ELABELA-APJ axis protects from pressure overload heart failure and angiotensin II-induced cardiac damage

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Aims

Elabela/Toddler/Apela (ELA) has been identified as a novel endogenous peptide ligand for APJ/Apelin receptor/Aplnr. ELA plays a crucial role in early cardiac development of zebrafish as well as in maintenance of self-renewal of human embryonic stem cells. Apelin was the first identified APJ ligand, and exerts positive inotropic heart effects and regulates the renin–angiotensin system. The aim of this study was to investigate the biological effects of ELA in the cardiovascular system.

Methods and results

Continuous infusion of ELA peptide significantly suppressed pressure overload-induced cardiac hypertrophy, fibrosis and impaired contractility in mice. ELA treatment reduced mRNA expression levels of genes associated with heart failure and fibrosis. The cardioprotective effects of ELA were diminished in APJ knockout mice, indicating that APJ is the key receptor for ELA in the adult heart. Mechanistically, ELA downregulated angiotensin-converting enzyme (ACE) expression in the stressed hearts, whereas it showed little effects on angiotensin-converting enzyme 2 (ACE2) expression, which are distinct from the effects of Apelin. FoxM1 transcription factor, which induces ACE expression in the stressed hearts, was downregulated by ELA but not by Apelin. ELA antagonized angiotensin II-induced hypertension, cardiac hypertrophy, and fibrosis in mice.

Conclusion

The ELA-APJ axis protects from pressure overload-induced heart failure possibly via suppression of ACE expression and pathogenic angiotensin II signalling. The different effects of ELA and Apelin on the expression of ACE and ACE2 implicate fine-tuned mechanisms for a ligand-induced APJ activation and downstream signalling.

Keywords

Elabela • Apelin • APJ • Angiotensin • ACE • ACE2

1. Introduction

Apelin is an endogenous peptide hormone, which exerts potent positive inotropic activity1,2 through binding to its cognitive receptor APJ/Apelin receptor/Aplnr. APJ is a G-protein coupled receptor which shares significant homology with the angiotensin II type 1 receptor (AT1R).3-5 Apelin peptide exerts load-independent positive inotropy and increases coronary blood flow by vascular dilation, thereby providing beneficial effects in failing hearts.5,6 The endogenous Apelin–APJ axis maintains heart contractility and is cardioprotective under conditions of aging, exercise, and pressure overload; as evidenced by the observation that mouse knockout of Apelin or APJ show reduced contractile functions and enhanced cardiac damage in such conditions.2,7 In addition, the Apelin–APJ system negatively regulates angiotensin II (Ang II)—AT1R signalling through heterodimeric interaction of APJ and AT1R in atherosclerosis or upregulation of angiotensin-converting enzyme 2 (ACE2) expression in pressure overload heart failure.9,10
mediated through an alternate cell-surface receptor, distinct from APJ. Several recent studies have shown that ELA peptide can activate mammalian APJ, regulates water balance, and is inotropic for contractility of the isolated heart.

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In this study, we show beneficial effects of ELA peptide (32 amino acids) on the pathology of pressure overload-induced heart failure in mice. We also demonstrate that endogenous APJ is an essential receptor for ELA to exert cardioprotective effects in failing hearts and that ELA antagonizes Ang II-induced hypertension and cardiac damage in mice.

2. Methods

2.1 Mice

C57BL/6j and C57BL/6N wild-type mice were purchased from Japan SLC and maintained at the animal facilities of Akita University Graduate School of Medicine. APJ knockout mice were generated and maintained as described previously. All animal experiments conformed to the Guide for the Care and Use of Laboratory Animals, Eighth Edition, updated by the US National Research Council Committee in 2011, and approvals of the experiments were granted by the ethics review board of Akita University.

2.2 Pharmacological intervention

The 32 amino-acid long human ELA peptide with the following primary sequence (NH2-GRP/VNLTRMRKLKHCNLCRQCMPLHRVPPF-COOH) comprising an intra-molecular disulfide bond was produced synthetically at >98% purity (BEX) and dialyzed twice into PBS. For ELA treatment, wild-type or APJ KO male mice at the age of 3-month old were subcutaneously infused with either saline or the ELA peptide at 1 mg/kg/day for 2 weeks by osmotic mini-pumps (Alzet model 1002, Alza Corp., Mountain View, CA, USA) using ChemiDoc Touch (Bio-Rad, Hercules, CA, USA). Image Lab software was used for quantification of bands.

