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Review

A promising therapeutic peptide and preventive/diagnostic biomarker for age-related diseases: The Elabela/Apela/Toddler peptide

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ABSTRACT

Elabela (ELA), Apela or Toddler peptide is a hormone peptide belonging to the adipokine group and a component of apelinergic system, discovered in 2013–2014. Given its high homology with apelin, the first ligand of APJ receptor, ELA likely mediates similar effects. Increasing evidence shows that ELA has a critical function not only in embryonic development, but also in adulthood, contributing to physiological and pathological conditions, such as the onset of age-related diseases (ARD). However, still little is known about the mechanisms and molecular pathways of ELA, as well as its precise functions in ARD pathophysiology. Here, we report the mechanisms by which ELA/APJ signaling acts in a very complex network of pathways for the maintenance of physiological functions of human tissue and organs, as well as in the onset of some ARD, where it appears to play a central role. Therefore, we describe the possibility to use the ELA/APJ pathway, as novel biomarker (predictive and diagnostic) and target for personalized treatments of ARD. Its potentiality as an optimal peptide candidate for therapeutic ARD treatments is largely described, also detailing potential current limitations.

1. Introduction

Despite recent advances (Balistreri, 2018; Campisi et al., 2019; Vaiserman et al., 2019), aging is still, in line with current data reported by the National Institute on Aging (NIA) (www.nia.nih.gov), the leading risk factor for a wide range of inflammatory diseases and chronic conditions, including Alzheimer's disease and related dementias (AD/ADRD), most types of cancer, many types of cardiovascular disease, osteoporosis and hip fractures, kidney failure, and diabetes (Chang et al., 2019). These diseases, also called age-related diseases (ARD), are rarely unavoidable and their incidence and prevalence are closely related to the increasing number of elderly individuals (Chang et al., 2019). Consequently, the increase in elderly in any population reflects (and will reflect) the number of individuals with ARD. This represents a real challenge because ARD is the cause of two-thirds of human deaths and 90% of all deaths in Western populations, as well as the main reason for the continued increase in disability in the elderly (Chang et al., 2019; Wu et al., 2023). Sociodemographic index (SDI) analysis shows that ARD levels range from 137.8 disability-adjusted life years (DALYs) per 1000 adults in high-SDI countries to 265.9 DALYs per 1000 adults in low-SDI countries (Chang et al., 2019; Wu et al., 2023). Another negative aspect of ARD is the enormous economic and psychological burden it places on patients, their caregivers, and societies worldwide (Wu et al., 2023). As a result, several strategies and interventions are being developed to reduce or prevent the burden of ARDs and the disorders and disabilities related to their complications (Balistreri, 2018; Campisi et al., 2019; Vaiserman et al., 2019). Among them, the potential use of peptides in the prevention, treatment, and diagnosis of ARD is currently being explored, as first suggested by Khavinson in 2013, during an interview conducted by Suresh I. S. Rattan: "*I think that the small peptides are the best for healthy ageing.*." (Khavinson, 2013). This is justified by the characteristics of peptides: a) good solubility; b) easy synthesis and low-cost (compared to protein production), and c) good pharmacokinetic properties (Balistreri, 2018; Balistreri, 2021; Olivieri et al., 2018). A

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well-known example is the peptide Irisin, which is becoming of great interest to aging researchers (Korta et al., 2019; Byun et al., 2020; Z. Zhang et al., 2022; H. Zhang et al., 2022). In addition, synthetic peptides and some derived structures are being investigated for use as theragnostic tools in neurodegenerative diseases, such as AD/ADRD (Ryan et al., 2018). They can bind typical AD/ADRD biomarkers and prevent their pathological self-assembly (Ryan et al., 2018). Peptides are also being studied for cancer treatment and diagnosis (Zhang et al., 2023). In addition, the ability of vaso-protective polypeptides (i.e., liraglutide, atrial natriuretic peptide, mimetics of relaxin, Ucn1, and adropin) to modulate the synthesis of molecules related to inflammaging and senescence-associated secretory phenotype (SASP)-forming cells of the cardiovascular system has been recently described (Balistreri, 2018; Balistreri, 2021) suggesting the possibility of developing peptide-based drugs for the treatment of age-associated cardiovascular diseases (Balistreri, 2018; Balistreri, 2021; Olivieri et al., 2018). Therefore, the search for natural or newly synthesized peptides is increasing. In this context, our interest is focused on a newly discovered (2013–2014) hormonal peptide, Elabela (ELA) (Chng et al., 2013; Pauli et al., 2014). Here, we summarize the current evidence on the physiological functions of the ELA peptide in promoting health aging, as well as being implicated in ARD, making it a potential target of their treatment and, simultaneously, a preventive/diagnostic biomarker.

2. A new hope for anti-ageing target: the ELA peptide

Discovered in 2013–2014 by two different groups (Chng et al., 2013; Pauli et al., 2014), and named *Elabela (ELA), Apela or Toddler*, ELA is a peptide hormone belonging to the group of adipokines (Balistreri et al., 2010), and a component of apelinergic system (Shin et al., 2017; Respekta et al., 2022; see Box 1), represented by ELA, apelin and a G-protein-coupled receptor, called APLNR, but also known as APJ.

In addition, ELA is encoded by a gene, initially observed to be involved in the development of the mouse endoderm, and consequently named *Ende*, and later shown in humans to encode for the other endogenous ligand of APJ, such that was, therefore, redefined *APELA* (**AP**elin receptor **E**arly endogenous **L**ig**A**nd) gene. In humans, *APELA* is composed by 3 exons and located on chromosome 4; the ELA peptide has been shown to be involved in embryonic tissue developmental processes and is expressed in adults in various tissues and organs (Jin et al., 2021; Respekta et al., 2022). Accordingly, many functions are attributed to ELA peptide, with crucial involvement in various physiological processes, including blood pressure control, heart morphogenesis, apoptosis, angiogenesis, cell proliferation, migration, etc. (Jin et al., 2021; Respekta et al., 2022). However, a key role of ELA is also emerging in pathological conditions, such as cardiovascular dysfunctions, heart failure, hypertension, kidney diseases, cancer, and central nervous system disorders (Sharma et al., 2022). This makes ELA a potential target for therapy and a diagnostic/preventive biomarker (Sharma et al., 2022). For a clear understanding of the multiple roles of ELA in human health and disease, such as ARD, the molecular characteristics of the ELA pathway, as well as of its isoforms, and the diversity of cellular responses mediated by the ELA pathway are outlined below.

3. The molecular characteristics of the ELA signaling pathway: the role of different isoforms with distinctive membrane-interactive properties

Current evidence suggests that ELA-induced signaling plays a critical role in several biological processes. This has led to an understanding of how ELA signaling can mediate multiple cellular responses. Thus, it has been observed that ELA can be processed into several active peptides, each of which binds to APLNR (see Box 2; Jin et al., 2021; Respekta et al., 2022), stimulating many pathways (see **Graphical abstract**), mainly the PI3K/AKT pathway. Precisely, the *APELA* gene encodes a 54 amino acid polypeptide with a signal peptide in its N-terminal region. It is cleaved in a pro-protein of 32 amino acid residues called ELA-32, which can be further cleaved in two active peptides called ELA-21 and − 11. ELA-32 and − 11 have different membrane interactive properties, which could represent the likely mechanisms used to induce distinct signaling effects (Huang et al., 2018). In addition, ELA has been shown to be the earliest ligand of APJ in humans (Chng et al., 2013), being expressed in embryonic stem cells (ESCs) and induced pluripotent stem cells, but also in the kidney, heart, and blood vessels during development. The ELA-APJ complex actives distinct downstream signaling cascades. For instance, ELA-32 through APLNR has been shown to mediate the early cardiovascular development at the embryonic stage (Chng et al., 2013; Wang et al., 2015). Zhang and coworkers (Zhang et al., 2019) have also demonstrated that ELA-32 regulates growth and decreases apoptosis by interacting with the PI3K/AKT/mTOR pathway, resulting in the prevention of diabetes-induced kidney disease. He and coworkers have proposed that internalization of APLNR through clathrin-mediated endocytosis after binding to its natural ligands, apelin and ELA-32, may result in cardiac hypertrophy (He et al., 2016). Further studies have also documented that ELA-32 and ELA-11 inhibit renal ischemia-reperfusion (I/R) injury, while ELA-21 significantly improves angiogenesis, promotes cardiomyocyte proliferation, and limits cardiac apoptosis and fibrosis near the infarct area (Xu, 2021).

In addition, Wang and coworkers (Wang et al., 2022) have demonstrated that ELA-11 protects the heart from oxidative stress-induced apoptosis through the ERK/MAPK and PI3K/AKT signaling pathway. In addition, Ye and coworkers have recently demonstrated that ELA-21 alleviates vascular remodeling through anti-inflammatory, anti-oxidative and anti-proliferative effects (Ye et al., 2022).

The PI3K-AKT pathway represents the signaling pathway that is principally activated by ELA-APLNR binding (Dagamajalu et al., 2022a

Box 1
The apelinergic system.

