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Effects of apelin on the cardiovascular system

Anna Folino¹, Pier Giorgio Montarolo¹, Michele Samaja², Raffaella Rastaldo³

¹Department of Neuroscience, University of Turin, C.so Raffaello 30, I – 10125 Torino, Italy

²Department of Health Science, University of Milan – San Paolo, via di Rudini 8, I - 20142 Milano, Italy

³Department of Clinical and Biological Sciences, University of Turin, Regione Gonzole 10, I – 10043 Orbassano, Italy

Corresponding author:

Raffaella Rastaldo, PhD

Department of Clinical and Biological Sciences

University of Turin

Regione Gonzole 10, 10043 Orbassano (TO) - Italy

e-mail: raffaella.rastaldo@unito.it

Phone number: +39 011 6705426

Fax number: +39 011 9038639

Abstract

Apelin is an endogenous peptide acting on the APJ receptor. It consists of several isoforms characterised by different numbers of aminoacids. The number of aminoacids in the active isoforms range from 36 to 12. Apelin-13 and, to a lesser extent, apelin-36 are considered the most active isoforms with the greatest activity on the cardiovascular homeostasis. The effects normally exerted by the basal level of endogenous apelin, can be enhanced not only by its up-regulation, but may also by its exogenous administration.

The present review considers the effects of apelin on various aspects of the cardiovascular function, such as cardiac development, vasomotor tone, angiogenesis, myocardial inotropy in healthy and failing hearts as well as the prevention of ischemia-reperfusion injury, cardiac fibrosis and remodeling. Also the biphasic changes of apelin level during the evolution of heart failure are considered. Although the positive inotropic effect exerted by apelin in normal and failing hearts would suggest the use of this peptide in the treatment of heart failure, the limited duration and extent of its effect do not support this possibility, unless a long lasting (6 hours) infusion is performed to overcome the limit of its short life. However, although the data on the characteristics of the inotropic activity do not provide a strong support for the treatment of active heart failure, apelin may be used in the prevention of heart failure because of its activity in limiting the consequences of myocardial ischemia such as infarct size and cardiac remodeling.

Keywords

Apelin/APJ system, heart failure, ischemia-reperfusion injury, contractility, cardiac protection, Renin-angiotensin system

The apelin/APJ system

Apelin

In 1998 Tatemoto et al. discovered the endogenous peptide capable of binding the G protein-coupled receptor (GPCR) APJ [1,2], which at that time was considered an orphan receptor because its ligand was unknown. The just discovered ligand was called, apelin e.g APj Endogenous LIgaNd. Apelin is expressed in hearts, lungs, kidneys, liver, adipose tissue, gastrointestinal tract, brain, adrenal glands, endothelium, and human plasma.

The gene of apelin is located on chromosome X [3] and encodes a 77 aminoacid sequence called pre-pro-apelin [4]. Upon cleavage by a family of endopeptidases, pre-pro-apelin generates several C-terminal fragments of various size, which are classified on the basis of the number of aminoacids. The active isoforms range from 36 to 12 aminoacids. As fragments shorter than 12 aminoacids are biologically inactive, it appears evident that the C-terminal 12 aminoacids are essential for receptor binding, whereas the N-terminal sequence modulates the interaction with the receptor [5].

At present it is recognized that, although different isoforms display similar functions, active isoforms differ in tissue distribution, potency and receptor binding affinity [6]. Apelin-13 and, to a lesser extent, apelin-36 have been considered the most active isoforms with the greatest activity on the cardiovascular apparatus [7,4]. Recently it has been demonstrated that, the predominant isoforms in the heart are apelin-13 and its post-transcriptionally modified form, pyroglutamyl apelin-13 ((pyr)apelin-13), which is obtained from apelin-13 by enzymatic conversion of the N-terminal glutamate residue into pyroglutamate, while apelin-36 is predominant in lung, testis and uterus [6,8].

Immunocytochemistry analysis of human hearts revealed that apelin is expressed in cardiomyocytes and in the vascular and endocardial endothelium [9,10]. Sartans are a group of hypotensive drugs reported to increase the expression of apelin [11].

The heart, especially atria, and adipose tissue are the predominant sources of plasma apelin in humans [12,8,6]. Apelin-17 and (pyr)apelin-13 have been considered the major isoforms present in human and rat plasma [13,14]. A large variability in plasma apelin concentration has been attributed to low specificity of the analysis procedures [6]. Recently, mass spectrometry analysis revealed that (pyr)apelin-13 is the major isoform present in healthy human plasma, with concentrations ranging 7.7-23.3 pg/ml [6].

Plasma half-life of apelin-13 and apelin-36 does not exceed 8 min [15]. The instability of apelin in plasma is attributed to its rapid degradation by endogenous circulating proteases [6]. Among the various isoforms, (pyr)apelin-13 is the most stable, perhaps because its cleavage is protected by the pyroglutamic acid residue located at the N-terminal [6].

The APJ receptor

The gene of the apelin receptor, APJ, was discovered before its ligand in 1993 and was mapped to chromosome 11 [1]. APJ is a 377 aminoacid G_i protein-coupled receptor with a 7 transmembrane domain, which was cloned from genomic human DNA [1]. The aminoacid sequences of APJ are well conserved in rats and humans [16]. Unlike its ligand, APJ occurs not only in human cardiomyocytes and endothelial cells, but also in vascular smooth muscle cells (VSMCs) [9]. At the subcellular level, APJ is localized in the T tubules and intercalated discs [17].

The availability of mice lacking either the ligand or the receptor helps to study the effects of alterations of the endogenous apelin-APJ signaling system. While apelin-deficient mice are viable, fertile and show normal development, APJ-deficient mice display cardiovascular development defects, suggesting the possibility of undiscovered APJ ligands or ligand-independent effects of APJ [18]. An example of such APJ ligand is the ELABELA hormone, which is involved in heart development [19,20] while a ligand-independent effect might consist in the initiation of cardiac hypertrophy in response to stretch [21].

General activity of the apelin-APJ system on cardiovascular homeostasis

Since the early studies on its effects on cardiovascular activity, the acute administration of apelin has been reported to reduce the vasomotor tone with increases in heart rate and myocardial contractility with a trend towards an increase in preload recruitable stroke work [22-24]. Apelin-induced vasodilation leads to a reduction of mean filling pressure, which in turn causes a decrease of preload and afterload. The acute administration of apelin may affect the left ventricular performance in one of the following ways: a) reduction of blood pressure, stroke volume and cardiac output at unchanged dP/dT despite increased contractility and heart rate [24], or b) a significant increase in stroke volume, P_{max} and dP/dT [25]. Moreover, the vasodilatation-dependent reduction of afterload can result in a reduction of cardiac work with increased cardiac efficiency.

When apelin was infused chronically over two weeks, the changes in blood pressure and heart rate observed with acute administration did not occur, whereas the increase in contractility led to increased stroke volume and cardiac output [24]. The occurrence of myocardial hypertrophy was not induced by neither type of apelin administration.

Apelin/APJ system vs renin-angiotensin system (RAS)

Note-worthy the aminoacidic compositions of pre-pro-apelin and APJ are similar to those of angiotensin (Ang) II and angiotensin II type 1 receptor (AT1R) respectively [3,1,2]. Although APJ shares a 40-50% identity with AT1R in the hydrophobic transmembrane region [1,2], the downstream effect of the two receptors is different. Ang II and apelin do not bind to APJ and AT1R respectively [1,2].

Angiotensin II is implicated in various cardiovascular diseases [26,27]. The apelin/APJ system acts as a counter-regulator of the renin-angiotensin system (RAS), in which a central role is played by Ang II. Two signalling mechanisms are involved in this antagonism: 1) the interaction between AT1 and APJ modulates the function of these receptors [28], and 2) the negative regulation of RAS by angiotensin-converting enzyme 2 (ACE2), a homologue of angiotensin converting enzyme (ACE) [29,30]. [24,25].

AT1Rs mediate major cardiovascular effects of Ang II, as induction of hypertension, myocardial hypertrophy and fibrosis. The binding of Ang II to its receptors is known to result in increased vasopressin secretion with relevant unwanted effects on the cardiovascular system [31]. Usually APJ and AT1R are co-expressed in various cardiovascular tissues [28]. Due to their co-expression, the two GPCRs may form a heterodimer which can be enhanced by apelin [32,28]. Apelin-13 binding to APJ forces allosteric formation of a heterodimer composed by APJ and AT1R in a dose-dependent manner. Upon formation of this heterodimer, the AT1R affinity for Ang II decreases markedly, thereby attenuating RAS. Interestingly, the reverse is not true, and AT1R binding to Ang II does not suppress the apelin-APJ axis. As a note of caution, the two techniques that have been used to assess this reciprocal effect, bioluminescence resonance energy transfer (BRET) and competitive binding assay, did not provide exactly convergent results, and this important point needs be further investigated by alternative, independent approaches [28]. By contrast, it has been proposed that inactivated APJ can form heterodimers with AT1 which suppresses RAS signalling. This inhibitory effect is reduced by the binding of apelin to APJ [33]. In addition to the modulation on AT1R signalling, apelin counteracts RAS by up-regulating ACE2 expression via APJ (Fig. 1). Unlike ACE, ACE2 is a negative regulator of RAS because it is responsible for the conversion of Ang II to Ang 1-7. The importance of apelin in ACE2 activation results from the observation that the latter is downregulated in apelin-deficient mice [30]. However, treatment with Ang 1-7 limits myocardial dysfunction, hypertrophy and fibrosis [30,34], as well as endothelial dysfunction [35]. Thus, it was thought that the axis apelin-ACE2-Ang 1-7 represents a signalling protective pathway and that angiotensin 1-7 does not simply indicate Ang II removal, but it also displays a therapeutic effect via its own G-protein coupled receptor, Mas, associated with cardiac protective responses [30].