2.3 Transverse aortic constriction (TAC)

Three-month old wild-type mice or APJ KO mice were subjected to pressure overload by transverse aortic constriction (TAC) as previously described. Briefly, mice were anesthetized via intraperitoneal injection of ketamine (100 mg/kg) and xylazine (20 mg/kg), and a longitudinal incision was made in the proximal portion of sternum. The aortic arch was ligated with an overlying 27-gauge needle by 7-0 silk. The needle was immediately removed leaving a discrete region of constriction. The sham-treated group underwent a similar procedure without ligation. Echocardiography was performed 2 weeks after TAC or sham surgery and mice were then sacrificed by cervical dislocation.

2.4 Echocardiography and blood pressure measurements

Echocardiographic measurements were performed as previously described. Briefly, after mice were anesthetized with isoflurane (1%)/oxygen, echocardiography was performed using Vevo770 equipped with a 30-MHz linear transducer. Fractional shortening (FS) was calculated as follows: FS = [(LVEDD – LVESD)/LVEDD] × 100. M-mode images were obtained for the measurement of wall thickness and chamber dimensions with the use of the leading-edge convention adapted by the American Society of Echocardiography. Blood pressures were measured in conscious mice by a programmable sphygmomanometer (BP-200, Softron, Tokyo, Japan) using the tail cuff method after 5 days of daily training, as described previously.

2.5 Histology

Heart tissues were fixed with 4% formalin and embedded in paraffin. Five μm thick sections were prepared and stained with Hematoxylin & Eosin or Masson & Trichrome.

2.6 Quantitative real-time PCR

RNA was extracted using TRizol reagent (Invitrogen, Waltham, MA, USA) and cDNA synthesized using the PrimeScript RT reagent kit (TAKARA, Berkeley, CA, USA). Sequences of the forward and reverse primers of the genes studied are shown in Supplementary material online, Table S1. Real-time PCR was run in 96-well plates using a SYBR Premix ExTaq II (TAKARA) according to the instructions of the manufacturer. Relative gene expression levels were quantified by using the Thermal Cycler Dice Real Time System II software (TAKARA).

2.7 Western blotting

Heart protein was extracted using a TNE lysis buffer (50 mM Tris, 150 mM NaCl, 1 mM EDTA, 1% NP40, protease inhibitor (complete Mini, Roche, Indianapolis, IN, USA), 100 mM NaF, 2 mM Na3VO4), and Microsmash (MS-100R, TOMY, Cologne, Germany). After sonication and denature with LDS sample buffer (Invitrogen) at 70 °C, proteins were electrophoresed on NuPAGE bis-tris precast gels (Invitrogen) and transferred to nitrocellulose membranes (0.45μm pore, Invitrogen). Anti-ACE2 antibody was used as described, angiotensin-converting enzyme (ACE) (Abcam ab75762, Cambridge, MA, USA), phsp-56 (Cell Signaling 9271, Beverly, MA, USA), Akt (Cell Signaling 9272), psp-ERK 1/2 (Cell Signaling 4370), psp-phospho-p38 (Cell Signaling 9211), and GAPDH (Cell Signaling 2118) anti-bodies were used and the bands were visualized with ECL prime reagent (GE Healthcare, Chicago, IL, USA) using ChemiDoc Touch (Bio-Rad, Hercules, CA, USA). Image Lab software was used for quantification of bands.

2.8 Luciferase reporter assays

HEK293T cells were seeded in 24-well plates (1.0×105 cells/well). Cells were transfected with 100 ng of APJ plasmid, 100 ng of AT1R plasmid, 300 ng of ACE (-230/+1) promoter- or ACE2 (-252/+103) promoter-reporter plasmid, and 30 ng of renilla-luciferase plasmid using lipofectamine.

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Lipofectamine 3000 (Invitrogen) 12 h before treatment. Twenty-four hours after PBS or peptide (ELA or Apelin with or without Ang II) treatment, cells were washed with D-PBS (Invitrogen) once, lysed with lysis reagent included in the Dual-luciferase assay system kit (Promega, Madison, WI, USA) and then luciferase assay was performed according to the instructions of the manufacturer. Luciferase activity was measured using GloMAX-Multi Detection System (Promega).