It has pleiotropic regulatory functions of regulation on many human tissues and organs (i.e., heart, blood vessels, adipose tissue, central nervous system, lungs, kidneys, and liver), where its components are expressed. Precisely, the apelinergic system regulates many physiological functions of these organs and tissues, but also shows a clear relationship to a variety of pathological conditions. For instance, apelinergic signaling is crucial for the functioning of the cardiovascular system, energy metabolism, fluid homeostasis, angiogenesis, human immunodeficiency virus-1 (HIV-1) infection, and the neuroendocrine stress response.This wide range of functions is related to the ability of apelin and ELA to be processed into multiple N-terminally truncated isoforms, in each case retaining the C-terminal residues of the respective preprotein to interact with their cell surface receptor (Chen et al., 2020). All the isoforms of two peptides probably interact with and stimulate APJ by a mechanism that seems similar, considering their common amino acid composition and relatively similar conformations. However, the influences and capacities differ between the isoforms. Many studies have tried to clarify these disagreements through the identification of important residues on the two peptides (apelin and ELA) and their receptor, sequence motifs that in some cases have clear structural properties in regulating receptor binding and downstream signaling, and pharmacological changes by comparing the diverse isoforms (Shin et al., 2017; Respekta et al., 2022).

Box 2
The apelin receptor (APLNR, also known as APJ) and a brief overview on apelin.

The apelin receptor (APLNR, also known as APJ) is a class A (rhodopsin-like) G protein-coupled receptor (GPCR), first discovered in 1993 (O'Dowd et al., 1993; Dagamajalu et al., 2022). The *APLNR* gene is located on chromosome 11q12 and encodes a protein of 380 amino acid residues (O'Dowd et al., 1993), which shows relatively high homology with angiotensin II receptor type-I (AT1), specifically in the transmembrane domain with 54% homology and ~28–50% identity for each of the seven transmembrane segments (Shin et al., 2017). Despite this, APLNR is not activated by angiotensin II and is expressed in various tissues and organs, and involved in their development, including heart, lung, kidney, brain, where it mediates many biological functions (Dagamajalu et al., 2022a and 2022b). Currently, its identified endogenous peptide ligands are two apelin (Dagamajalu et al., 2022a and 2022b) and ELA. Apelin represents the most widely studied member of the apelinergic system, plays a key role particularly in the cardiovascular system and exerts a pleiotropic effect in several tissues. Accordingly, apelin is an endogenous peptide produced from a 77-amino acid precursor, pre-pro-apelin, which can be cleaved by endopeptidases to form C-terminal biologically active peptides, including apelin-13, – 16, – 17, – 19, and – 36. Apelin-13 is the most potent activator of cell lines expressing the APJ receptor and is susceptible to additional posttranslational modifications, which result in the production of its more stable, pyroglutaminated form, called [Pyr1]-apelin-13, which is the most abundant form in cardiac tissue. Shin et al. have shown that the bioactivity of apelin-55 isoform greatly increases the number of potential therapeutic targets for the apelinergic system (Shin et al., 2017). Apelin-13, – 16, – 17, – 19, and – 36 are widely expressed in various types of human tissue, predominantly in adipose tissue, as well as in the central nervous system, heart, lungs, kidneys, and liver. However, apelin-13 and apelin-17 have been proven to show the most significant activity on cardiovascular problems and have been researched most (widely quoted in Zhong et al., 2020 and Rozwadowski et al., 2022).

and 2022b), where PI (3, 4, 5) P_3 is an intracellular second messenger in the cell required to transfer protein kinase B (AKT) to the membrane for activation (Dagamajalu et al., 2022a and 2022b). Phosphorylation of AKT is mediated by insulin and various growth factors to induce cell growth and promote cell survival through numerous channels (Dagamajalu et al., 2022a and 2022b). ELA-11 induced ERK/MAPK is another activated pathway, specifically the classic anti-apoptotic signaling pathway. The downstream phosphorylation of ERK results in inhibition of the apoptotic process. However, other pathways participate in the ELA-APLNR induced signaling or ELA signaling independent of APLNR binding. Prasad's group has recently developed a map on the network of ELA signaling pathway, which is freely available through the WikiPathways Database (https://classic.wikipathways.org/index.php/Pathway:WP5100; Dagamajalu et al., 2022a and 2022b). This map offers a clear description on the multiple roles of ELA signaling pathway, and exactly the signaling evoked by the three ELA-32, – 21 and – 11 isoforms of the ELA peptide and by a mechanism dependent or not on APLNR receptor binding. In addition, all the involved pathways and transcriptional factors are descripted in detail, offering the possibility of identifying the distinctive functions of the ELA signaling pathway, ranging from a protective role in fetal heart and blood vessels development, and vascular tension regulation in adults to improve cell viability, migration, cardio-protection, inflammation, oxidative stress, apoptosis, fibrosis, mitochondrial dysfunction, and prevention of pre-eclampsia during pregnancy (Dagamajalu et al., 2022a and 2022b). Such information could make it possible to provide a platform that could accelerate further research on this pathway and, consequently, enable a deeper understanding of the biological role of ELA under normal and pathological conditions.

4. ELA as a regulatory/protective factor

Growing evidence attributes multiple regulatory functions to the ELA signaling pathway (as abovementioned and shown in (Fig. 1) with protective biological effects, limiting, or blocking dangerous conditions linked to the onset of several diseases, such as ARD, which are the most

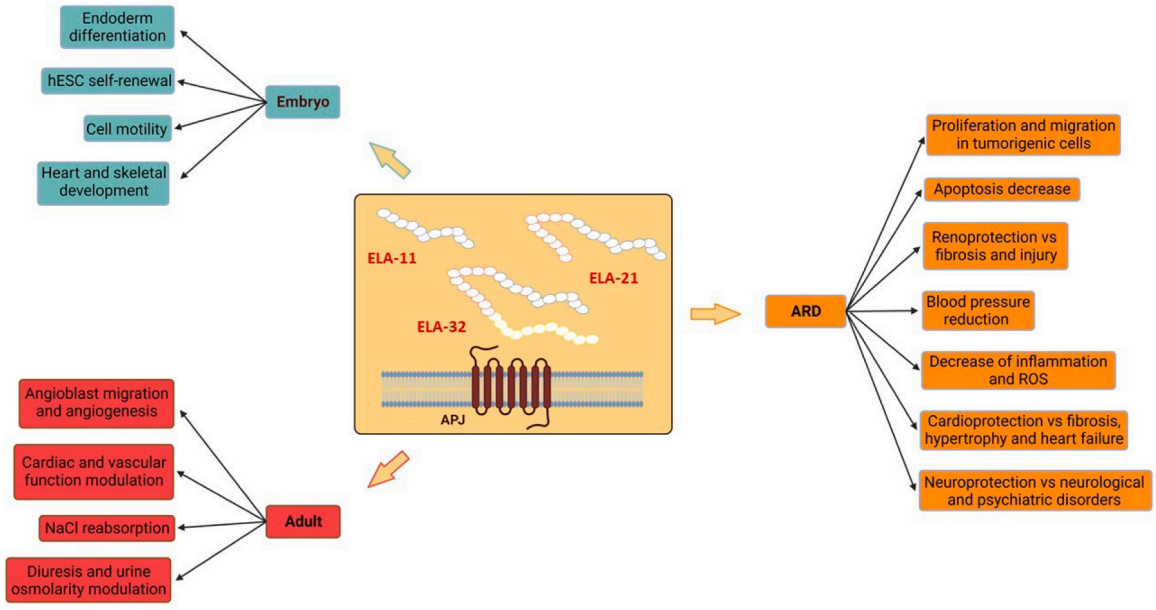

Fig. 1. Physiological roles of ELA peptides during embryogenesis and adult life, as well as protective actions in age-related diseases. Abbreviations: ROS, reactive oxygen species.

common diseases in the elderly population. The ELA signaling appears to exert protective roles, such as heart-brain-kidney protective roles, by regulating innate immune responses (i.e., inflammatory responses), apoptosis, oxidative stress, and fibrosis. Consequently, ELA has been described as an essential regulator of heart development (Pauli et al., 2014; Kuba et al., 2019). In embryos, ELA mediates, as an early developmental signal, the migration of mesodermal cells. Furthermore, $ELA^{-/-}$ knock-out mice have been found to exhibit cardiac agenesis or develop only a rudimentary heart (Yang et al., 2017; Liu et al., 2019). ELA also appears to regulate endothelial cell function. Studies in human pulmonary microvascular endothelial cells (HPMECs) and human umbilical vein endothelial cell lines (HUVECs), namely HUVECs and EA. hy926 respectively, have shown that ELA can improve endothelial cell function via the ELA-APJ axis, by activating the PI3K/Akt signaling pathway, or attenuate the lipopolysaccharide-induced endothelial damage (Wang et al., 2015; Wang et al., 2022; Zhang et al., 2018).