ACE2 counteracts RAS also by transforming Ang I into Ang 1-9 by the hydrolysis of the carboxy terminal leucine [36,37] (Fig. 1). In turn, Ang 1-9 can be converted to Ang 1-7 by ACE [36]. Angiotensin 1-9 exerts beneficial effects on the heart, which consist in reduction of blood pressure, improvement of endothelial function and limitation of hypertrophy and fibrosis [38]. It has been suggested that these beneficial effects of Ang 1-9 can be achieved via angiotensin II type 2 receptor (AT2R), independently of its conversion to Ang 1-7 [38,39].

In addition to the favorable activity on cardiac dysfunction, ACE2 was reported to play a role in the hydrolysis of a large number of peptides, including apelin-13 and apelin-36, which are thus degraded to ineffective forms [40]. So far, ACE2 has been considered the only enzyme able to cleave the C-terminal residue [40]. However, plasma apelin instability has been attributed to its rapid degradation carried out by endogenous circulating proteases [6].

The antagonism of apelin towards RAS indicates that the former displays an important protective effect against cardiac remodelling after myocardial infarction (MI). As it will be discussed later in the present review, the treatment with apelin immediately after an ischemic insult can attenuate the injury during early reperfusion [41]. Thus, the fact that apelin can limit the immediate and delayed consequences of myocardial ischemia underscores its possible role the treatment of coronary artery disease.

Regulation of cardiovascular development and repair

The involvement of apelin in cardiac development was first identified in the frog [42]. Later on the effect of apelin on the differentiation of embryonic stem cells (ESCs) along cardiac lineage was studied in mice and humans [43]. More than one half of APJ-null mouse embryos die *in utero* due to cardiovascular developmental defects that range from impaired maturation of yolk sac and embryo vasculature to aberrantly formed right ventricles and defective atrio-ventricular cushion formation [44]. It was also observed that apelin, if combined with mesodermal differentiation factors, not only increases the percentage of contractile embryonic bodies derived from ESCs, but also up-regulates various specific markers of differentiation into cardiomyocytes [43].

Apelin is also required for normal vascular development. In frogs and mice, APJ expression was detected in the endothelium of primary blood vessels and newly forming hearts [45-48]. Moreover during angiogenesis apelin is involved in the adaptation of blood vessel size to the tissue demand for oxygen and nutrients in mouse embryos. In mice, the formation of retinal vessels during the foetal period and the first two postnatal weeks is characterized by a transient up-regulation of apelin/APJ mRNAs in retinal endothelial cells [49,45,50]. This

intervention of the apelin/APJ system was confirmed in apelin-null mice, in which the impairment of retinal vascularization in the early postnatal period was detected [51].

In vitro studies confirmed the role of apelin in vascular development. Apelin promotes proliferation, migration and capillary-like formation of retinal endothelial RF/6A cell line with high expression of apelin and APJ transcripts [51]. Identical effects were also produced by apelin-13 in myocardial microvascular endothelial cells [52].

In addition to cardiovascular development, apelin contributes to the post-ischemic vascular regeneration. In mouse ischemic hind limbs, transgenic apelin overexpression together, with vascular endothelial growth factor (VEGF), promotes the development of relatively large non-leaky vessels. This does not necessarily mean that a synergy between apelin and VEGF is required for any apelin activity, because this peptide is known to prevent the pro-oedemigenic hyper-permeability by inhibiting VEGF effect [53]. On the other hand, a reduction in capillary density and vessel integrity was observed in the heart of apelin-knockout mice after MI [54].

Molecular mechanisms. Myocyte enhancer factor 2 (MEF2) plays a role in cardiac development [55,56] and activated by G α 13 protein [44]. While in mice apelin administration or APJ overexpression increase class II histone deacetylases (HDAC) phosphorylation and translocation from nucleus to cytoplasm, in APJ-null animals not only the response is abrogated, but the phosphorylation of HDAC is reduced even in baseline conditions. The response is also abrogated in human umbilical vein endothelial cell (HUVEC) by knockdown of G α 13 protein [44]. Taken together, these data indicate that the apelin/APJ system regulates cardiac development via a G α 13-HDAC-MEF2 cascade. In zebrafish a role in cardiac development has also been attributed to ELABELA, the newly discovered hormone acting as a second ligand of APJ receptors [19,20].

Since apelin induces endothelial nitric oxide synthase (eNOS) phosphorylation in endothelial cells and nitric oxide (NO) participates to the process of angiogenesis [57,58], the role of apelin in vessel formation is expected to be mediated by NO.

It was observed that apelin stimulates angiogenesis in myocardial microvascular endothelial cells via Thr-172 phosphorylation of AMP-activated protein kinase (AMPK) and via Ser-1179 phosphorylation of eNOS [52]. The pro-angiogenic effect was abolished by compound C, inhibitor of AMPK, and LY294002, inhibitor of phosphoinositide 3-kinase (PI3K), indicating that apelin promotes angiogenesis through the AMPK and Akt/eNOS signaling pathways [52]. Interestingly, apelin induces proliferation in HUVEC only after the up-regulation of APJ level by VEGF [59]. However, the pro-angiogenic effect of apelin is independent of growth factors [45,46].

Vasomotor tone

Earlier studies on the cardiovascular activity of apelin revealed its vasodilator and hypotensive effects [3,2,60,15]. Initially, the intravenous injection of apelin in the rat at the dose of 1-2 μ g/300 g reduced systolic and diastolic blood pressure by 10-13% [3]. In another study in the rat, doses of 10 nmol/kg of apelin-12, apelin-13 and apelin-36 given separately decreased arterial blood pressure by 26, 11 and 5 mmHg, respectively, i.e. inversely proportional to the molecular weight of the administered compounds [2]. In the rat, the hypotensive effect lasted only a few minutes (2 –3 min) [3,22]. In APJ-deficient mice, basal blood pressure was found to be equivalent to that of wild-type mice [60] suggesting, the presence of endogenous apelin plays a poor, if any, role in the regulation of basal blood pressure. Exogenous apelin, which transiently reduced blood pressure not only in wild type mice, but also in spontaneously hypertensive rats, was ineffective in APJ-deficient mice [60], so that the role of APJ in the hypotensive response was underlined.

Hypotension is responsible for a reduction of the baroreceptor stimulation with decrease of the vagal and increase of the sympathetic tones. Tachycardia is a result of these changes in the autonomic activity. In the rat, blood pressure reduction is accompanied by a transient (3 - 4 min) increase in heart rate [3]. Such increase has been attributed to enhanced sympathetic discharge rather than to reduced vagal tone, which is low in this species. In fact, apelin injection was followed by a decrease in both arterial and venous filling pressures without changes in heart rate, if the sympathetic ganglia had previously been blocked with mecamylamine [23]. The reduction in the overall vascular filling pressure shows that apelin-induced vasodilatation is almost simultaneously extended to systemic arteries and veins. However, there is a limit to homogeneous distribution of the vasodilatation throughout the entire vascular system. In fact, intravenous bolus injections of apelin in the dog reduces pressure in the systemic circulation but not in the pulmonary vascular bed [61]. Hypotension is not the unique response to the administration of apelin *in vivo*. Intravenous injections of 20 and 50 nmol of (pyr)apelin-13 induces dose-dependent increases in arterial pressure and heart rate in conscious rats [62].

The presence of endothelium seems to be necessary for the vasodilator effect of apelin. In fact, in human mammary arteries and saphenous veins, apelin reverses its effect into vasoconstriction after removal of the endothelium [63]. This latter effect can contribute to the hypertension observed in endothelial dysfunction [64,65]. However, the absence of vasoconstriction when the denuded rat portal vein was treated with the peptide is intriguing [66]. The discrepancy between the behaviors of portal and saphenous veins may be attributed to

differences in type of vessels and in species. Due to the dysfunctional endothelium and the marked increase of apelin in their plaques, the human isolated atherosclerotic coronary arteries easily undergo vasoconstriction [67].

Molecular mechanisms. The hypotensive effect of apelin is mediated by endothelium-derived NO. It was not observed in mice [60] and humans [15] after eNOS inhibition or in mice when it was injected in APJ-deficient animals [60]. These findings confirm the intervention of APJ in the phosphorylation of eNOS and production of NO, the latter exerting its well known relaxing effect on the vascular musculature. In brief, after binding to APJ on endothelial cells, apelin induces phosphorylation of eNOS via PI3K/Akt activation [68]. The endothelium-derived NO diffuses into the VSMCs where it displays its well known vasodilator effect via cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) pathway. The consequent increase in cGMP level activates PKG which induces the re-uptake of calcium by the sarcoplasmic reticulum (SR) via sarco/endoplasmic reticulum calcium ATPase (SERCA) activation. PKG also favors dephosphorylation of myosin light chains which is responsible for a reduction in number of the cross bridges [69-71].

In the absence of a functional endothelium, apelin binds to APJ of VSMCs causing their contraction. The pathway leading to apelin-induced constriction of denuded vessels was studied in rat VSMCs and in isolated thoracic aorta after endothelium removal [72]. The binding of apelin to APJ of VSMCs causes phospholipase C (PLC) activation with production of inositol 1,4,5 phosphate (IP₃) and diacylglycerol (DAG). These mediators lead to contraction via an increase in intracellular Ca²⁺ concentration and a phosphorylation of myosin light chains (MLC) [72].