2.9 Primary cardiomyocyte cultures
Primary cardiomyocytes were isolated from prenatal mouse hearts of wild type mice as described previously. Briefly, prenatal mice (E17.5) were removed from pregnant mice euthanized by cervical dislocation, prenatal mouse hearts were harvested and rapidly minced into 3 or 4 pieces in MSS buffer (30 mM HEPES, 120 mM NaCl, 4 mM glucose, 2 mM KCl, 1 mM KH2PO4, pH 7.6). After digestion with collagenase (Wako, Richmond, VA, USA) for 45 min at 35°C, cardiomyocytes were collected, pre-plated to exclude non-cardiomyocytes, and plated on gelatinized culture dishes or plates with DMEM/F-12 (Gibco, Waltham, MA, USA) supplemented with 10% foetal bovine serum (Equitech Bio, Kerrville, TX, USA). For qRT-PCR, cardiomyocytes and non-cardiomyocytes were harvested 24 h after plating with Trizol. For Western-blots, the cells were serum-starved for 24 h with DMEM/F-12 containing 0.01% bovine serum albumin (Sigma) and were subsequently treated with 1 µM of ELA or Apelin-13 for 15 min and harvested with lysis buffer. For immunocytochemistry, cardiomyocytes on Lab-Tek chamber slides (1.0 x 10⁵ cells/well) were fixed with 4% paraformaldehyde and stained with Alexa Fluor 546 Phalloidin (Thermo Fisher Scientific, Waltham, MA, USA). The cells were observed by using a BIOREVO microscope (BZ9000, Keyence) and analyzed using the BZII analyzer software (Keyence, Osaka, Japan).

2.10 Statistical analyses
Data are presented as mean values ± SEM. Statistical significance between two experimental groups was determined using Student’s two-tailed t-test. Comparisons of parameters among more than three groups were analyzed by one-way ANOVA, followed by Turkey’s post-hoc test. When a comparison is done for groups with two factor levels, two-way ANOVA with Sidak’s correction for post-hoc multiple comparisons were used. P < 0.05 was considered significant.

3. Results

3.1 Injected ELA rescues cardiac dysfunction and hypertrophy in mice under TAC pressure overload
To examine the functional role of ELA in the heart, we first conducted continuous infusions of ELA peptide into wild type mice (C57/BL6N) under pressure overload induced by TAC (transverse aortic constriction) surgery. After 2 weeks of TAC, % fractional shortening (%FS) was significantly decreased in the vehicle treatment group, while %FS was preserved in ELA-treated mice (Figure 1A–Q). Heart weight to body weight ratio (HW/BW) was also significantly decreased in the ELA treated group as compared with the vehicle-treated control cohort (Figure 1D,E). Similarly, ELA treatment significantly suppressed the increased expression of mRNA associated with cardiac hypertrophy, such as brain natriuretic peptide (BNP), atrial natriuretic factor (ANF), and β-myosin heavy chain (β-MHC) (Figure 1F–H), in the TAC mice.

Histological analysis further revealed that ELA treatment reduced the area of cardiac fibrosis in the interstitial space and perivascular region in the hearts of TAC mice (Figure 2A,B). Consistently, while the expression of the pro-fibrotic genes TGFβ2 (Tgfβ2), Latent TGFβ binding protein 2 (Ltbp2), Periostin (Postn), and Collagen 8a (Col8a) were increased in the hearts of vehicle-treated mice with TAC, ELA treatment markedly downregulated the expression of those pro-fibrotic genes (Figure 2C–F). These results indicate that exogenous ELA peptide treatment can protect mice from pressure overload-induced cardiac dysfunction, hypertrophy, and fibrosis.