A similar action of ELA has been observed in kidney development and homeostasis in adult mammals, including humans. ELA shows a reno-protection, contributing to physiological diuresis, reduction of TGF-β, resulting in inhibition of renal fibrosis through reduction of inflammation and ROS release and, consequently, inhibiting kidney remodeling and injury (Lu et al., 2017; Xu et al., 2018).

Interestingly, the potential role of ELA-APJ signaling in neurogenesis has recently been investigated, as well as its possible contribution to affecting the brain and the pathophysiology of cerebral ARD. Precise, molecular, and histo-anatomical investigations have identified the expression of APJ signaling in the nervous system, as well as its multiple roles in neurogenesis, pituitary hormone release, body fluid homeostasis, blood pressure regulation, and feeding behaviour. However, APJ signaling is expressed in restricted areas of the cerebral cortex such as the frontal, temporal, occipital, piriform, and entorhinal cortices. Furthermore, current evidence suggests the involvement of APJ signaling in glioblastoma multiforme, as well as protective biological effects on certain neurological and psychiatric disorders (i.e., ischemic stroke, epilepsy, Parkinson's, and Alzheimer's disease; see description below) (Ivanov et al., 2022).

5. "The paradigm on versatility of cell responses mediated" by ELA/APJ pathway: similarities and differences with apelin/APJ pathway

As above described, the ELA/APJ signaling receptor pathway plays an important role in both the physiological development of embryonic and homeostasis of adult tissues. This denotes its ability to mediate a multitude of cellular responses and pleiotropic effects, which certainly reflect not only the complexity and key features of the APJ pathway, but also the ability of ELA, as well as apelin (the most extensively studied member of the apelinergic system), to be processed into multiple N-terminal truncated isoforms, in each case retaining the C-terminal residues of the respective preprotein to interact with their receptor on the cell surface (Read et al., 2019; Chen et al., 2020). All the isoforms of two peptides, ELA and apelin, probably interact and stimulate APJ through a mechanism that appears similar, considering their common amino acid composition and relatively similar conformations. However, influences and capacities differ between the isoforms (see Table 1).

Many studies have tried to clarify these differences through the identification of important residues on the two peptides (apelin and ELA) and their receptor, i.e., on amino-acid sequences, called sequence motifs. In some cases, these latter have shown to have clear structural properties in regulating receptor's binding and downstream signaling, as well as pharmacological changes, comparing the different isoforms (Shin et al., 2017; Couvineau et al., 2020; Respekta et al., 2022). Worthly of interest is the recent study conducted by Couvenau and colleagues (Couvineau et al., 2020). They have explored possible structural and functional similarities between apelin and ELA, by performing in *vitro* pharmacological characterization and biased signaling

Table 1
Variables involved in the multiple cellular responses of APJ/ELA and APJ/apelin pathway.

Variables involved in the multiple cellular responses of APJ/ELA and APJ/apelin pathway	
Aminoacidic residues modification or mutation on APJR	Effects
C-terminus $pSer^{335}$	Reduced interaction of APJR with β-arrestin 1/2 and AP2
C-terminus $pSer^{339}$	Increased interaction of APJR with GRK2 with β-arrestin 1/2
I109A mutation on TM3	Loss of the ability of APJ to recruit β-arrestin but still able to activate G-protein mediated. c-AMP signaling
C-terminus Asp^{282} (human) and Asp^{284} (rat)	Involved in APJ/apelin binding and activity
Variation in expression levels of APJR dependent on cell context	Different downstream transduction and diverse signaling profiles of activation or desensitization
Modification in ligands or pathway components	**Effects**
Tyr^{47} β-arrestin 1 and Tyr^{48} β-arrestin 2	Receptor endocytosis impairment
apelin-13 and apelin-36	Induce activation of Gαq signaling
Variation in expression levels of ligands dependent on cell context	Different downstream transduction and desensitization

analysis in CHO cells, measuring binding affinities, inhibition of cyclic adenosine monophosphate (cAMP) production, and activation of β-arrestin − 2 intakes. Alanine scanning and structure function studies based on site-directed mutagenesis of the rat and human APJ receptor have been also performed by these researchers for evaluating the binding modes of apelin and ELA. Alanine scanning of K22P has established that none of the APJ receptor cysteine residues participate in peptide binding or activity, and that its C-terminus includes the major pharmacophore for receptor binding and activation. Specifically, they have showed that Asp^{282} and Asp^{284} of the rat and human APJ receptor, respectively, are not involved in ELA activity, while they constitute the key residues for apelin binding and activity. Furthermore, the analyses executed have also evidenced that the structural features of ELA and apelin differe, resulting in different types of binding to the APJ receptor. These differences should be taken into consideration for the development of metabolically stable analogues of ELA and apelin to use in the ARD treatment. Accordingly, Yue and colleagues (2022), using the cryogenic electron microscopy (cryo-EM) technique, have specifically highlighted the complex structure of APJ binding to the endogenous ligand ELA-32, providing further evidence, that they may contribute to the development of more selective drugs for APJ in the treatment of CVD, such as heart disease, as well as of other ARD.

Furthermore, the isoforms of the two peptides, having different amino acid lengths, can activate different downstream pathways after binding to the APJ receptor, leading to different signaling biases. This feature also needs to be further explored to understand any differences aimed at improving the production of an isoform in each tissue or at a given time. Furthermore, the observed endogenous bias could facilitate the development of synthetically biased molecules. Current evidence, however, attributes this role to phosphorylation of the C-terminal region of APJ-mediated G protein-dependent and β-dependent signaling. This mechanism has been investigated by Chen and collaborators, using some techniques (Chen et al., 2020), such as mass spectrometry (MS), mutation analysis and bioluminescence resonance energy transfer (BRET), and highlighted APJ phosphorylation at five serine residues in the C-terminal region (i.e., Ser^{335}, Ser^{339}, Ser^{345}, Ser^{348}, and Ser^{369}). Additional phosphorylation, including three previously identified residues (Ser^{412}, Ser^{361}, and Thr^{383}) and two new sites, Tyr^{47} in β-arrestin1 and Tyr^{48} in β-arrestin2, has been also identified at two sites in β-arrestin1

and three in β-arrestin2. Furthermore, it reports that APJ mutations do not alter β-arrestin phosphorylation, but rather such alteration may affect the β-arrestin signaling pathway, acting specifically at Ser^{335} and Ser^{339} residues. Accordingly, mutations in Ser^{335} have been demonstrated to reduce the ability of the receptor to interact with β-arrestin 1/2 and AP2, indicating that APJ influences the β-arrestin signaling pathway by stimulating ELA. While mutations in Ser^{339} have showed the ability of the receptor to interact with GRK2 and β-arrestin1/2 after stimulation with apelin-36 and disrupted receptor internalization and β-arrestin-dependent ERK1/2 activation. Thus, the five peptides are involved in distinct APJ C-terminal phosphorylation sites, differentially setting APJ signal transduction and resulting in different biological effects. These findings may accelerate drug screening for the treatment of cardiovascular and metabolic diseases.

In addition to the aspects described above, an important point in the different cellular response lies in the functional selectivity of the APJ and its responsiveness to mechanical stretching observed in cardiovascular cells due to the ability of ELA, (and in particular of apelin, given the current robust evidence on this peptide respect to that on ELA), of signaling via both G protein-dependent mechanisms (isoforms of Gαi protein (Chapman et al., 2014) or of Gαo and Gαq (Bai et al., 2014); see Box 3) and G protein-independent mechanisms (with the particular involvement in this case of the multifunctional proteins, β-arrestins; see Box 4). APJR, upon binding to all the apelin isoforms, commonly activates Gαi- and Gαq-mediated signaling in various cell types, inducing ERK, PKB/Akt, and p70S activation in a PTX-sensitive Gαi-mediated pathway (Chapman et al., 2014). In both neurons and HEK293 cells, apelin-13 and apelin-36 induce the activation of Gαq signaling (Murali and Aradhyam, 2023). Specificity for Gαi protein subtypes appears to be cell line dependent (Murali and Aradhyam, 2023). For example, in CHO and HEK293 cells, APJR prefers association with Gαi1 and Gαi2, whereas in HUVEC cells, APJR preferentially pairs with Gαi3 (Murali and Aradhyam, 2023).

β-arrestin-mediated signaling participates in receptor internalization, but it has been also demonstrated that it exhibits other functions (Murali and Aradhyam, 2023). Consequently, this requires further studies to understand its role in downstream signaling and physiology.

