indicate that BKCa, Kv, KATP, 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 BKCa 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. determined that irisin exerts a vasodilator effect through potassium channels in the rat thoracic
vasodilation, contrary to our study. However, in contrast to the present study, SKCa potassium channels were also found to be effective in irisin-induced vasodilation. The data we obtained in this study suggest that SKCa 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 effect mechanisms of elabela is of great importance, elucidating the vasorelaxant effect in the precontracted endothelium and in vivo disease models are needed.

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. 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. 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.

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