3.2 ELA downregulates ACE expression in the hearts under TAC
Enhanced activity of renin–angiotensin system (RAS) is a key pathway involved in the progression of heart failure in mice with TAC. Since we had previously shown that Apelin upregulates ACE2 expression in the heart, we examined ACE2 expression in ELA-treated TAC mice. Levels of ACE2 showed a trend for increased, albeit not significantly increased, expression in ELA-treated TAC mice (Figure 2G). However, ELA treatment downregulated the increased expression of ACE in TAC mice (Figure 2H) resulting in a reduced ACE to ACE2 ratio in TAC mice (Figure 2I). Angiotensinogen mRNA levels appeared unaffected by ELA treatment (Figure 2J). On the other hand, ELA treatment did not significantly affect mRNA expression of APJ and ELA (Figure 2K,L). These data suggest that ELA treatment can reduce RAS activity under pathogenic TAC conditions.

3.3 ELA exerts its cardioprotective effects in an APJ-dependent manner
While ELA has been identified as a second endogenous ligand for the APJ receptor, it was recently suggested that an alternate receptor for ELA exists, and is implicated in the self-renewal of human embryonic stem cells. We thus examined whether the cardioprotective effects of ELA depend on the APJ receptor. We subjected wild type mice and APJ knockout (KO) mice (Figure 3A) (both on a C57/BL6J genetic background) to TAC and treated the mice with vehicle or ELA peptide by continuous infusion. After 2 weeks of TAC, loss of APJ significantly reduced cardiac hypertrophy (Figure 3B,C), whereas cardiac contractility was not significantly altered at this stage in the C57/BL6J background (Figure 3D,E), consistent with previous reports. On the other hand, ELA treatment did not affect heart weight and LV wall thickness in APJ knockout mice with TAC (Figure 3B,C), also shown by echocardiography that there were no alterations in the thickness of interventricular septum (IVS) or posterior wall dimension (PwD) (Figure 3F,G). In addition, the observed interstitial fibrosis in the hearts of APJ KO mice with TAC was not improved by ELA treatment (Figure 3H). Consistently, mRNA expressions of genes associated with hypertrophy or fibrosis were not further altered by ELA treatment in APJ KO mice under TAC (Figure 3I). These data indicate that ELA exerts its inhibitory effects on TAC-induced cardiac hypertrophy and fibrosis in an APJ receptor-dependent manner.

3.4 Differential effects of ELA and Apelin on expression of ACE and ACE2
To address how ELA regulates ACE, we determined the mRNA expression levels of ACE and ACE2 in the hearts of C57/BL6J wild-type mice at baseline treated with either Apelin-13 or ELA peptide. Consistent with our previous data, continuous infusion of Apelin did not affect ACE
mRNA expression (Figure 4A) but upregulated ACE2 mRNA expression in the hearts (Figure 4B). By contrast, ELA treatment did not alter ACE2 mRNA expression but downregulated ACE mRNA levels (Figure 4A,B). Consistently, the protein expression of ACE but not ACE2 was significantly downregulated in ELA-treated hearts (Figure 4C). Notably, when we compared the effects of ELA and Apelin on cardiac dysfunction under TAC, there was no apparent difference between ELA and Apelin on heart function, cardiac hypertrophy and heart failure gene expressions (see Supplementary material online, Table S2 and Figure S1A,B). Thus, ELA and Apelin differentially regulate ACE and ACE2 expressions but exert equivalent cardioprotective effects under TAC stress.

Since Apelin–APJ axis activates ACE2 promoter activity,10 we next compared the effect of ELA on ACE2 promoter activation with the Apelin effect by co-transfecting the ACE2 promoter (-252/+103)-luciferase reporter with the APJ receptor plasmids in HEK293T cells. While Apelin potently activated ACE2 promoter in APJ-transfected HEK293T cells as described,10 ELA showed modest effects on ACE2 promoter activation, in which the maximal effect was approximately half of Apelin (Figure 4D). To clarify whether ELA transcriptionally regulate ACE expression, we further conducted ACE-promoter reporter assays with the ACE promoter (-230/+1)-luciferase reporter and the APJ plasmids. ELA dose dependently decreased ACE promoter activity in APJ-transfected HEK293T cells, whereas ELA did not affect the activity in the absence of APJ (Mock-transfected cells) (Figure 4E). On the other hand, Apelin did not affect ACE promoter activity consistent with previous data.10 Since Ang II is a potent inducer of ACE expression,23–25 we further examined the effects of ELA on ACE expression in Ang II-treated hearts. Continuous infusion of Ang II increased both mRNA and protein levels of ACE expression in the hearts of wild-type mice (Figure 4F,G). By contrast, co-infusion of ELA with Ang II
significantly downregulated Ang II-induced ACE expression in the hearts (Figure 4F,G). Moreover, ELA but not Apelin decreased Ang II-triggered ACE promoter activation in HEK293T cells transfected with APJ and AT1R (Figure 4H).