5.1. Biased Signaling

To conclude our considerations on the versatile activity of the ELA and apelin/APJ pathways, it is imperative to better describe another reasonable aspect, just above mentioned, namely the ability of APJR to activate many downstream pathways, which result in the activation of a particular pathway selectively and preferentially at a precise moment and in particular cellular conditions. This phenomenon is known as *functional selectivity or biased signaling* (above mentioned), and such bias exists, for example and as above mentioned, between the G-protein and β-arrestin-mediated pathways (extensively cited in Murali and Aradhyam, 2023). In this case, each pathway has a different function for different physiological conditions, and the biased ligands may be more effective in their therapeutic activity with reduced side effects, as observed in the case of many GPCR A classes (Murali and Aradhyam, 2023). Many studies (extensively cited in Murali and Aradhyam, 2023) on biased signaling have suggested that mutations in receptor residues or differences in their ligands cause the receptor to preferentially activate G-protein or β-arrestin-mediated pathways. For example, in APJR, the *I109A* mutation in TM3 eliminates the receptor's ability to recruit β-arrestin, while remaining capable of activating G protein-mediated cAMP signaling (Murali and Aradhyam, 2023). The synthetic analogue MM07 has shown functional selectivity towards the Gα pathway, compared to β-arrestin-mediated internalization (Murali and Aradhyam, 2023). In addition, analogues of apelin, and particularly of apelin-36, have been developed with the *L28A* and *L28C* substitution bound to 30 kDa polyethylene glycol (PEG). They have evidenced the ability to compete with the apelin-13 ligand in rat hearts and to be influenced by G protein-mediated signaling (cited in Murali and Aradhyam, 2023). Consequently, functional selectivity constitutes an important aspect to consider in the design of targeting APJR drugs.

The next section will highlight other relevant aspects that have emerged from recent studies, in an attempt to clarify how apelin and ELA interact constructively or not, before discussing both the role of ELA in ARD and ageing, and the potential treatments. Advances in the understanding of these cascades are highly relevant, not only for basic science, but also for clinicians, since they offer a concrete potential in developing therapeutic strategies to counteract alterations in this signaling, and consequently to retard or delay ARD.

5.2. ELA/apelin APJ signaling as a rheostat?

The evidence reported in current literature is not adequate to clarify this aspect extensively. However, the more acceptable opinion is that such pathway may function as a rheostat, which can reset to produce different intensities in the signaling output. In particular, the intensity of signaling output mediated by such pathway can derive by the diverse density of expression of two endogenous ligands, or by varying levels of APJ pathway. Therefore, such pathway can influence the fate and decision of a variety of cell types, and it can contribute to alter tissue patterning and morphogenesis, such as the physiological development of heart, by affecting cell differentiation, proliferation, survival, and apoptosis (Respekta et al., 2023).

To give some examples and better understand, deficiency of apelin expression in mice has been shown to cause vascular stiffening through extracellular matrix remodeling of the aortic wall (Romier et al., 2021). ELA deficiency in $ELA^{+/-}$ mice has been found to significantly enhance diabetic glomerular damage, as evidenced by exacerbation of glomerular morphological damage, increased serum creatine and blood urea nitrogen, and elevated 24-h urinary albumin excretion. In addition, in *vivo* overexpression of ELA has been demonstrated to result in the prevention of diabetic glomerular injury, reduced von Willebrand factor expression, restored endothelial marker CD31 expression, and attenuated the production of adhesive molecules, such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (Chen et al., 2023). Another study conducted by Nielsen group (Adhicary et al., 2023) reports that endothelial recombination signal-binding protein for immunoglobulin *J*κ regions (Rbpj), a transcriptional regulator of Notch

Box 3
Ligand binding and activation of G proteins.

To be precise, ligand binding to the classical G protein-mediated pathway results in the activation of several downstream molecules. Precisely, Gαq coupling to the activated receptor leads to the activation of phosphoinositide-specific phospholipase Cβ (PLCβ), which increases intracellular calcium that drives the phosphorylation of ERK, AKT, and NO production. Gαi coupling to the receptor also mediates ERK, AKT, and NOS pathways and inhibits cAMP production. After activation, phosphorylation of the C-terminal chain of APJR recruits β-arrestins that drive the receptor's endocytosis-mediated internalization. The internalized receptor is either recycled back to the membrane or directed to proteolysis (Murali and Aradhyam, 2023).

Box 4
Ligand independent signaling.

APJ receptor is also activated by mechanical stretch, by leading to the activation of β-arrestin mediated signaling, as shown in endothelial cells, by consequently regulating polarization. In endothelial cells, shear stress has been demonstrated to increase apelin/APJR expression and cause the release of nitric oxide, which results further amplified when exposed to apelin-12 peptide. Further studies have shown that APJR regulates the cytoskeletal organization in HUVEC, and knockdown of APJR in these cells determines disturbance in the cytoskeleton arrangement in response shear-flow. A study on mechanosensitive properties of histamine H1 receptors (H1R) has revealed that helix 8 results to be involved in the signal transduction of mechanical signals in endothelial cells. H1R receptors lacking helix 8 has been reported to be unable to respond to shear flow, indicating its essential role in this process. The molecular mechanism of mechanical shear induced APJR signaling remains elusive and is a compelling area for future research.

signaling (Balistreri et al., 2016), promotes rearrangement of brain endothelial cells (EC) during cerebrovascular remodeling, through apelin/APJ-mediated small GTPase activity, and prevents brain arteriovenous malformations (bAVM). By inhibiting apelin/APJ signaling *in vivo*, Nielsen and coworkers have evidenced a pharmacological prevention of Rbpj-mediated bAVM. In addition, it has now been described that in heterozygous ELA knockout mice ($ELA^{+/-}$), ELA deficiency has remarkably accelerated the onset of deoxycorticosterone acetate (DOCA)/salt-induced hypertension. Thus, ELA prevents DOCA/salt-induced hypertension by inhibiting NADPH oxidase/ROS/NLRP3 pathway in the kidney, which is APJ independent. Pharmacological targeting of ELA may serve as a novel therapeutic strategy for the treatment of hypertensive kidney disease (Chen et al., 2020).

5.3. Considerations: Is the regulation of ELA/ apelin signaling rheostat "cell-context-dependent"? and is it mediated by different mechanisms?

The limited number of researchers involved in the ELA/apelin studies often evade the complicated discussions of versatility paradigm of mediated cell responses by using a common expression or saying: "*but likely, this aspect of ELA or apelin signaling is cell-context-dependent.*" The few examples above described elicit the same observation. However, it is important to point out that this is not always just an explanation of convenience, and that ELA/apelin APJ signaling induces different responses, depending on the cellular context, as described in the examples above reported. It is, therefore, important to understand how such signaling is tuned-up, both in the normal development and diseased conditions, and whereas ELA may be redundant in a tissue when compared to expressed apelin levels and *vice versa,* or if their concomitant expression is imperative. Certainly, the identification of mechanisms involved in the regulation of ELA/apelin APJ signaling is important. Thus, further studies are imperative. Currently, it is possible to state that ELA/apelin APJ signaling is complex and appears to be context dependent, since its contribution is elicited by multiple ligand isoforms, and mediated by several downstream molecules. Although the isoforms differ in length, they demonstrate similar or different modes of binding and activation of the receptor. The presence of two agonists bound APJR structures will serve as a guide for detailed studies into the activation mechanisms of the receptor, as well as the development of agonists/antagonists with increased precision. The signaling profile of ELA/apelin APJR is also diverse, and non-canonical signaling pathways, such as response to mechanical stretch and β-arrestin-mediated signaling following internalization are exciting avenues to be explored. ELA-mediated signaling, although recently discovered, has shed light on the multifaceted nature of APJR. Accordingly, Prasad's group, as above mentioned, has provided a good instance on the number of the pathways activated by ELA isoforms, and thereby, on the complexity and potential functions of the network activated by ELA/APJ signaling pathway or ELA/independent APJ pathway (Dagamajalu et al., 2022a and 2022b). All the involved pathways and transcriptional factors explicit the distinctive functions of the ELA signaling pathway, ranging from a protective role in fetal heart and blood vessels development, and vascular tension regulation in adults to improve cell viability, migration, cardio-protection, inflammation, oxidative stress, apoptosis, fibrosis, mitochondrial dysfunction, and prevention of preeclampsia during pregnancy (Dagamajalu et al., 2022a and 2022b). Such information could help to accelerate further investigations on this pathway and, consequently, to understand how and why ELA and apelin act, the typical phenotypes related to overexpression, redundancy, deficiency of either or both the two peptides, and the related cell context dependent factors, regulation's mechanisms, and conditions. Thus, numerous efforts need to be performed to achieve such complex object, which could, however, facilitate the development of APJ agonists or antagonists for the treatment of ARD.

6. ELA-APJ signaling in ARD

Other functions to be attributed to ELA are its involvement in pathological conditions, such as ARD, as above mentioned, which makes ELA a potential target for therapy of such diseases. A growing number of research groups are focusing their attention on this topic, documented by the increasing evidence in literature, which is described below. To facilitate understanding of the concepts, that follow, a brief description on cellular senescence and SASP, which represent one of the obscure sides of the senescence response in aging, is provided, as well as an illustration on their potential relationship with ELA, taking in account the very limited amonut of available evidence.