The increase in cellular calcium level is attributed to the opening of IP₃ receptor-channels (IP₃R) of SR and then to Ca²⁺-induced Ca²⁺ release from ryanodine receptor-channels (RyR) [69]. Also DAG contributes to the increase Ca²⁺ level via protein kinase C (PKC) mediated activation of sarcolemmal Na⁺/H⁺ (NHE) and reverse mode Na⁺/Ca²⁺ (NCH) exchangers [69,72]. As it was seen in VSMCs obtained from isolated rat middle cerebral arteries, Ca²⁺ concentration may also increase in response to an apelin-induced inhibition of Ca²⁺-activated K⁺ channels and the consequent increase in the activity of voltage-dependent L-type Ca²⁺ channels [73]. Finally, apelin also inhibits of MLC-phosphatase activity thus inducing a further increase in the phosphorylation of MLC [72].

Inotropic effect

Apelin is reported to potentiate myocardial inotropy in isolated preparations and intact animals [74,24,8,25,17,75,41,69]. For this reason it has been suggested as a tool for the treatment of heart failure (HF) [76,70,24,67,77,78]. However, the results of various investigations arise some doubts about this proposal.

In isolated perfused rat hearts, infusion of apelin-16 produced a progressive dose-dependent (from 1 pM to 10 nM) increase in developed tension which reached its peak (69%) in 24 min [74]. On the contrary, in similar rat heart preparations, a 20 min apelin-13 infusion (500 nM) produced an immediate increase by about 18% in developed pressure, which was back to the control 3 min later only [41]. These data are consisted with what observed in trabeculae, where apelin-12 induced a transient (1 - 2 min) increase in developed force by about 7,4% [79]. A short lasting inotropic effect was also seen in isolated adult rat cardiomyocytes, where the sarcomere shortening increased for 1 - 2 min only in response to apelin-16 superfusion (1-10 nM) and returned to the control before the superfusion was discontinued [17].

The different duration of the effect does not seem to be depend on the different isoforms, because (pyr)apelin-13, apelin-13 and apelin-36 show comparable potency and efficacy in inducing positive inotropic effect [8,75]. On the other hand the importance of different procedures of contractility assessment cannot be disregarded.

The inotropic effect carried out by apelin is not always accompanied by similar haemodynamic effects. In anesthetized mice, increases in contractility were observed, either when 100 nM apelin was infused acutely via the internal jugular vein at 5 µl/min for 20 min, or when apelin was infused chronically at 2 mg/kg/day with a subcutaneous scruff minipump and hemodynamic measurements were performed after 7 and 14 days of treatment [24]. The inotropic effect was revealed by the changes in the slope and intercept of the end-systolic pressure-volume relationship after apelin administration. Interestingly, in these animals the acute apelin administration was not followed by increases in stroke volume, cardiac output and systolic pressure, i.e by the changes that one would expect because of the enhanced contractility [24]. The apparent contradiction between increased contractility and unchanged stroke volume and systolic pressure may be attributed to decreases in preload resulting from apelin-induced venous dilatation. It cannot be excluded that, in the absence of anesthesia which impairs the sympathetic tone, vasodilatation would have been replaced by vasoconstriction, paralleled by an increase in pressure, as reported by Kagiya and his co-workers [62]. It is evident that the uncertainty of the hemodynamic changes compromises the possibility to treat heart failure with apelin.

Unlike what obtained with acute administration, mice heart showed significant increases in cardiac output and circumferential shortening after 14 days of apelin chronic administration [24]. In spite of the long treatment with apelin, no cardiac hypertrophy was observed. These results of the chronic administration suggested the possibility of a therapeutic use of the peptide in the failing heart, which in fact was seen to exhibit improvements

of contractility in response to apelin infusion (0.01 $\mu\text{g}/\text{min}$ for 20 min) performed 6 weeks after ligation of the left descending coronary artery [25]. It may be argued that the reduction in filling pressure after acute administration was curtailed with time in chronically repeated infusions [74]. This hypothesis seems to be confirmed by experiments performed *ex vivo* or *in vitro*, i.e. in the absence of any variable distension force. In these experiments, force development increased after apelin in trabeculae of the failing right ventricle of the rat [79] and in cardiomyocytes from chronic post-ischemic rat heart [17]. It is note-worthy that in both preparations the inotropic effect was stronger in failing than in healthy hearts, so that various authors support the hypothesis that apelin should contribute to the treatment of patients with heart failure .

Molecular mechanisms. Usually a positive inotropic effect is attributed to an increase in intracellular calcium level. Activations of L type Ca^{2+} current or of NHE and reverse mode NCE exchangers are considered responsible for such an increase [80-83,74]. However, the mode of action of apelin on myocardial calcium movement is a matter of debate.

Szokodi and his coworkers demonstrated that apelin does not modulate L-type Ca^{2+} current in isolated adult rat cardiomyocytes. Since in isolated rat hearts the separated inhibition of PLC, PKC, NHE and reverse mode NCE limited the increase in myocardial inotropy, they attributed the improvement of contractility to the activation of these factors [74]. Thus, they proposed that DAG, obtained from phosphoinositide hydrolysis by PLC, is responsible for the activation of NHE via PKC. The resulting increases in intracellular Na^+ concentration lead to an increase in intracellular Ca^{2+} level via the reverse mode of NCE [74] (Fig. 2). The involvement of NHE and NCE in the inotropic activity of apelin has been confirmed by Wang et al. [84] who also demonstrated that apelin increases the SERCA-regulated re-uptake of Ca^{2+} into the SR in a PKC-dependent manner, thus adding the lusitropic effect to the inotropic one. Independently of the way by which apelin induces the entrance of Ca^{2+} into the cell, it can be argued that the initial increase in calcium level can also take place via the Ca^{2+} -induced Ca^{2+} release through the opening of RyR of SR. Moreover, a release of Ca^{2+} from SR also results from the activation of IP_3R by IP_3 , which results from phosphoinositide hydrolysis [69].

Since the apelin-induced increase in contractility was not totally abolished by the blockade of the two exchangers, the authors suggested the possibility that an increase in troponin C affinity for Ca^{2+} or in the cyclin rate of actomyosin cross-bridges could contribute to the inotropic effect [74] (Fig. 3).

An increased myofilament sensitivity to Ca^{2+} in the absence of any change in Ca^{2+} level was later attributed to the alkalinization of sarcoplasm following the loss of H^+ because of an increased NHE activity. This hypothesis found support when measurements of the sarcomere shortening in isolated cardiomyocytes showed that the positive inotropic effect was not accompanied by any increase in Ca^{2+} concentration [17,85].

Recently, Perjés et al. [75] demonstrated that the raises in myofilament Ca^{2+} sensitivity and cross-bridge kinetics is due to the phosphorylation of regulatory myosin light chain (RMLC) via myosin light chain kinase (MLCK) (Fig. 3). The inotropic response was suppressed by the inhibition of the parallel PKC ϵ and mitogen-activated protein kinase kinase (MEK) – extracellular signal-regulated kinases 1/2 (ERK 1/2) pathways. Although cardiac MLCK and RMLC have been proposed as potential downstream targets of PKC , the real cascade leading to MLCK is still elusive.

Finally, although low concentrations of nitric oxide can increase the force of contraction [86,87], NOS inhibition has not been seen to alter the apelin-induced increase in contractility [74].

Apelin in heart failure

In HF, apelin and AJP expressions vary in a biphasic mode, i.e they are unchanged or upregulated if HF is compensated, while they are downregulated if HF is decompensated [77,70]. In particular, in the rat the elevation of apelin gene expression *in vivo* was seen to take place within 24 hours after MI, when an increased apelin release contributes to compensate the sudden HF [76]. Consistently, apelin deficiency in humans worsens the damage of ischemia-reperfusion (I/R), included extension of infarct size, related inflammation and mortality [54]. Taken together, these data indicate that, whereas apelin/APJ expression increases in response to pathological stimuli, its deficiency worsens the effects of these stimuli.

Apelin production falls when HF becomes chronic or more severe [76]. An example of this fall is the reduction of the myocardial level of apelin observed in the dog with an advanced HF induced with microembolization of a coronary artery [78]. The biphasic changes in apelin release are in line with the general opinion that in the early stages of HF, factors activating the force of contraction, as e.g. enhanced sympathetic activity, can improve the cardiac function, while later they become progressively maladaptive and lead to a worsening of the cardiac conditions.

In apparent contrast with the above results, in humans it was seen that left ventricular apelin mRNA did not decrease, but increased in the last stage of chronic HF secondary to coronary disease or idiopathic dilated cardiomyopathy [12]. Such increase was not observed in atria, which are the main source of cardiac-generated apelin in normal conditions and was accompanied by reduction in ventricular APJ mRNA level. Moreover, in spite of the elevated ventricular apelin expression, apelin-like immunoreactivity was remarkably reduced in

plasma. Thus, the overall scenario is in favor of a reduced rather than an increased apelin activity. In the absence of any change in apelin level, a reduction in density of APJ receptors was observed in another investigation on chronically failing human left ventricles [67]. Although the authors argued that this reduction might contribute to the contractile dysfunction, it may represent a protective limitation of the cardiac responsiveness to the inotropic effect of apelin.

A decreased of apelin content in plasma, as well as in atrial and ventricular myocardium, may be obtained in rats with heart failure produced with repeated high doses of isoproterenol [88]. In this model, the heart failure is significantly improved by apelin administration.

Molecular mechanisms. Myocardial ischemia by coronary disease, together with post-infarction remodelling, is considered the most frequent cause of HF [89,54]. The increase in apelin expression in response to acute hypoxia is mediated by hypoxia-inducible factor-1 (HIF-1) [76]. In fact, hypoxia has been found to be responsible for HIF-1 stabilization and nuclear translocation resulting in a 27-fold increase in apelin RNA level [76]. It may then be argued that also when a HF is the results of a chronic coronary artery disease, HIF-1 mediates apelin production during the first phase of the failure.