To gain more mechanistic insight into the different effects of ELA and Apelin on ACE expression, we compared the effects of ELA and Apelin on activation of APJ receptor in in vitro heterologous expression system with cAMP production, receptor internalization and β-arrestin recruitment as readouts, but no apparent differences between ELA and Apelin effects were detected (see Supplementary material online, Figure S1C).

We next examined cellular signalling in the hearts treated with ELA or Apelin. Again there was no difference in phosphorylation status of Akt, ERK1/2 or p38 MAPK between ELA and Apelin-treated hearts or cardiomyocytes (see Supplementary material online, Figure S1D–G and not shown). Since Brg1-FoxM1 transcription factor complex was recently reported to upregulate ACE expression in the stressed hearts, we measured the mRNA expression levels by qRT-PCR. FoxM1 but not Brg1 expression was upregulated under TAC stress, whereas ELA but not Apelin significantly downregulated FoxM1 expression in the TAC hearts (Figure 4I and see Supplementary material online, Figure S1H). Similarly, Ang II-induced FoxM1 mRNA expression was significantly downregulated by ELA (Figure 4J), while ELA did not affect Brg1 expression (see Supplementary material online, Figure S1I). Thus, ELA transcriptionally downregulates ACE expression possibly via negative regulation of FoxM1 transcription factor in the heart.

3.5 The ELA-APJ axis antagonizes angiotensin II-induced hypertension and cardiac damage

To further address the effects of ELA on the RAS, we assessed the effects of ELA peptide on Ang II-induced cardiovascular dysfunctions. Continuous infusion of Ang II elevated, as expected, blood pressure in C57/BL6J wild-type mice, whereas co-treatment of ELA and Ang II
significantly downregulated Ang II-induced hypertension (Figure 5A–C), indicating that ELA antagonized the vasopressor effect of Ang II. In addition, ELA suppressed Ang II-induced cardiac hypertrophy and upregulation of heart to body weight ratios (Figure 5D,E). Consistently, Ang II-induced LV wall thickening was significantly downregulated by ELA treatment as shown by echocardiography (Figure 5F,G). %FS (D), LVEDD (E), IVS (F), and PWd (G) are shown. Heart weight to body weight ratio. (H, I) Histology of hearts stained with Masson-Trichrome (H) and qRT-PCR analysis of mRNA expression of BNP, β/α-MHC, Tgb2 (I) in APJ KO mice under TAC treated with either vehicle (n = 6) or ELA peptide (n = 5). All values are means±SEM. n.s. not significant, *P < 0.05, **P < 0.01, ***P < 0.001, unpaired t-test (A) and two-way ANOVA with Sidak’s post hoc test (D-G, I) were performed.

4. Discussion

In this study, we demonstrate that ELA peptide improves cardiac dysfunction, hypertrophy, and fibrosis induced by pressure overload in mice. These effects are contingent on the presence of ELA’s cognate receptor, since APJ-knockout mice could not respond to ELA treatment. Unexpectedly, the ELA-APJ axis was found to down-regulate transcription of ACE, whereas it had little effects on ACE2 expression in the heart. Downregulation of ACE expression was correlated with downregulation of FoxM1 transcription factor. Moreover, we report that ELA, similar to Apelin, antagonizes Ang II-induced hypertension and cardiac damage and remodelling. We thus conclude that ELA, signalling via APJ, exhibits potent cardioprotective effects in failing hearts.