6.1. Cellular senescence and the SASP: the beneficial effects of ELA

Cellular senescence is the typical feature, which heralds inevitable ageing process in the tissues, organs and systems of the organisms, humans included (Olivieri et al., 2018). It occurs in response to the actions and biological effects of endogenous and exogenous stressors, and is characterized by several cellular alterations, including telomere dysfunction, oncogene activation and persistent DNA damage (Balistreri et al., 2014). Furthermore, senescent cell extrinsic activities, largely associated with the SASP development, fruit of the activation of NF-kB pathway (Balistreri et al., 2013), acerbate the impact of cell-intrinsic proliferative arrest and contribute to impaired tissue regeneration and the organismal ageing followed by the ARD onset. Sex specificity has been also affirmed for cell senescence, as evidenced in male mice until the end of life (amply quoted in Balistreri, 2023). Recently, it has been demonstrated that appropriate therapies can delay or retard the cellular senescence and the related SASP. Of particular interest is a new branch of the medical science, called *anti-ageing medicine.,* which is developing the so called *senotherapeutics*. This last represents an emerging anti-cellular senescence treatment and includes three therapeutic approaches: (a) molecules to selectively kill senescent cells (SC), defined *senolytics*; (b) compounds able in reducing evocated SC SASP, acting hence as SASP suppressors, or capable to change the senescent phenotype, called *senomorphics*; (c) inhibition of increase of the number of SC

in the tissues. Many molecules are emerging, including two senolytics, dasatinib and quercetin, which have been recently demonstrated by Salerno and coworkers (Salerno et al., 2022) improve, when used in combination, cardiac remodeling, and function after myocardial infarction in female aged mice. Interesting also are the effects of other senolytics in reducing senescence and cardiac dysfunction in diabetic organisms, as reported in the investigations conducted by Molinaro and coworkers (Molinaro et al., 2022) and Marino group (Marino et al., 2023), in C57BL/6 J mice treated with streptozotocin to evocate the onset of type 1 or 2 diabetes.

Of note also appear small peptides, as promising anti-ageing drugs. Among these, there are the bioactive peptides (biopeptides)/hydrolysates, obtained from various food sources. Biopeptides are contemplated as interesting treatments for industrial application, because they have diverse functional properties (e.g., anti-aging, antioxidant, anti-inflammatory, and antimicrobial properties) and technological properties (e.g., solubility, emulsifying, and foaming). However, it has been tested that they have fewer side effects than synthetic drugs, likely due to some challenges linked to administration via the oral route. Precisely, gastric, pancreatic, and small intestinal enzymes and acidic stomach conditions can affect their bioavailability and the levels that can reach the site of action. Some delivery systems have been studied to avoid these problems (e.g., microemulsions, liposomes, solid lipid particles; Scola et al., 2019; Balistreri, 2021). Therefore, the search for natural or newly synthesized peptides is increasing. In this context, our interest is focused on ELA as synthetic or endogenous drug, which plays, as above-mentioned important roles, in various physiological processes, as well as in pathological conditions, like ARD, as below reported. The association of ELA with these disease conditions makes it a potential target for their therapy, even if the current evidence is limited and further investigations are needed. Nevertheless, the development of peptide analogues that have shown promise as potential pharmacological agents is increasing. Here, it reports their description and the related benefits and limitations (see paragraph 7).

6.2. ELA blood levels and its relationship with ARD

The close association of the ELA-APJ axis with ARD has been confirmed in some studies by evaluating and comparing circulating levels of ELA in ARD patients compared to healthy controls (Xu et al., 2018; Sharma et al., 2022). On the other hand, ELA is physiologically expressed in the systems and organs of the human body, where the onset and progression of ARD occur (Lu et al., 2017; Xu et al., 2018). Consequently, changes in the circulating levels of ELA measured in plasma or serum samples may reflect the onset and degree of severity of damage to the systems/organs and, consequently, could represent an optimal biomarker for the diagnosis and prognosis of ARD. Accordingly, Li and co-workers reported a decrease in circulating ELA levels in patients with essential hypertension that correlated with the degree of impaired vascular function (Li et al., 2019). Onalan and colleagues (Onalan et al., 2020) determined the relationship of ELA levels with diabetic nephropathy and metabolic indices by measuring ELA levels in a healthy control group and type 2 diabetic (T2D) patients with and without diabetic nephropathy. The results showed higher ELA levels in healthy individuals than in patients with T2D (Onalan et al., 2020). Furthermore, patients with advanced albuminuria and reduced estimated glomerular filtration rate (eGFR) values showed significantly lower levels of ELA than patients without microalbuminuria. Therefore, these data suggest that ELA levels could be an important clinical prognostic biomarker, and simultaneously ELA could be used as a promising agent for the treatment of patient with diabetic nephropathy (Onalan et al., 2020). Similar data were obtained by Zhang and Yang's groups (Zhang et al., 2018; Yang et al., 2017). In addition, the researchers' group from Wroclaw Medical University evaluated apelin, ELA and APJ-receptor levels in the plasma of patients with acute (ACS) and chronic coronary syndromes (CCS) in a pilot sample, including 30 CCS and 84 ACS cases and 33 healthy controls. CCS had significantly lower plasma levels of both apelin and ELA than healthy controls and ACS. Moreover, the ACS cases surprisingly showed higher levels of both molecules than healthy controls and CCS, suggesting their involvement in compensatory up-regulation mechanisms (Zhou et al., 2019). This could help to make a differential diagnosis between the two forms. On the other hand, a positive correlation has also been shown between ELA and apelin and biochemical biomarkers of ischemia and left ventricular ejection fraction.

An increasing number of research groups have also evaluated ELA levels as a diagnostic biomarker for pre-eclampsia, and these data were conflicting. This was taken care of by van Dijk's team (Ho et al., 2017; Georgiadou et al., 2019a). They analysed ELA measurements using commercial disposable kits and observed conflicting data regarding peptide extraction failure. Consequently, van Dijk's team recommends the use of peptide extraction to obtain accurate and unambiguous data (Ho et al., 2017; Georgiadou et al., 2019b). This also leads to the suggestion of the need to standardise ELA quantification methods by reaching standard methodological goal, as well as further studies on larger samples.

6.3. ELA in cancer as a diagnostic biomarker and therapeutic agent

In recent years, the clinical application of peptides in the diagnosis and treatment of cancer is increasing, because peptides show optimal solubility, simple low-cost synthesis, and promising pharmacokinetic proprieties. As a novel peptide, ELA has been documented to be implicated in the carcinogenetic process of five types of cancer, including ovarian cancer, glioma, leukaemia, thyroid and kidney cancer (Liet et al., 2021). In ovarian cancers, ELA is overexpressed in adenocarcinoma, where it takes on the role of regulator of tumor cell proliferation and migration by influencing the expression of p53 (Yi et al., 2017). Consequently, disruption of ELA expression reduces the tumorigenesis of such cells in vivo. ELA also shows an essential role in glioblastoma initiation and/or development and could also be considered a biomarker of high-grade glioblastoma (Artas et al., 2018). Furthermore, elevated ELA levels in the blood characterize chronic lymphocytic leukemia (CLL) patients and correlate with CCL prognosis and treatment responses (Acik et al., 2019). In benign and malignant thyroid tumours, ELA expression has been shown to play a differential role in diagnosis (Bankir et al., 2021). Higher ELA expression has been observed in macro-carcinomas than the micro-carcinomas (Bankir et al., 2021). In contrast, reduced ELA expression levels were found in kidney tumours, although further studies are needed for a clearer understanding of the ELA role in these cancers (Artas et al., 2019).

However, the role of ELA has so far been studied in a limited number of tumour tissues, and further investigations could make it possible to establish the exact role of the ELA peptide in a very wide range of tumours, as well as to differentially select its application as a potential diagnostic biomarker or therapeutic agent.

6.4. ELA in kidney diseases

The two more common kidney diseases in elderly people are acute kidney injury (AKI) (Fang et al., 2020) and chronic kidney disease (CKD) (Nitta et al., 2013), which are characterized by functional decline of varying degrees. Precisely, AKI is a condition indicated by an immediate onset of kidney malfunction and defined clinically by increased serum creatinine levels and reduced urinary output. By contrast, CKD is characterized by changes in renal structure and function lasting for more than 3 months (e.g., by a decreased glomerular filtration rate [<60 ml/min/1.73 m2], elevated levels of blood urea nitrogen, uric acid, creatinine, and abnormal urinary albumin excretion). Several studies have also shown that ELA plays a crucial role in the pathogenesis of the two kidney diseases. ELA has been shown to be an important mediator of ischemia/reperfusion (I/R) injury, hypertensive

nephropathy, renal fibrosis, diabetic nephropathy and cardiorenal syndrome. Furthermore, ELA may also be a promising biomarker for differentiating renal tumour types. However, little is known about the molecular mechanisms and precise functions of ELA in these different kidney diseases. Further investigation of the mechanistic role of ELA in kidney diseases is mandatory to clarify its potential role (Tejeswin et al., 2023).