The mechanisms of the positive inotropic activity displayed by apelin in HF are considered to be the same that improve the contractility of normal hearts, via the intervention of NHE and NCE [17]. Moreover, also the antagonizing effect of apelin/APJ system on RAS might be a support to the beneficial effect. Conversely, in the late phase of failure an increased RAS activation would be responsible for the reduction of apelin expression [70].

Apelin in myocardial protection

Protection against ischemia-reperfusion injury

Heart may be protected against I/R injury by ischemic pre- (PreC) or postconditioning (PostC). While PreC is performed with brief (2 - 2.5 min) coronary occlusions 5 – 10 min before a heavy ischemic challenge, PostC is obtained with even shorter (seconds) occlusions, starting a few seconds after the end of the ischemia. The largest part of the reperfusion injury occurs in the first minutes after the end of the ischemia in the correspondence of ROS burst [90,91]. Consequently, the first minutes of reperfusion are a good window for the PostC interventions. However this is not a strict limit, because sometimes the infarct size may increase for 2-12 hours during reperfusion after ischemia in the rat heart [92].

In the isolated rat heart, the size of a MI caused by 35 min of occlusion of the left main coronary artery was significantly reduced if an 8 μ M exogenous apelin solution was infused for 20 min starting 5 min before the onset of reperfusion [93]. On the contrary, it remained unchanged if the infusion started 5 min before the onset of reperfusion and ended before removing the occlusion, suggesting that apelin mimics PostC rather than PreC. The hypothesis was confirmed by Rastaldo et al. [41], in Langendorff isolated rat heart preparation perfused with 0.5 μ M apelin-13 buffer for 20 min before or after the end of a 30 min global ischemia. While no protection was seen when apelin was given before ischemia, significant reductions of infarct size and improvement of post-ischemic mechanical recovery were obtained when apelin was given at the beginning of reperfusion. It is likely that the ineffectiveness of apelin before ischemia is due to its short half life [15] which does not allow the peptide to reach the level required for protection at the onset of reperfusion.

The expression of endogenous apelin mRNA was significantly increased at the end of ischemia and returned to control value after 60 min of reperfusion [93]. By contrast, the expression of APJ mRNA was unchanged at the end of ischemia [41,93]. These data confirm that apelin mimics PostC rather than PreC and that an endogenous production of apelin during ischemia might represent a process of myocardial self-protection. The fact that the increased expression of apelin mRNA was no longer present after 60 min of reperfusion is not in contrast with this opinion, because it is accepted that usually most of I/R injury takes place during the first minutes after the end of ischemia.

In mouse hearts, a reduction in IS occurred to a greater extent with apelin-13 and to a lesser extent with apelin-36 [4]. A similar reduction was also seen in mouse *in vivo* with occlusion of the left descending coronary artery, and the administration of apelin-13 and apelin-36 [4]. In this model the administration of apelin-13 resulted in a transient initial well evident recovery of mean arterial pressure, which however did not exceed the value recorded before the onset of ischemia. Since the effect of apelin on the resistance and capacitance vessels is dilatatory, the pressure increase should be solely attributed to the increase of cardiac performance. It is intriguing that such increase was not observed with apelin-36 [4]. The cardioprotective effect against I/R in *in vivo* rat model was also obtained by apelin-12 administration [94].

I/R-induced myocardial dysfunction consists in an increase of left ventricle diastolic pressure (i.e. contracture) accompanied by reduction of systolic and developed pressures. In isolated perfused rat hearts, Zeng et al. [95] observed that after 40 min of global ischemia and 30 min of reperfusion, the mechanical performance was highly ameliorated if 30 pM apelin was infused throughout the entire experimental period.

From the above data, apelin protection against I/R injury clearly consists in reduction of the infarct size and improvement of the post-ischemic mechanical recovery. However, it remains unclear whether the improved mechanical recovery is simply the consequence of the reduction of the infarct size or it is also due to the direct inotropic effect of apelin. In Langendorff rat heart preparations [41], the improvement of post-ischemic mechanical recovery took the developed pressure back to almost the value recorded before ischemia, where it remained throughout the entire period of reperfusion. Since when apelin was infused before ischemia, only a slight and brief increase in developed pressure was obtained [41], the hypothesis that a direct inotropic effect of apelin contributed to post-ischemic mechanical recovery must be refused.

Molecular mechanisms. Apelin protects the heart against I/R injury via either a NO-dependent or a NO-independent pathway, which are both components of the Reperfusion Injury Salvage Kinase (RISK) cascade. NO-dependent pathway begins with the activation of PI3K-Akt system which is responsible for the activation of eNOS. In fact, in *in vivo* mice with LAD occlusion, the inhibition of PI3K with LY294002 abolished apelin-induced reduction of the infarct size [4]. The role of PI3K was confirmed by the observation that the protection was accompanied by enhanced Akt phosphorylation in mice isolated hearts during reperfusion after global ischemia [96] and in neonatal rat cardiomyocytes during reoxygenation after hypoxia [95]. Moreover, the blockade of NOS prevented the protective effect of apelin-13 in the rat [41].

In a study no impairment of apelin-induced cardioprotection was observed in isolated rat hearts after inhibition of PI3K [93]. This finding is consistent with the possibility that apelin leads to myocardial protection also via a NO-independent pathway. In fact, phosphorylation of ERK 1/2 and the effect of its blockade [4] suggest that a relevant NO-independent cascade elicited by G-protein activation is responsible for the apelin protective activity via the downstream regulation of Bax/Bad pro-apoptotic proteins [97]. Both these protective pathways prevent the opening of mitochondrial permeability transition pore (mPTP) which is considered the key factor of I/R injury. In contrast, no data are available on the role of the Survivor Activating Factor Enhancement (SAFE) pathway in apelin-induced myocardial protection.

Protection against apoptosis

Apoptosis, a complex process of programmed cell death, occurs in all multicellular organisms. Although still debated whether apoptosis represents a protective or an aggressive phenotype, it is likely that attenuation of apoptosis in organs with low regenerative potential, such as the myocardium, is protective. In I/R injury and in dilated cardiomyopathy, apoptosis may also be triggered by the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), or ER stress [98,92].

Earlier observations obtained in mouse osteoblastic MC3T3-E1 cells show that apelin suppresses apoptosis induced by serum deprivation [99]. Later it was demonstrated that the anti-apoptotic effect of apelin concerns also the cardiovascular system [92,95]. In *in vivo* rat hearts exposed to 30 min of ischemia followed by reperfusion, the activation of ER stress-dependent apoptosis was attenuated by the administration of 1 µg/kg apelin [92]. Similar results were found in Langendorff isolated hearts of the same species. Although in reperfusion APJ is over-expressed at both mRNA and protein levels (by 7-fold and 35%) during I/R, pre-administration of 30 pmol/L apelin reduces apoptosis thereby ameliorating heart function, in conjunction with reduced generation of reactive oxygen species [95].

Patients with pulmonary artery hypertension have lower levels of plasma apelin and decreased apelin expression in pulmonary endothelial cells [100]. These apelin-deficient cells are more prone to undergo apoptosis and to promote proliferation of pulmonary arterial smooth muscle cells. On the other side, apelin administration can reverse pulmonary artery hypertension in mice that show a reduced production of apelin [101]. Furthermore, apelin suppresses serum deprivation-induced apoptosis of human VSMCs [102] and of rat bone marrow-derived mesenchymal stem cells [103], the latter bearing great promise for ischemic tissue repair, despite their poor viability within ischemic tissues.

Of interest, the apelin/APJ system has positive effects on the apoptotic potential in organs other than the cardiovascular system [104-108], further supporting the beneficial systemic effects of this drug.

Molecular mechanisms. In general, the two main pathways of apoptosis regulation include targeting of mitochondria functionality (intrinsic pathway), and external signal transduction via adaptor proteins or increased intracellular calcium concentration (extrinsic pathway).

In the latter pathway, PI3K/Akt stimulation plays a pivotal role in the beneficial activity of apelin against apoptosis as demonstrated by its impairment by PI3K inhibitor LY294002 [102,109]. Moreover, in mouse osteoblastic MC3T3-E1 cells the suppression of serum deprivation-induced apoptosis by apelin is also mediated by the activation of c-Jun N-terminal kinase (JNK) pathway [99]. In the case of mesenchymal stem cells, the protection from apoptosis is achieved by apelin via the usual PI3K/Akt signaling pathways coupled to the inhibition of the mitogen activated protein kinases (MAPK)/ERK 1/2 pathway [103].

In rat hearts, ER stress-dependent apoptosis was attenuated by PI3K/Akt, AMPK and ERK. In particular, using specific inhibitors, it was demonstrated that PI3K/Akt and AMPK are reciprocally dependent for their activation and exert a stimulating effect on eNOS, while ERK did not show any interaction with the other signals suggesting to belong to a NO-independent pathway to apelin-induced protection [92]. It may then be argued that NO-dependent and NO-independent pathways concern not only the protection against I/R injury in general, but also in the specific outcome that is apoptosis.

Protection against cardiac remodelling

Cardiac fibrosis and remodelling lead HF to end-stage. The process requires the differentiation of cardiac fibroblasts into myofibroblasts and is mediated by transforming growth factor- β (TGF- β). Experiments were performed *in vitro* and *in vivo* [110]. *In vitro* it was demonstrated that pretreatment of mouse fibroblasts with apelin prevents TGF- β from inducing both the expression of myofibroblast marker α -smooth muscle actin (α -SMA) and collagen production [110]. *In vivo*, it was seen that in mouse remodelling and ventricular dysfunction are impaired either if apelin is given before or 2 weeks after aortic banding [110].