ELA and Apelin, which are phylogenetically unrelated, share the same receptor APJ, and show similar effects in cell migration during zebrafish
Figure 4 ELA and Apelin differentially regulate ACE and ACE2 expressions in the hearts. (A,B) qRT-PCR analysis of mRNA expression of ACE (A) and ACE2 (B) in the hearts of wild type mice treated with ELA (n = 6) or Apelin (n = 5–7) for 2 weeks. (C) Western-Blot for ACE and ACE2 protein in the hearts of wild-type mice treated with ELA or Apelin (n = 5–6 per group). (D) ACE2 promoter assay using the luciferase reporter plasmids ACE2 (-252/+103)-luc in combination with APJ expression vectors in HEK293T cells. (E) Effects of ELA or Apelin on ACE promoter (-230/+1) activity in HEK293T cells transfected with control mock vectors (Mock) or APJ expression vectors (APJ). (F,G) Measurements of ACE expression, qRT-PCR analysis for ACE mRNA (F) and Western-Blot for ACE protein (G) in the hearts of wild-type mice treated with vehicle, Ang II (1 mg/kg/day), ELA (1 mg/kg/day), or a combination of Ang II and ELA (Ang II + ELA) for 2 weeks (n = 6–7 per group). (H) ACE reporter assay with ACE (-230/+1)-luc reporter plasmids in combination with APJ and AT1R in HEK293T cells. Cells were treated with vehicle, ELA (1 µM), Apelin (1 µM), Ang II (1 µM), or combinations of Ang II plus ELA or Ang II plus Apelin. For each transfection, a total of three wells for each condition were collected and measured individually. A total of three independent experiments were assayed and averaged (n = 3). (I,J) qRT-PCR analysis for FoxM1 mRNA expression. mRNA expression levels were measured for the hearts of sham-operated (n = 5) or TAC-operated mice treated with vehicle (n = 14), ELA (n = 5), or Apelin (n = 5) (I), and for the hearts of wild-type mice treated with vehicle (n = 7), ELA (n = 8), Ang II (n = 6), or Ang II plus ELA (n = 6) (J). All values are means ± SEM, n.s. not significant, *P < 0.1, **P < 0.05, ***P < 0.01, one-way ANOVA with Turkey’s post-hoc test (A, B, D–G), unpaired t-test (C) or two-way ANOVA with Sidak’s post hoc test (H–J) were performed.
Figure 5 ELA peptide attenuated Ang II effects in cardiovascular system. (A–C) Blood pressure measurements for wild-type mice treated with vehicle, Ang II, ELA or Ang II plus ELA. Systolic (A), diastolic (B), and mean (C) blood pressure were measured by tail-cuff system (n = 5–7 per group). (D–H) Combination treatment Ang II with ELA peptide reduced cardiac hypertrophy. Macroscopic heart images (D) and HW/BW (E) are shown. Bars indicate 2 mm. (F–H) Echocardiography parameters of IVS (F), PWd (G), and %FS (H) in the mouse hearts. (I) qRT-PCR analysis of BNP mRNA in the hearts (n = 5–7 per group). (J) Reduced fibrosis in the hearts of ELA peptide-treated mice. Histology of hearts stained with Masson-Trichrome (J). (K–N) qRT-PCR analysis of pro-fibrotic gene expressions in the hearts (n = 5–6 per group): Tgfb2 (K), Periostin (L), ColIIa (M), and Ltbp2 (N) normalized with TBP. All values are means ± SEM. n.s. not significant, *P < 0.1, **P < 0.05, ***P < 0.01, ****P < 0.001, two-way ANOVA with Sidak’s post-hoc test were performed.
development and in diuresis and water intake of rats in vivo. It has been recently shown that ELA can competitively replace Apelin bound to rat cardiomyocytes and that the perfusion of ELA increases contractility of ex vivo isolated rat hearts in an MAPK kinase 1/2 (MEK1/2)-dependent manner. The effect of ELA in the heart is thus most likely mediated through APJ. On the other hand, it has been recently suggested that ELA can function through an alternate receptor in human embryonic stem cells. Our genetic study utilizing APJ-knockout mice, however, indicate that, in the stressed hearts, APJ is the key receptor for ELA to mediate cardioprotective signalling.