6.5. ELA in cardiovascular diseases and its beneficial effects

ELA-APJ signaling seems to be also involved in cardiovascular diseases (CVD), in addition to renal disorders, above described (Rozwadowski et al., 2022). However, the two systems are strongly interconnected, and this has led some authors to integrate cardiac and renal aspect in the ELA-APJ signaling' s impact on cardio-renal syndrome. (Nyimanu et al., 2022). In this context, it is reasonable, the role of apelin in the chronic inflammatory process related to onset of the atherosclerosis. The activation of the apelin- APJ system has been shown to increase the expression of adhesion molecules, such as an Intracellular Adhesion Molecule 1 (ICAM-1), Vascular Adhesion Molecule 1 (VCAM-1) and chemokines, including Monocyte Chemoattractant Protein-1 (MCP-1), involved in monocyte recruitment and in the development of vascular inflammatory states predisposing to atherosclerosis. (Bäck et al., 2019). It is very likely that the pharmacological silencing of APJ pathway and lowering the plasma apelin levels would be beneficial in preventing atherosclerosis. On the other hand, Donmez and coworkers have described an increasing of Elabela levels in patients with acute ST segment elevation myocardial infarction (Dönmez and Acele, 2019). Furthermore, they have demonstrated a moderate positive correlation between the levels of troponin I, NT-ProBNP and Elabela.

Recent studies have also analysed the cardioprotective activity of apelin in coronary artery diseases (CAD) related to its vasodilatory effects on coronary arteries (Pisarenko et al., 2013). Accordingly, the circulating apelin concentration have been detected significantly lower in patients with CAD than in controls, and the plasma apelin concentration has been found to augment from the fifth day after percutaneous coronary intervention (Chen et al., 2017a; Chen et al., 2017b). Akboga and coworkers have reported that the plasma apelin concentration appears to achieve higher levels in patients with partial or complete filling of epicardial artery by the collateral vessels than in patients with no filling of any collateral vessels (Akboga et al., 2014). These authors have concluded that higher apelin plasma concentration associated to a wider coronary collateral development is a promising target strategy for anti-ischemic treatment. Accordingly, it has been demonstrated that the exogenous ELA treatment appears significantly to reduce myocardial fibrosis and cellular apoptosis in heart and kidney tissue in mice with acute myocardial infarction (MI) (Pan et al., 2020). Even, such treatment seems to improve cardiac and renal function in mice with acute MI. Jin group (Jin et al., 2021) has shown the capacity of ELA to activate VEFG/ VEGFR2 and Jagged1/Notch3 pathways through APJ, promoting angiogenesis after MI infarction. Such data underline the importance to use in a near future ELA, as well as its gene, as potential candidates to develop promising therapies, molecular and genetic, in the treatment of ischemic cardiomyopathy.

Apelin/ELA have been also demonstrated to play an important role in the treatment of hypertension. Circulating ELA and Apelin levels have been detected to be decreased in patients with essential hypertension and overall hypertension, respectively (Xie et al., 2017). Apelin has an antihypertensive effect in both peripheral and central nervous system (CNS) (Geng et al., 2020). In CNS, Apelin has been found to activate APJ receptors in the hippocampal gyrus and hypothalamus. The activation of such receptor has been assessed to reduce the blood pressure via vasopressin release reduction and plasma adrenocorticotropin and adrenocorticotropin synthesis rise (Newson et al., 2009). In peripheral system, apelin has been to observe to neutralize the activity of Angiotensin II and increases nitric oxide synthase (NOS) expression, resulting in decreased profibrotic gene expression, including plasmin- gen activator inhibitor-1 (PAI-1) (Newson et al., 2009). Furthermore, ELA levels have been detected to decrease in hypertensive patients with atrial fibrillation (AF) and further lowered in the persistent AF subgroup. There also is the evidence of growing levels of blood ELA in the first and second trimesters of pregnancy in women, who have developed gestational hypertension, even if there is not any significant association between the ELA levels and preeclampsia during any stage of pregnancy (Huang et al., 2019). Apelin has been also reported to exert a protective effect on the development of heart failure through inhibition of adverse cardiac remodeling and a reduction of the extent of myocardial fibrosis. In *vitro* experiments performed in mouse cardiac fibroblasts, obtained from normal and pressure-overloaded hearts, have showed that the pre-treatment of naive cardiac fibroblasts with apelin results to able to inhibit the production of collagen and decrease the spontaneous production of collagen in cardiac fibroblasts isolated from the hearts after aortic banding (Pchejetski et al., 2012). Finally, in a recent study has been investigated the vasorelaxant effect mechanism of ELA in the thoracic rat aorta mediated by the potassium channel. This appears to be a promising protective mechanism against thoracic aneurysm progression into rupture and dissection (Şahintürk and İşbil, 2022).

Several beneficial effects mediated by ELA and apelin APJ signaling report the studies above described, which suggest such molecule as promising targets for developing anti age-related CVD therapies. Certainly, further studies are necessary to achieve this important challenge.

6.6. ELA in brain diseases

Multiple actions are also attributed to ELA in the brain, from contributing to the neurogenesis in embryos to regulating in adult several pathways, being expressed the ELA/APJ signaling in central and peripheral nervous systems,: a) PI3K/Akt/mTOR pathways determining cell cycle progression, inhibition of autophagy, and cell survival and response to injury; b) MEK1/2/ERK1/2 leading to an increase in cell survival due to inhibition of apoptosis; c) RhoGEF/Rhoa pathway evocating cytoskeleton remodeling; d) activation of N-methyl-D-aspartate receptor (NMDAR) signaling inducing mitochondrion permeabilization, decreasing of the generation of ROS, Cytochrome C, and Caspase-3, thus, inhibiting apoptosis; d) Ca2 + -dependent Casein kinase-2 (CK2) phosphorylation of The N-methyl-D-aspartate receptor subunit 2B subunit (NR2B) at S1480, leading to decreased activity of NMDAR, and inhibiting the effect of the GRP78/CHOP pathway and caspase-12 cascade associated with ER stress. In addition, the apelinergic system has been also demonstrated to be expressed in the hippocampus and the subventricular zone along the lateral wall of the cerebral lateral ventricle suggesting that alterations in apelinergic system may also impact neural regeneration. Consequently, the multiple roles of such system justify its involvement in several neuronal and mental diseases, such as glioblastoma (as abovementioned), ischemic stroke, Alzheimer's disease, and Parkinson's disease, epilepsy, among others. Here, particular attention has been given on the protective role of ELA in the stroke.

Recent studies have shown the ELA beneficial effect on ischemic stroke (IS), a cerebrovascular disease associated with high disability, morbidity, and mortality. The ischemic event and the infarct cause a loss of neurons causing cerebral functional deficit followed by the inhibition of axonal signals. Kang-long-Zhang group (Zhang et al., 2023) has experimented the beneficial effects of ELA on neuron survival after ischemia and the underlying molecular mechanisms in C57BL/6 J mice. Precisely, these researchers, using flow cytometry and immunofluorescence, have evidenced the role of ELA to inhibit oxygen–glucose deprivation (OGD) -induced apoptosis and axonal damage in vitro. Other assays, from molecular to gene expressions and epigenetic analysis, have demonstrated that ELA modulates neuronal apoptosis and axonal damage by upregulating miR-124–3p and activating the C-terminal domain small phosphatase 1 (CTDSP1)/AKT signaling pathway,

suggesting the potential use of ELA as a therapeutic agent in the treatment of ischemic stroke.

Another study has focused its attention on ferroptosis, a form of non-apoptotic cell death caused by iron-dependent peroxidation of lipids, that contributes to ischemic stroke-induced neuronal damage. Its regulation likely mediated by ELA/APJ signaling could be a promising strategy for the treatment of stroke. On the other hand, a recent study performed in the mice middle cerebral artery occlusion (MCAO) models has observed the protective role of ELA-APJ axis in ischemic stroke, after the ELA-32 treatment. Precisely, a reduced brain infarction, an amelioration of neurobehavioral deficits and cognitive dysfunction have been detected. Moreover, ELA-32 administration has been also shown to be able to meliorate neuronal ferroptosis, accompanied by reduced iron deposition, decreased mitochondrial damage, relieved relived lipid peroxidation and glutathione reduction. These findings suggested that the ELA-APJ axis mitigates neuronal ferroptosis after ischemic stroke, and ELA-32 peptide may be a putative therapeutic avenue for ischemic stroke (Xu et al., 2023).

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder in the elderly, whose hallmark neuropathological features are extracellular amyloid beta (Aβ) deposition and the presence of intracellular neurofibrillary elbows, resulting in progressive neurodegeneration that macroscopically results in brain atrophy (Calsolaro and Edison, 2016). In addition to the neurodegenerative process, the disease is associated with neuroinflammation Calsolaro and Edision, 2016), and oxidative and nitrative damage (Mangialasche et al., 2009), pathological processes that occur even in the early stages of the disease. Few data have described the role of the apelinergic system in Alzheimer's disease. Aminyavari et al. in rats with Aβ25–35-induced toxicity and memory deficits have shown that the concomitant treatment with Apelin-13 improves working and spatial memory performance (Aminyavari et al., 2019). This protective effect would be attributable to inhibition of autophagy processes and suppression of apoptotic processes, probably mediated by the mTOR signaling pathway (Calsolaro and Edison, 2016). Other authors found that apelin-13 significantly improved performance in the streptozotocin (STZ)-induced sporadic AD mouse model, including improved cognitive performance, improved cholinergic dysfunction and synaptic plasticity (Luo et al., 2019). In addition, the above authors described that apelin-13 was found to attenuate neuroinflammatory processes by reducing cytokine levels. Finally, Yang et al. (2018) (suggested that apelin, by regulating the PI3K/AKT/GSK-3β signaling pathway, may play a significant role in modulating the pathological aggregation of tau, Aβ and α-synuclein.