In high salt loaded Dahl salt-sensitive hypertensive rats, a 7 day intraperitoneal administration of (pyr)apelin-13 at the dose of 200 μ g/kg/day suppressed the expression of inflammation factors, such as tumor necrosis factor- α and interleukin-1 β protein [111], which can induce myocardial fibrosis and remodeling [112].

Molecular mechanisms. TGF- β stimulates the differentiation of fibroblasts into myofibroblasts via the intervention of a sphingosine kinase 1 (Sphk 1). Thus, it has been proposed that the reduction of the activity of Sphk 1 is the starting point of apelin cascade in protecting the heart against fibrosis and remodelling. Once Sphk 1 is inhibited, the absence of α -SMA expression and of collagen production is consistent with the impossibility for fibroblasts to differentiate into myofibroblasts [110]. Moreover, in Dahl salt-sensitive hypertensive rats the up-regulation of ACE, AT1R and nuclear factor- κ B (NF- κ B) was inhibited by apelin-13 treatment [111]. These results confirm the hypothesis that RAS and NF- κ B can lead to cardiac fibrosis and remodelling [113-115].

Is apelin a therapeutic tool?

As the inotropic effect of apelin is well evident in failing hearts, a potential role of exogenous apelin for the treatment of HF has been suggested [24,15,29,25,116]. Although based on consolidated opinions including the simultaneous reduction of afterload, this point needs careful considerations.

A limitation to the use of apelin for pharmacological treatment of HF is the short (1 - 2 min) duration of its inotropic effect. The short-lasting effect also concerns vasodilatation, i.e. the reduction of afterload which otherwise should be expected to ameliorate cardiac efficiency. Thus, effective treatments would require chronic administration, consisting in repeated subcutaneous injections or prolonged infusions, because a peptide cannot be administered successfully via oral administration. However, the increase in cardiac index that was observed in humans with chronic heart failure throughout a six hours infusion of (pyr)apelin-13 suggests that at the moment APJ agonism may hold promise to complement the current medical therapies [116].

Moreover, in our experiments a modest increase in pressure suggests that apelin is not always one of the strongest inotropic compounds as sometimes it has been reported [74,41]. On the other hand, chronic apelin administration may be a key factor leading to tumorigenesis [117,118]. Independently of the duration and intensity of the effect on contractility, it must be kept in mind that at present the use of inotropic drugs is quite limited, because of their poor long term therapeutic effectiveness.

Rather than contractility, the beneficial effect of apelin mainly concerns myocardial protection against I/R injury, post-ischemic remodeling and myocardial fibrosis. The limitation of I/R injury offers the chance to set up a pharmacological post-conditioning procedure by intracoronary injection of apelin during post-infarction angioplasty. Since I/R may result in a rapid irreversible deterioration of myocardial contractility, apelin administration may represent a valid upstream prevention of HF, independently of the short duration of the effect of apelin on myocardial contractility.

Heart failure may be prevented by contrasting myocardial remodeling and fibrosis. The question then arises whether this prevention is produced not only by exogenous, but also by endogenous apelin. The production of endogenous apelin may be enhanced by *sartans*, a group of drugs which are used successfully against hypertension [11]. With sartans the risk of an unwanted hypotensive effect must not be disregarded before the treatment is undertaken. Studies should be carried on to see whether a well modulated use of these drugs could prevent cardiac remodelling, fibrosis and apoptosis by a non-excessive increase of plasma and tissue apelin concentration.

Conclusion

In conclusion, while the chance to treat failing hearts with the inotropic activity of apelin is still doubtful, there is the possibility that apelin could curb the onset or progression of a postischemic heart failure. This prevention is

the result of the protection against I/R injury, apoptosis, fibrosis and remodelling. As a consequence, the use of apelin as exogenous pharmacological intervention appears a promising and worth pursuing preventing tool.

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Conflicts of interest

None

References

1. O'Dowd BF, Heiber M, Chan A, Heng HH, Tsui LC, Kennedy JL, Shi X, Petronis A, George SR, Nguyen T (1993) A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene* 136 (1-2):355-360
2. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, Kurokawa T, Onda H, Fujino M (1998) Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochemical and biophysical research communications* 251 (2):471-476. doi:10.1006/bbrc.1998.9489
3. Lee DK, Cheng R, Nguyen T, Fan T, Kariyawasam AP, Liu Y, Osmond DH, George SR, O'Dowd BF (2000) Characterization of apelin, the ligand for the APJ receptor. *Journal of neurochemistry* 74 (1):34-41
4. Simpkin JC, Yellon DM, Davidson SM, Lim SY, Wynne AM, Smith CC (2007) Apelin-13 and apelin-36 exhibit direct cardioprotective activity against ischemia-reperfusion injury. *Basic Res Cardiol* 102 (6):518-528. doi:10.1007/s00395-007-0671-2
5. Zhou N, Zhang X, Fan X, Argyris E, Fang J, Acheampong E, DuBois GC, Pomerantz RJ (2003) The N-terminal domain of APJ, a CNS-based coreceptor for HIV-1, is essential for its receptor function and coreceptor activity. *Virology* 317 (1):84-94
6. Zhen EY, Higgs RE, Gutierrez JA (2013) Pyroglutamyl apelin-13 identified as the major apelin isoform in human plasma. *Analytical biochemistry* 442 (1):1-9. doi:10.1016/j.ab.2013.07.006
7. Masri B, Knibiehler B, Audigier Y (2005) Apelin signalling: a promising pathway from cloning to pharmacology. *Cellular signalling* 17 (4):415-426. doi:10.1016/j.cellsig.2004.09.018
8. Maguire JJ, Kleinz MJ, Pitkin SL, Davenport AP (2009) [Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease. *Hypertension* 54 (3):598-604. doi:10.1161/HYPERTENSIONAHA.109.134619
9. Kleinz MJ, Davenport AP (2005) Emerging roles of apelin in biology and medicine. *Pharmacology & therapeutics* 107 (2):198-211. doi:10.1016/j.pharmthera.2005.04.001
10. Kleinz MJ, Davenport AP (2004) Immunocytochemical localization of the endogenous vasoactive peptide apelin to human vascular and endocardial endothelial cells. *Regulatory peptides* 118 (3):119-125. doi:10.1016/j.regpep.2003.11.002
11. Fukushima H, Kobayashi N, Takeshima H, Koguchi W, Ishimitsu T (2010) Effects of olmesartan on Apelin/APJ and Akt/endothelial nitric oxide synthase pathway in Dahl rats with end-stage heart failure. *Journal of cardiovascular pharmacology* 55 (1):83-88. doi:10.1097/FJC.0b013e3181c87a82
12. Foldes G, Horkay F, Szokodi I, Vuolteenaho O, Ilves M, Lindstedt KA, Mayranpaa M, Sarman B, Seres L, Skoumal R, Lako-Futo Z, deChatel R, Ruskoaho H, Toth M (2003) Circulating and cardiac levels of apelin, the novel ligand of the orphan receptor APJ, in patients with heart failure. *Biochemical and biophysical research communications* 308 (3):480-485
13. De Mota N, Reaux-Le Goazigo A, El Messari S, Chartrel N, Roesch D, Dujardin C, Kordon C, Vaudry H, Moos F, Llorens-Cortes C (2004) Apelin, a potent diuretic neuropeptide counteracting vasopressin actions through inhibition of vasopressin neuron activity and vasopressin release. *Proceedings of the National Academy of Sciences of the United States of America* 101 (28):10464-10469. doi:10.1073/pnas.0403518101