Inotropic actions of Apelin–APJ have been reported to be mediated by protein kinase C (PKC), Na⁺/H⁺ exchanger, Na⁺/Ca²⁺ exchanger, and extracellular signal-regulated kinase 1/2 (ERK1/2). On the other hand, ELA has been recently shown to induce cardiac contractility independently of PKC, implicating that ELA and Apelin exert similar cardioprotective effects through different signalling pathways. It is known that pressure overload-induced cardiac hypertrophy leads to a mismatch between supply and requirement of blood in the heart, and results in systolic dysfunction. Apelin exerts vasodilatory effects via the PI3K/Akt/eNOS pathway in vascular endothelium, and increased blood flow in coronary artery and vaso vasorum by Apelin has been linked to the cardioprotective response. A recent study reported that ELA also activates the PI3K/Akt pathway in human embryonic stem cells, suggesting that ELA-induced activation of PI3K/Akt may also be operational in the cardiac endothelium. Consistently, ELA has been suggested to be vasodilatory in the vasculature of isolated rat hearts. Thus, the vasodilatory effect of ELA may also contribute to the beneficial effects in pressure overloaded and Ang II-challenged hearts.

The Apelin–APJ system plays a counter-regulatory role to the RAS. Several signalling mechanisms are known to be critical for this antagonism, including oligomeric interaction of AT1R and APJ to inhibit the activation of AT1R. We have previously shown that endogenous Apelin upregulates ACE2 levels transcriptionally and thereby decreases Ang II levels in the heart. However, our current results indicate that ELA peptide treatment has little effects on ACE2 expression in the heart, whereas ELA downregulates ACE2 transcription. Increased ratio of ACE to ACE2 leads to RAS acceleration and cardiac remodelling such as hypertrophy and fibrosis, and clinical relevance of balancing ACE/ACE2 in heart function has been reported. In the stressed hearts, Ang II upregulates ACE expression level via the p38 MAPK or HIF1α/STAT3 pathways, and this positive feed-forward loop results in cardiac remodelling. Brg1-FoxM1 transcription complex functions as both a transactivator and a repressor for transcription regulation, and it is recently reported to induce ACE expression by a transcription activator but suppress ACE2 expression by a repressor in the TAC-stressed hearts. We found that ELA, but not Apelin, decreases TAC stress-induced upregulation of FoxM1 expression. Such differential effect of ELA on signalling but similar cardioprotective effect has been also observed in ex vivo rat heart function measurements in that ELA induces cardiac contractility independently of PKC signalling, which is distinct from Apelin’s signalling. The reason why ELA has no significant effects on ACE2 expression in the hearts may be explained by minor effects of ELA on the repressor activity of FoxM1 for transcription regulation. Indeed, pharmacological inhibition of transactivator activity of FoxM1 was not enough to induce ACE2 expression in the hearts. Together with ELA’s weak effect on activation of ACE2 promoter in vitro, the findings may implicate biased inhibitory effects of ELA on transactivator activity of FoxM1. Thus, although further studies are needed, it can be speculated that Brg1-FoxM1 transcription complex is negatively regulated by ELA through PKC-independent mechanisms, leading to downregulation of ACE expression.

Despite the difference in amino acid sequence, both ELA and Apelin peptides are rich in basic residues and have similar high isoelectric points. Earlier studies have shown that the C-terminal Phenylalanine (Phe) residue of Apelin-13 peptide is crucial for activation of APJ as shown by deletion- or alanine-mutant peptides, and that chemical modifications of C-terminal Phe of Apelin can modulate the potency of Apelin to activate APJ. On the other hand, although ACE2 efficiently cleaves the C-terminus Pro–Phe bond of Apelin, the C-terminal Pro–Phe–Pro of ELA is resistant to cleavage by ACE2 (unpublished results). It has been recently reported that prolonged half-life of Apelin peptide by nano-liposomal encapsulation or Apelin analogues resistant to ACE2-mediated inactivation enhanced cardioprotective effects of Apelin. Thus, ELA and Apelin are differentially processed and hence their potency and half-lives might be different which could affect them in vivo cardioprotective effects.

In summary, our results reveal that the ELA–APJ axis is protective against pressure overload-induced heart failure and antagonizes the effects of Ang II in the heart. ELA has similar beneficial roles to Apelin in heart failure but has distinct structures and regulations. Utilizing ELA peptide as a therapeutic compound could be another option to activate APJ in cardiovascular diseases. Further analyses for ELA and Apelin signalling should provide more mechanistic insights into regulation of APJ activation in cardiovascular diseases.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

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Conflict of interest: none declared.

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