Future work must address the role of ELA in relation to gene regulation, stem cell biology, neuronal development, and the related implications. This might also be of help in the development of other strategies of treatment in the periclinal face of patients at high risk to AD or Parkison onset. In addition, the recent identification and characterization of novel analogues and ligands (see next paragraph), having an increased half-life, specificity, and binding strength might facilitate the research of novel therapeutic approaches regarding the neuropsychiatric disorders and permit to clear how the components of the apelinergic system interact, and the related mechanisms and pathways.

7. Therapeutic Importance of ELA: exogenous or synthetic/recombinant nature

The strong association of ELA with the abovementioned diseases makes it a potential key candidate for their therapy. On the other hand, the beneficial effects of ELA/apelin-APJ system on hypertension, vasodilation, fluid and cardiovascular homeostasis, kidney injury, are becoming well documented. Based on such growing evidence, several works, recently published, have analysed the helpful effects evocated by the exogenous ELA-32, − 11, − 21 administration in subjects affected by above-described diseases. A brief report on current literature data is below illustrated (see Table 2).

7.1. Therapy on exogenous ELA

First evidence arrives from animal studies, whose data suggest a beneficial effect of exogenous ELA treatment in counteracting the onset and the progression of kidney diseases. Precisely, Chen et al. have demonstrated the capacity of exogenous Ela to significantly decrease tubular injuries in rat renal tubular cells subject to a hypoxia-reperfusion (H/R) injury, by mediating several actions: a) inhibiting apoptosis; b) downregulating of factors involved in the DNA damage response (DDR) pathway; c) decreasing the expression of inflammatory mediators (IL-6, IL-8) and fibrogenic factors (TGF- β, fibronectin and collagen 1a) in mice renal I/R models (Chen et al., 2017a; Chen et al., 2017b). In addition, Xu et al., have experimented a recombinant form of ELA 21, called Fc-ELA-21 (an IgG-based chimeric fusion protein, having a prolonged half-life), and showed its capacity to evocate an anti-apoptotic action by activating PI3K/AKT and mTOR pathways *in vivo* and *in vitro* models of renal IRI, and inhibiting FoxO. Thus, these data have reported a similar effect of the ELA 11 above described (Xu et al., 2021).

Advantageous effects were also obtained from Zhang's group by testing exogenous ELA 32 in streptozotocin (STZ)-induced mouse model of type 1 diabetes mellitus with the object to limit inflammation, fibrosis, and apoptosis of kidney tissues, as diabetes complications (Zhang et al., 2019).

The treatment with exogenous ELA administration has also reported to delay the progression of cardio-renal syndrome and the renal functional decline in patients with heart failure, during septic shock, by reducing inflammation and infection, as well as decreasing vascular permeability trough the maintenance of a constant circulatory blood volume and hemodynamic stability (Coquerel et al., 2017).

Moreover, Zhang and colleagues have evidenced in a hypertensive mice model that exogenous ELA administration can significantly prevent the Ang II-mediated pathological myocardial remodelling, by limiting the expression of inflammation-, hypertrophy-, and fibrosis-related genes. Particularly, ELA can significantly reduce the Ang II-induced upregulation of iron levels and lipid peroxidation in hypertensive mice, by inhibiting cardiac interleukin-6 (IL-6)/STAT3 signaling and activating the xCT/glutathione peroxidase (GPX4) signaling (Zhang et al., 2022a; Zhang et al., 2022b). Similar effects have been described after the administration of exogenous ELA-32 in hypertensive rats with pulmonary artery hypertension (PAH). Precisely, this treatment has consented to prevent the remodelling of pulmonary vasculature and hypertrophy of right ventricular (RV) cardiomyocytes (Yang et al., 2017). In addition, Ye et al., have described in a recent work a protective effect in limiting VSMC (Vascular smooth muscle cell) proliferation and vascular remodeling in spontaneously hypertensive rats (SHRs) after the exogenous ELA-21 infusion which reduces inflammation and oxidative stress (Ye et al., 2022). Helpful actions, mediated by the treatment with an adeno-associated plasmid vector coding for ELA-32, have been also detected by Jin et al., 2021 et al. Precisely, they have observed, after the injection of the abovementioned vector in myocardial infarction murine models, a greater improvement in angiogenesis process and cardiac function. It makes ELA-21 a serious candidate for the treatment of ischemic cardiomyopathy in the future (Jin et al., 2021).

Finally, exogenous ELA- 32 administration has shown the capacity to ameliorate cardiotoxicity induced by doxorubicin treatment in mice models (Chen et al., 2022).

7.2. Agonists and antagonists of APJ signaling

ELA has been proven to exert a great therapeutic potential for several age-related diseases, as briefly above described. However, to increase its half-life *in vivo* and improve its therapeutic effects, many synthetic molecular analogues have been developed. Among these, ALX40–4 C (AcNH-(D-Arg)9-COOH), a nine-arginine-residue polypeptide (Hu et al.,

Table 2
The use of exogenous ELA for therapeutic purpose.

Model	Pathologic condition	Molecule	Mechanism	Effect	Reference
Rat renal tubular cells	Hypoxia reperfusion injury	ELA-32 and ELA-11	Unknown	↓ Apoptosis ↓ DNA damage response	Chen et al. (2017a); Chen et al. (2017b)
C57/BL6 mouse renal I/R model	Ischemia/reperfusion injury	ELA-32 and ELA-11	↓ IL6, MCP1, IL8, Tgfb1 fibronectin, and collagen1a	↓ Inflammation and fibrosis	Chen et al. (2017a); Chen et al. (2017b)
C57/BL6 mouse model I/R model	Ischemia/reperfusion injury	Fc ELA-21	PI3K/AKT/mTOR pathway activation	↓ Apoptosis ↓ ROS production	Xu et al., 2021
Streptozotocin (STZ)-induced mouse model of type 1 diabetes mellitus	Type 1 diabetes related renal damages	ELA-32	PI3K/AKT/mTOR pathway activation ↓ Renal collagen deposition	↓ Apoptosis ↓ Renal fibrosis	Zhang et al. (2019)
Rat model of septic shock	Cardiorenal syndrome	ELA-32	Neutralization of pro-inflammarory cytokines	↑ Renal function ↓ Inflammation ↓ Vascular permeability	Coquerel et al., 2022
Ang II treated-C57/BL6-model	Hypertension Myocardial hypertrophy	ELA-32	Cardiac interleukin-6 (IL6) inhibition xCT/glutathione peroxidase (GPX4) signalling activation	↓ Iron levels ↓ Lipid peroxidation	Zhang et al. (2022a); Zhang et al. (2022b)
Monocrotaline exposed rat	Pulmunary arthery hypertension	ELA-32	Unknown	↓ Right ventricle hypertrophy	Yang et al. (2017)
Spontaneously hypertensive rats (SHRs)	Hypertension Remodelled and stiffened vessels	ELA-21	↓ Matrix metalloproteinase 2 and 9 ↑ Nuclear translocation of nuclear factor erythroid 2-related factor (Nrf2) in VSMCs ↓ Inflammatory cytokines and NADPH oxidase 1 expression	↓ Vascular remodelling ↓ Inflammation ↓ Oxidative stress	Chao et al., 2022
Myocardial infarction mouse model	Post-infarction heart damage	AAV containing ELA-32 sequence	↑ VEGF/VEGFR2 ↑ Expression of Jagged1/Notch3	↑ Angiogenesis ↑ Cardiac function	Jin et al. (2021)
Doxorubicin induced cardiomiopathy model	Miocardial injury	ELA-32	PI3K/AKT/mTOR pathway activation ↑ Trascription factor EB expression	↓ Apoptosis ↑ Autophagic flux	Chen et al. (2022)

2016), having the capacity to directly bind to APJ and prevent internalization and intracellular calcium mobilization (Zhou et al., 2003). Another APJ antagonist is the cyclo(1−6)CRPRLC-KHcyclo(9−14) CRPRLC, (Macaluso et al., 2011), having high affinity to APJ in the human left ventricle, and a longer half-life and resistance to proteolytic degradation. Promising also is the MM07 (cyclo(1−6) CRPRLCHKGPMPF), that has been also developed by using molecular dynamics simulations (Brame et al., 2015). MM07 has revealed to be able to promptly induce vasodilation and increase blood flow, as confirmed in a study where MM07 significantly reduced hypertrophy induced by monocrotaline (Yang et al., 2019).

Many efforts in this field have been performed to develop peptide ELA agonists with high potency and metabolic stability for improving the therapeutic effects on cardiac hypertrophy. Among these, The N-terminal lipid conjugation of apelin-13 derivative has been proposed as the best analogue with a half-life of 29 h and clearance of 0.049 $Lh^{-1}kg^{-1}$ in *vivo* pharmacokinetics (Reed et al., 2020), and having a high plasma protein binding capacity (Reed et al., 2020).