14. Azizi M, Iturrioz X, Blanchard A, Peyrard S, De Mota N, Chartrel N, Vaudry H, Corvol P, Llorens-Cortes C (2008) Reciprocal regulation of plasma apelin and vasopressin by osmotic stimuli. *Journal of the American Society of Nephrology : JASN* 19 (5):1015-1024. doi:10.1681/ASN.2007070816
15. Japp AG, Cruden NL, Amer DA, Li VK, Goudie EB, Johnston NR, Sharma S, Neilson I, Webb DJ, Megson IL, Flapan AD, Newby DE (2008) Vascular effects of apelin in vivo in man. *Journal of the American College of Cardiology* 52 (11):908-913. doi:10.1016/j.jacc.2008.06.013
16. Hosoya M, Kawamata Y, Fukusumi S, Fujii R, Habata Y, Hinuma S, Kitada C, Honda S, Kurokawa T, Onda H, Nishimura O, Fujino M (2000) Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *The Journal of biological chemistry* 275 (28):21061-21067. doi:10.1074/jbc.M908417199
17. Farkasfalvi K, Stagg MA, Coppens SR, Siedlecka U, Lee J, Soppa GK, Marczin N, Szokodi I, Yacoub MH, Terracciano CM (2007) Direct effects of apelin on cardiomyocyte contractility and electrophysiology. *Biochemical and biophysical research communications* 357 (4):889-895. doi:10.1016/j.bbrc.2007.04.017
18. Charo DN, Ho M, Fajardo G, Kawana M, Kundu RK, Sheikh AY, Finsterbach TP, Leeper NJ, Ernst KV, Chen MM, Ho YD, Chun HJ, Bernstein D, Ashley EA, Quertermous T (2009) Endogenous regulation of cardiovascular function by apelin-APJ. *American journal of physiology Heart and circulatory physiology* 297 (5):H1904-1913. doi:10.1152/ajpheart.00686.2009
19. Chng SC, Ho L, Tian J, Reversade B (2013) ELABELA: a hormone essential for heart development signals via the apelin receptor. *Developmental cell* 27 (6):672-680. doi:10.1016/j.devcel.2013.11.002
20. Xie F, Lv D, Chen L (2014) ELABELA: a novel hormone in cardiac development acting as a new endogenous ligand for the APJ receptor. *Acta biochimica et biophysica Sinica* 46 (7):620-622. doi:10.1093/abbs/gmu032
21. Scimia MC, Hurtado C, Ray S, Metzler S, Wei K, Wang J, Woods CE, Purcell NH, Catalucci D, Akasaka T, Bueno OF, Vlasuk GP, Kaliman P, Bodmer R, Smith LH, Ashley E, Mercola M, Brown JH, Ruiz-Lozano P (2012) APJ acts as a dual receptor in cardiac hypertrophy. *Nature* 488 (7411):394-398. doi:10.1038/nature11263
22. Tatemoto K, Takayama K, Zou MX, Kumaki I, Zhang W, Kumano K, Fujimiya M (2001) The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regulatory peptides* 99 (2-3):87-92
23. Cheng X, Cheng XS, Pang CC (2003) Venous dilator effect of apelin, an endogenous peptide ligand for the orphan APJ receptor, in conscious rats. *European journal of pharmacology* 470 (3):171-175
24. Ashley EA, Powers J, Chen M, Kundu R, Finsterbach T, Caffarelli A, Deng A, Eichhorn J, Mahajan R, Agrawal R, Greve J, Robbins R, Patterson AJ, Bernstein D, Quertermous T (2005) The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading in vivo. *Cardiovascular research* 65 (1):73-82. doi:10.1016/j.cardiores.2004.08.018
25. Berry MF, Pirolli TJ, Jayasankar V, Burdick J, Morine KJ, Gardner TJ, Woo YJ (2004) Apelin has in vivo inotropic effects on normal and failing hearts. *Circulation* 110 (11 Suppl 1):II187-193. doi:10.1161/01.CIR.0000138382.57325.5c
26. Ferrario CM (2006) Role of angiotensin II in cardiovascular disease therapeutic implications of more than a century of research. *Journal of the renin-angiotensin-aldosterone system : JRAAS* 7 (1):3-14. doi:10.3317/jraas.2006.003
27. Higuchi S, Ohtsu H, Suzuki H, Shirai H, Frank GD, Eguchi S (2007) Angiotensin II signal transduction through the AT1 receptor: novel insights into mechanisms and pathophysiology. *Clinical science* 112 (8):417-428. doi:10.1042/CS20060342
28. Siddiquee K, Hampton J, McAnally D, May L, Smith L (2013) The apelin receptor inhibits the angiotensin II type 1 receptor via allosteric trans-inhibition. *British journal of pharmacology* 168 (5):1104-1117. doi:10.1111/j.1476-5381.2012.02192.x
29. Kazemi-Bajestani SM, Patel VB, Wang W, Oudit GY (2012) Targeting the ACE2 and Apelin Pathways Are Novel Therapies for Heart Failure: Opportunities and Challenges. *Cardiology research and practice* 2012:823193. doi:10.1155/2012/823193

30. Sato T, Suzuki T, Watanabe H, Kadowaki A, Fukamizu A, Liu PP, Kimura A, Ito H, Penninger JM, Imai Y, Kuba K (2013) Apelin is a positive regulator of ACE2 in failing hearts. *The Journal of clinical investigation* 123 (12):5203-5211. doi:10.1172/JCI69608
31. Catt KJ, Mendelsohn FA, Millan MA, Aguilera G (1984) The role of angiotensin II receptors in vascular regulation. *Journal of cardiovascular pharmacology* 6 Suppl 4:S575-586
32. Chun HJ, Ali ZA, Kojima Y, Kundu RK, Sheikh AY, Agrawal R, Zheng L, Leeper NJ, Pearl NE, Patterson AJ, Anderson JP, Tsao PS, Lenardo MJ, Ashley EA, Quertermous T (2008) Apelin signaling antagonizes Ang II effects in mouse models of atherosclerosis. *The Journal of clinical investigation* 118 (10):3343-3354. doi:10.1172/JCI34871
33. Sun X, Iida S, Yoshikawa A, Senbonmatsu R, Imanaka K, Maruyama K, Nishimura S, Inagami T, Senbonmatsu T (2011) Non-activated APJ suppresses the angiotensin II type 1 receptor, whereas apelin-activated APJ acts conversely. *Hypertension research : official journal of the Japanese Society of Hypertension* 34 (6):701-706. doi:10.1038/hr.2011.19
34. Zhong J, Basu R, Guo D, Chow FL, Byrns S, Schuster M, Loibner H, Wang XH, Penninger JM, Kassiri Z, Oudit GY (2010) Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation* 122 (7):717-728, 718 p following 728. doi:10.1161/CIRCULATIONAHA.110.955369
35. Beyer AM, Guo DF, Rahmouni K (2013) Prolonged treatment with angiotensin 1-7 improves endothelial function in diet-induced obesity. *Journal of hypertension* 31 (4):730-738. doi:10.1097/HJH.0b013e32835ecbe5
36. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S (2000) A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circulation research* 87 (5):E1-9
37. McKinney CA, Fattah C, Loughrey CM, Milligan G, Nicklin SA (2014) Angiotensin-(1-7) and angiotensin-(1-9): function in cardiac and vascular remodelling. *Clinical science* 126 (12):815-827. doi:10.1042/CS20130436
38. Ocaranza MP, Moya J, Barrientos V, Alzamora R, Hevia D, Morales C, Pinto M, Escudero N, Garcia L, Novoa U, Ayala P, Diaz-Araya G, Godoy I, Chiong M, Lavandero S, Jalil JE, Michea L (2014) Angiotensin-(1-9) reverses experimental hypertension and cardiovascular damage by inhibition of the angiotensin converting enzyme/Ang II axis. *Journal of hypertension* 32 (4):771-783. doi:10.1097/HJH.0000000000000094
39. Flores-Munoz M, Work LM, Douglas K, Denby L, Dominiczak AF, Graham D, Nicklin SA (2012) Angiotensin-(1-9) attenuates cardiac fibrosis in the stroke-prone spontaneously hypertensive rat via the angiotensin type 2 receptor. *Hypertension* 59 (2):300-307. doi:10.1161/HYPERTENSIONAHA.111.177485
40. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Baronas E, Hsieh F, Acton S, Patane M, Nichols A, Tummino P (2002) Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *The Journal of biological chemistry* 277 (17):14838-14843. doi:10.1074/jbc.M200581200
41. Rastaldo R, Cappello S, Folino A, Berta GN, Sprio AE, Losano G, Samaja M, Pagliaro P (2011) Apelin-13 limits infarct size and improves cardiac postischemic mechanical recovery only if given after ischemia. *American journal of physiology Heart and circulatory physiology* 300 (6):H2308-2315. doi:10.1152/ajpheart.01177.2010
42. Inui M, Fukui A, Ito Y, Asashima M (2006) Xapelin and Xmsr are required for cardiovascular development in *Xenopus laevis*. *Developmental biology* 298 (1):188-200. doi:10.1016/j.ydbio.2006.06.028
43. Wang IN, Wang X, Ge X, Anderson J, Ho M, Ashley E, Liu J, Butte MJ, Yazawa M, Dolmetsch RE, Quertermous T, Yang PC (2012) Apelin enhances directed cardiac differentiation of mouse and human embryonic stem cells. *PloS one* 7 (6):e38328. doi:10.1371/journal.pone.0038328
44. Kang Y, Kim J, Anderson JP, Wu J, Gleim SR, Kundu RK, McLean DL, Kim JD, Park H, Jin SW, Hwa J, Quertermous T, Chun HJ (2013) Apelin-APJ signaling is a critical regulator of endothelial MEF2

- activation in cardiovascular development. *Circulation research* 113 (1):22-31. doi:10.1161/CIRCRESAHA.113.301324
45. Saint-Geniez M, Masri B, Malecaze F, Knibiehler B, Audigier Y (2002) Expression of the murine msr/apj receptor and its ligand apelin is upregulated during formation of the retinal vessels. *Mechanisms of development* 110 (1-2):183-186
46. Devic E, Rizzoti K, Bodin S, Knibiehler B, Audigier Y (1999) Amino acid sequence and embryonic expression of msr/apj, the mouse homolog of Xenopus X-msr and human APJ. *Mechanisms of development* 84 (1-2):199-203
47. Devic E, Paquereau L, Vernier P, Knibiehler B, Audigier Y (1996) Expression of a new G protein-coupled receptor X-msr is associated with an endothelial lineage in *Xenopus laevis*. *Mechanisms of development* 59 (2):129-140
48. Cox CM, D'Agostino SL, Miller MK, Heimark RL, Krieg PA (2006) Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. *Developmental biology* 296 (1):177-189. doi:10.1016/j.ydbio.2006.04.452
49. Saint-Geniez M, Argence CB, Knibiehler B, Audigier Y (2003) The msr/apj gene encoding the apelin receptor is an early and specific marker of the venous phenotype in the retinal vasculature. *Gene expression patterns : GEP* 3 (4):467-472
50. Kojima Y, Quertermous T (2008) Apelin-APJ signaling in retinal angiogenesis. *Arteriosclerosis, thrombosis, and vascular biology* 28 (10):1687-1688. doi:10.1161/ATVBAHA.108.174847
51. Kasai A, Shintani N, Oda M, Kakuda M, Hashimoto H, Matsuda T, Hinuma S, Baba A (2004) Apelin is a novel angiogenic factor in retinal endothelial cells. *Biochemical and biophysical research communications* 325 (2):395-400. doi:10.1016/j.bbrc.2004.10.042
52. Yang X, Zhu W, Zhang P, Chen K, Zhao L, Li J, Wei M, Liu M (2014) Apelin-13 stimulates angiogenesis by promoting crosstalk between AMP-activated protein kinase and Akt signaling in myocardial microvascular endothelial cells. *Molecular medicine reports* 9 (5):1590-1596. doi:10.3892/mmr.2014.1984
53. Kidoya H, Naito H, Takakura N (2010) Apelin induces enlarged and nonleaky blood vessels for functional recovery from ischemia. *Blood* 115 (15):3166-3174. doi:10.1182/blood-2009-07-232306
54. Wang W, McKinnie SM, Patel VB, Haddad G, Wang Z, Zhabyeyev P, Das SK, Basu R, McLean B, Kandalam V, Penninger JM, Kassiri Z, Vederas JC, Murray AG, Oudit GY (2013) Loss of Apelin exacerbates myocardial infarction adverse remodeling and ischemia-reperfusion injury: therapeutic potential of synthetic Apelin analogues. *Journal of the American Heart Association* 2 (4):e000249. doi:10.1161/JAHA.113.000249
55. Lin Q, Schwarz J, Bucana C, Olson EN (1997) Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. *Science* 276 (5317):1404-1407
56. Naya FJ, Black BL, Wu H, Bassel-Duby R, Richardson JA, Hill JA, Olson EN (2002) Mitochondrial deficiency and cardiac sudden death in mice lacking the MEF2A transcription factor. *Nature medicine* 8 (11):1303-1309. doi:10.1038/nm789
57. Matsunaga T, Weihrauch DW, Moniz MC, Tessmer J, Warltier DC, Chilian WM (2002) Angiostatin inhibits coronary angiogenesis during impaired production of nitric oxide. *Circulation* 105 (18):2185-2191
58. Cooke JP, Losordo DW (2002) Nitric oxide and angiogenesis. *Circulation* 105 (18):2133-2135
59. Kidoya H, Ueno M, Yamada Y, Mochizuki N, Nakata M, Yano T, Fujii R, Takakura N (2008) Spatial and temporal role of the apelin/APJ system in the caliber size regulation of blood vessels during angiogenesis. *The EMBO journal* 27 (3):522-534. doi:10.1038/sj.emboj.7601982
60. Ishida J, Hashimoto T, Hashimoto Y, Nishiwaki S, Iguchi T, Harada S, Sugaya T, Matsuzaki H, Yamamoto R, Shiota N, Okunishi H, Kihara M, Umemura S, Sugiyama F, Yagami K, Kasuya Y, Mochizuki N, Fukamizu A (2004) Regulatory roles for APJ, a seven-transmembrane receptor related to angiotensin-type 1 receptor in blood pressure in vivo. *The Journal of biological chemistry* 279 (25):26274-26279. doi:10.1074/jbc.M404149200

61. Feng JH, Li WM, Wu XP, Tan XY, Gao YH, Han CL, Li SQ, Xie HN (2010) Hemodynamic effect of apelin in a canine model of acute pulmonary thromboembolism. *Peptides* 31 (9):1772-1778. doi:10.1016/j.peptides.2010.06.004
62. Kagiya S, Fukuhara M, Matsumura K, Lin Y, Fujii K, Iida M (2005) Central and peripheral cardiovascular actions of apelin in conscious rats. *Regulatory peptides* 125 (1-3):55-59. doi:10.1016/j.regpep.2004.07.033
63. Katugampola SD, Maguire JJ, Matthewson SR, Davenport AP (2001) [(125)I]-(Pyr(1))Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. *British journal of pharmacology* 132 (6):1255-1260. doi:10.1038/sj.bjp.0703939
64. Nagano K, Ishida J, Unno M, Matsukura T, Fukamizu A (2013) Apelin elevates blood pressure in ICR mice with LNAME-induced endothelial dysfunction. *Molecular medicine reports* 7 (5):1371-1375. doi:10.3892/mmr.2013.1378
65. Han X, Zhang DL, Yin DX, Zhang QD, Liu WH (2013) Apelin-13 deteriorates hypertension in rats after damage of the vascular endothelium by ADMA. *Canadian journal of physiology and pharmacology* 91 (9):708-714. doi:10.1139/cjpp-2013-0046
66. Gurzu B, Petrescu BC, Costuleanu M, Petrescu G (2006) Interactions between apelin and angiotensin II on rat portal vein. *Journal of the renin-angiotensin-aldosterone system : JRAAS* 7 (4):212-216. doi:10.3317/jraas.2006.040
67. Pitkin SL, Maguire JJ, Kuc RE, Davenport AP (2010) Modulation of the apelin/APJ system in heart failure and atherosclerosis in man. *British journal of pharmacology* 160 (7):1785-1795. doi:10.1111/j.1476-5381.2010.00821.x
68. Zhong JC, Yu XY, Huang Y, Yung LM, Lau CW, Lin SG (2007) Apelin modulates aortic vascular tone via endothelial nitric oxide synthase phosphorylation pathway in diabetic mice. *Cardiovascular research* 74 (3):388-395. doi:10.1016/j.cardiores.2007.02.002
69. Rastaldo R, Cappello S, Folino A, Losano G (2011) Effect of apelin-apelin receptor system in postischemic myocardial protection: a pharmacological preconditioning tool? *Antioxidants & redox signaling* 14 (5):909-922. doi:10.1089/ars.2010.3355
70. Japp AG, Newby DE (2008) The apelin-APJ system in heart failure: pathophysiologic relevance and therapeutic potential. *Biochemical pharmacology* 75 (10):1882-1892. doi:10.1016/j.bcp.2007.12.015
71. Ladeiras-Lopes R, Ferreira-Martins J, Leite-Moreira AF (2008) The apelinergic system: the role played in human physiology and pathology and potential therapeutic applications. *Arquivos brasileiros de cardiologia* 90 (5):343-349
72. Hashimoto T, Kihara M, Ishida J, Imai N, Yoshida S, Toya Y, Fukamizu A, Kitamura H, Umemura S (2006) Apelin stimulates myosin light chain phosphorylation in vascular smooth muscle cells. *Arteriosclerosis, thrombosis, and vascular biology* 26 (6):1267-1272. doi:10.1161/01.ATV.0000218841.39828.91
73. Modgil A, Guo L, O'Rourke ST, Sun C (2013) Apelin-13 inhibits large-conductance Ca²⁺-activated K⁺ channels in cerebral artery smooth muscle cells via a PI3-kinase dependent mechanism. *PloS one* 8 (12):e83051. doi:10.1371/journal.pone.0083051
74. Szokodi I, Tavi P, Foldes G, Voutilainen-Myllyla S, Ilves M, Tokola H, Pikkarainen S, Piihola J, Rysa J, Toth M, Ruskoaho H (2002) Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circulation research* 91 (5):434-440
75. Perjes A, Skoumal R, Tenhunen O, Konyi A, Simon M, Horvath IG, Kerkela R, Ruskoaho H, Szokodi I (2014) Apelin increases cardiac contractility via protein kinase C ϵ - and extracellular signal-regulated kinase-dependent mechanisms. *PloS one* 9 (4):e93473. doi:10.1371/journal.pone.0093473
76. Ronkainen VP, Ronkainen JJ, Hanninen SL, Leskinen H, Ruas JL, Pereira T, Poellinger L, Vuolteenaho O, Tavi P (2007) Hypoxia inducible factor regulates the cardiac expression and secretion of apelin. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 21 (8):1821-1830. doi:10.1096/fj.06-7294.com