By using protein bioconjugation with a Fc-fragment has been also extended its peptide stability. This recombinant protein has offered a major half-life of about 33 h and exercised several benefits in limiting, for example, heart fibrosis, improving glucose disposal and increasing cardiac stroke volume and output. An analogous approach has been utilized for the exogenous ELA to expand its half-life *in vivo*. The Fc- ELA-32 conjugate (abovementioned) has been observed to have a half-life of approximately 44 h, providing high therapeutic potential (Xi et al., 2019).

7.3. Small molecule modulators of APJ

Small molecule modulators of APJ are developing in recent years, and some appear to reveal as clinical candidates, such as ML221, a kojic acid-based non-peptide APJ antagonist identified using high-throughput screening. By selectively (IC50–1.7 μM) inhibiting APJ, ML221 stops the generation of apelin-mediated cAMP and the recruitment of β-arrestin. The first developed nonpeptidic APJ agonist is, while, the E339–3D6 (Iturrioz et al., 2010), with a moderate affinity for APJ and acceptable stability in plasma (Margathe et al., 2014). The ML233 (C19H21NO4S) has been secondly developed as agonist, and has a molecular weight of 359 g/mol. It has shown stability in human plasma, but a short half-live of about 3 h, as well as hepatotoxicity (Fischer, 2020). Consequently, further efforts have been made to develop next-generation drug-like small molecules as APJ agonists, including in *primis* the CMF-019, a benzimidazole-derived compound. Evidence *in vivo* has demonstrated that CMF-019 can represent a tool compound for designing biased agonists with high pharmacokinetics to treat cardiac-renal pathological conditions (Read et al., 2016). More recently, AMG-986 and BMS-986224 have been developed and having orally bioavailability and potentiality as clinical candidates. They have been demonstrated, for example, to stimulate a sustained cardiac output, and have beneficial effects in the treatment of numerous cardiac and renal diseases (Ason et al., 2020; Gargalovic et al., 2021; Goldfogel et al., 2021; Narayanan et al., 2022; Pi et al., 2021; Tora et al., 2021; Winkle et al., 2022). However, their clinical application and the appropriate dosing still require to be defined in the future (Winkle et al., 2022).

7.4. Considerations

Many research and efforts have been devoted to developing analogues and small molecule modulators targeting ELA/APJ signaling. This has led, in recent decades, to make emerged a growing number of such molecules, prevalently tested in animal models, and only some are currently being validated in humans. In addition, the features of such molecules, including orally or plasma bioavailability (half-live), stability, doses, and effects are expected to be experimented and applied after clinical trials in humans soon. They will provide new therapeutic approaches for treating the age-related diseases and are assumed to be applied to the clinic one day.

8. Conclusions

The apelinergic system exerts numerous physiological effects on organs, including the regulation of fluid homeostasis, food intake, apoptosis, regeneration, ROS generation, inflammation, glucose

metabolism, regulation of renal and cardiovascular function, angiogenesis, cardiac-renal-brain development, cardiac contractility, vascular tone, cardiac hypertrophy, as well as in their disorders. Given the high homology with the first ligand apelin of APJ, ELA likely mediates similar effects. Accordingly, growing evidence demonstrates that ELA has a critical function not only in embryonic development, but also in adulthood, such as vasculogenesis, alleviating food intake, regulating vascular, cardiac, and renal functions, promoting the angiogenesis, relaxing mouse aortic blood vessel and exerting an antihypertension effect, inhibiting renal remodeling, suppressing fibrotic effects, and regulating water homeostasis, among others (see Fig. 2). The discovery of ELA supports why apelin knock-out mice has significant modifications in early embryonic development, phenotype, and pathogenesis of CVD and kidneys diseases (Xu et al., 2017), compared with apelin knock-out mice. Going advances on ELA evidence is ulteriorly underscoring the role of ELA in both embryonic development and adulthood. However, yet little is proven about the related ELA molecular mechanisms and pathways, as well as its precise functions in various pathophysiological events. Thus, a complete and systematic biological study of ELA/APJ signaling still needs to be done, as well as experimentations on its potential as optimal candidate for diagnostic/preventive ARD biomarker and therapeutic agent.

CRediT authorship contribution statement

Prof Balistreri firstly contributed to conception and design, and in drafting the major number of the sections of paper. Prof Pisano cured the section on ELA in CVD; Dr Magro was involved in drafting part of the 6 section and creating figure and table, and Dr Venezia in drafting the section on the ELA role in brain diseases with the cooperation and specialist supervision of Prof Monastero. Profs Balistreri and Monastero equally contributed to the critical revision. All authors participated in the study, and they read and approved the final version of the paper.

Declaration of Competing Interest

All authors declare that:

2) the paper is not under consideration elsewhere,
3) none of the paper's contents have been previously published,
4) the manuscript is original, and it is free from any form of plagiarism,
5) to have read and approved the manuscript,
6) to have not conflict of interests

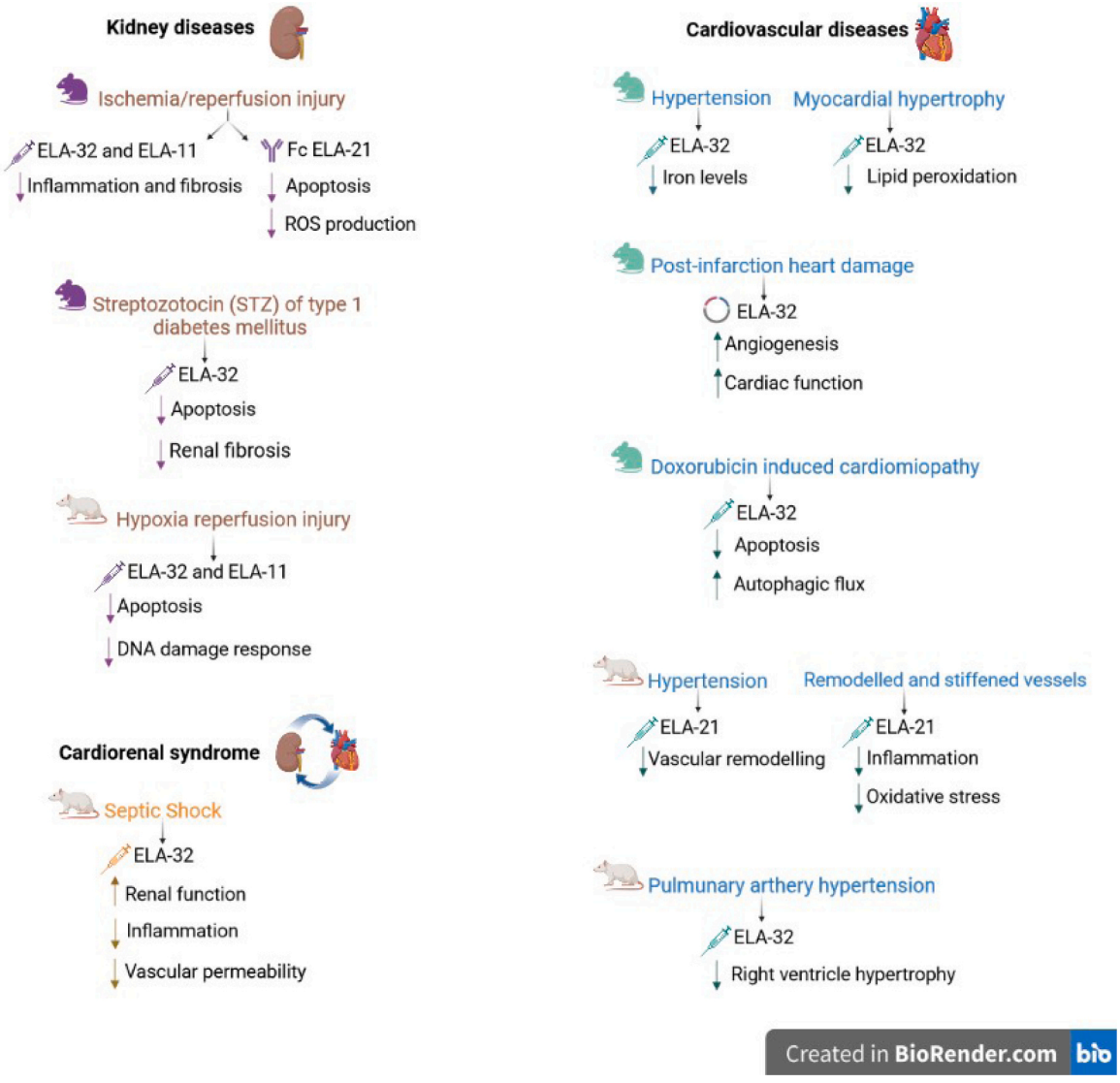

Fig. 2. The two disease's models mainly experimented for testing the biological effects of exogenous ELA treatment as anti-ageing/ARD peptide.

Data Availability

No data was used for the research described in the article.

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