77. Chong KS, Gardner RS, Morton JJ, Ashley EA, McDonagh TA (2006) Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. *European journal of heart failure* 8 (4):355-360. doi:10.1016/j.ejheart.2005.10.007
78. Wang M, Gupta RC, Rastogi S, Kohli S, Sabbah MS, Zhang K, Mohyi P, Hogue M, Fischer Y, Sabbah HN (2013) Effects of acute intravenous infusion of apelin on left ventricular function in dogs with advanced heart failure. *Journal of cardiac failure* 19 (7):509-516. doi:10.1016/j.cardfail.2013.05.004
79. Dai T, Ramirez-Correa G, Gao WD (2006) Apelin increases contractility in failing cardiac muscle. *European journal of pharmacology* 553 (1-3):222-228. doi:10.1016/j.ejphar.2006.09.034
80. Mathar I, Kecskes M, Van der Mieren G, Jacobs G, Camacho Londono JE, Uhl S, Flockerzi V, Voets T, Freichel M, Nilius B, Herijgers P, Vennekens R (2014) Increased beta-adrenergic inotropy in ventricular myocardium from *Trpm4*^{-/-} mice. *Circulation research* 114 (2):283-294. doi:10.1161/CIRCRESAHA.114.302835
81. Bers DM (2002) Cardiac excitation-contraction coupling. *Nature* 415 (6868):198-205. doi:10.1038/415198a
82. Katz AM, Lorell BH (2000) Regulation of cardiac contraction and relaxation. *Circulation* 102 (20 Suppl 4):IV69-74
83. Dorn GW, 2nd (2010) Adrenergic signaling polymorphisms and their impact on cardiovascular disease. *Physiological reviews* 90 (3):1013-1062. doi:10.1152/physrev.00001.2010
84. Wang C, Du JF, Wu F, Wang HC (2008) Apelin decreases the SR Ca²⁺ content but enhances the amplitude of [Ca²⁺]_i transient and contractions during twitches in isolated rat cardiac myocytes. *American journal of physiology Heart and circulatory physiology* 294 (6):H2540-2546. doi:10.1152/ajpheart.00046.2008
85. Karmazyn M, Gan XT, Humphreys RA, Yoshida H, Kusumoto K (1999) The myocardial Na⁽⁺⁾-H⁽⁺⁾ exchange: structure, regulation, and its role in heart disease. *Circulation research* 85 (9):777-786
86. Kojda G, Kottenberg K, Nix P, Schluter KD, Piper HM, Noack E (1996) Low increase in cGMP induced by organic nitrates and nitrovasodilators improves contractile response of rat ventricular myocytes. *Circulation research* 78 (1):91-101
87. Rastaldo R, Pagliaro P, Cappello S, Penna C, Mancardi D, Westerhof N, Losano G (2007) Nitric oxide and cardiac function. *Life sciences* 81 (10):779-793. doi:10.1016/j.lfs.2007.07.019
88. Jia YX, Pan CS, Zhang J, Geng B, Zhao J, Gerns H, Yang J, Chang JK, Tang CS, Qi YF (2006) Apelin protects myocardial injury induced by isoproterenol in rats. *Regulatory peptides* 133 (1-3):147-154. doi:10.1016/j.regpep.2005.09.033
89. Tendera M (2004) The epidemiology of heart failure. *Journal of the renin-angiotensin-aldosterone system : JRAAS* 5 Suppl 1:S2-6. doi:10.3317/jraas.2004.020
90. Piper HM, Abdallah Y, Schafer C (2004) The first minutes of reperfusion: a window of opportunity for cardioprotection. *Cardiovascular research* 61 (3):365-371. doi:10.1016/j.cardiores.2003.12.012
91. Zweier J, Rayburn B, Flaherty J, Weisfeldt M (1987) Recombinant superoxide dismutase reduces oxygen free radical concentrations in reperfused myocardium. *The Journal of clinical investigation* 80:1728-1734
92. Tao J, Zhu W, Li Y, Xin P, Li J, Liu M, Li J, Redington AN, Wei M (2011) Apelin-13 protects the heart against ischemia-reperfusion injury through inhibition of ER-dependent apoptotic pathways in a time-dependent fashion. *American journal of physiology Heart and circulatory physiology* 301 (4):H1471-1486. doi:10.1152/ajpheart.00097.2011
93. Kleinz MJ, Baxter GF (2008) Apelin reduces myocardial reperfusion injury independently of PI3K/Akt and P70S6 kinase. *Regulatory peptides* 146 (1-3):271-277. doi:10.1016/j.regpep.2007.10.002
94. Pisarenko OI, Lankin VZ, Konovalova GG, Serebryakova LI, Shulzhenko VS, Timoshin AA, Tskitishvili OV, Pelogeykina YA, Studneva IM (2014) Apelin-12 and its structural analog enhance antioxidant defense in experimental myocardial ischemia and reperfusion. *Molecular and cellular biochemistry* 391 (1-2):241-250. doi:10.1007/s11010-014-2008-4
95. Zeng XJ, Zhang LK, Wang HX, Lu LQ, Ma LQ, Tang CS (2009) Apelin protects heart against ischemia/reperfusion injury in rat. *Peptides* 30 (6):1144-1152. doi:10.1016/j.peptides.2009.02.010

96. Smith CC, Mocanu MM, Bowen J, Wynne AM, Simpkin JC, Dixon RA, Cooper MB, Yellon DM (2007) Temporal changes in myocardial salvage kinases during reperfusion following ischemia: studies involving the cardioprotective adipocytokine apelin. *Cardiovascular drugs and therapy / sponsored by the International Society of Cardiovascular Pharmacotherapy* 21 (6):409-414. doi:10.1007/s10557-007-6054-y
97. Hausenloy DJ, Yellon DM (2007) Preconditioning and postconditioning: united at reperfusion. *Pharmacology & therapeutics* 116 (2):173-191. doi:10.1016/j.pharmthera.2007.06.005
98. Hamada H, Suzuki M, Yuasa S, Mimura N, Shinozuka N, Takada Y, Suzuki M, Nishino T, Nakaya H, Koseki H, Aoe T (2004) Dilated cardiomyopathy caused by aberrant endoplasmic reticulum quality control in mutant KDEL receptor transgenic mice. *Molecular and cellular biology* 24 (18):8007-8017. doi:10.1128/MCB.24.18.8007-8017.2004
99. Tang SY, Xie H, Yuan LQ, Luo XH, Huang J, Cui RR, Zhou HD, Wu XP, Liao EY (2007) Apelin stimulates proliferation and suppresses apoptosis of mouse osteoblastic cell line MC3T3-E1 via JNK and PI3-K/Akt signaling pathways. *Peptides* 28 (3):708-718. doi:10.1016/j.peptides.2006.10.005
100. Andersen CU, Hilberg O, Mellekjaer S, Nielsen-Kudsk JE, Simonsen U (2011) Apelin and pulmonary hypertension. *Pulmonary circulation* 1 (3):334-346. doi:10.4103/2045-8932.87299
101. Alastalo TP, Li M, Perez Vde J, Pham D, Sawada H, Wang JK, Koskenvuo M, Wang L, Freeman BA, Chang HY, Rabinovitch M (2011) Disruption of PPARgamma/beta-catenin-mediated regulation of apelin impairs BMP-induced mouse and human pulmonary arterial EC survival. *The Journal of clinical investigation* 121 (9):3735-3746. doi:10.1172/JCI43382
102. Cui RR, Mao DA, Yi L, Wang C, Zhang XX, Xie H, Wu XP, Liao XB, Zhou H, Meng JC, Yuan LQ, Liao EY (2010) Apelin suppresses apoptosis of human vascular smooth muscle cells via APJ/PI3-K/Akt signaling pathways. *Amino acids* 39 (5):1193-1200. doi:10.1007/s00726-010-0555-x
103. Zeng X, Yu SP, Taylor T, Ogle M, Wei L (2012) Protective effect of apelin on cultured rat bone marrow mesenchymal stem cells against apoptosis. *Stem cell research* 8 (3):357-367. doi:10.1016/j.scr.2011.12.004
104. Khaksari M, Aboutaleb N, Nasirinezhad F, Vakili A, Madjd Z (2012) Apelin-13 protects the brain against ischemic reperfusion injury and cerebral edema in a transient model of focal cerebral ischemia. *Journal of molecular neuroscience : MN* 48 (1):201-208. doi:10.1007/s12031-012-9808-3
105. Yang Y, Zhang X, Cui H, Zhang C, Zhu C, Li L (2014) Apelin-13 protects the brain against ischemia/reperfusion injury through activating PI3K/Akt and ERK1/2 signaling pathways. *Neuroscience letters* 568:44-49. doi:10.1016/j.neulet.2014.03.037
106. Gu Q, Zhai L, Feng X, Chen J, Miao Z, Ren L, Qian X, Yu J, Li Y, Xu X, Liu CF (2013) Apelin-36, a potent peptide, protects against ischemic brain injury by activating the PI3K/Akt pathway. *Neurochemistry international* 63 (6):535-540. doi:10.1016/j.neuint.2013.09.017
107. Antushevich H, Pawlina B, Kapica M, Krawczynska A, Herman AP, Kuwahara A, Kato I, Zabielski R (2013) Influence of fundectomy and intraperitoneal or intragastric administration of apelin on apoptosis, mitosis, and DNA repair enzyme OGG1,2 expression in adult rats gastrointestinal tract and pancreas. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society* 64 (4):423-428
108. Xie H, Yuan LQ, Luo XH, Huang J, Cui RR, Guo LJ, Zhou HD, Wu XP, Liao EY (2007) Apelin suppresses apoptosis of human osteoblasts. *Apoptosis : an international journal on programmed cell death* 12 (1):247-254. doi:10.1007/s10495-006-0489-7
109. Zhang Z, Yu B, Tao GZ (2009) Apelin protects against cardiomyocyte apoptosis induced by glucose deprivation. *Chinese medical journal* 122 (19):2360-2365
110. Pchejetski D, Foussal C, Alfarano C, Lairez O, Calise D, Guilbeau-Frugier C, Schaak S, Seguelas MH, Wanecq E, Valet P, Parini A, Kunduzova O (2012) Apelin prevents cardiac fibroblast activation and collagen production through inhibition of sphingosine kinase 1. *European heart journal* 33 (18):2360-2369. doi:10.1093/eurheartj/ehr389
111. Koguchi W, Kobayashi N, Takeshima H, Ishikawa M, Sugiyama F, Ishimitsu T (2012) Cardioprotective effect of apelin-13 on cardiac performance and remodeling in end-stage heart failure. *Circulation journal : official journal of the Japanese Circulation Society* 76 (1):137-144

112. Siwik DA, Chang DL, Colucci WS (2000) Interleukin-1beta and tumor necrosis factor-alpha decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro. *Circulation research* 86 (12):1259-1265
113. Kim S, Iwao H (2000) Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. *Pharmacological reviews* 52 (1):11-34
114. Sopel MJ, Rosin NL, Lee TD, Legare JF (2011) Myocardial fibrosis in response to Angiotensin II is preceded by the recruitment of mesenchymal progenitor cells. *Laboratory investigation; a journal of technical methods and pathology* 91 (4):565-578. doi:10.1038/labinvest.2010.190
115. Gordon JW, Shaw JA, Kirshenbaum LA (2011) Multiple facets of NF-kappaB in the heart: to be or not to NF-kappaB. *Circulation research* 108 (9):1122-1132. doi:10.1161/CIRCRESAHA.110.226928
116. Barnes GD, Alam S, Carter G, Pedersen CM, Lee KM, Hubbard TJ, Veitch S, Jeong H, White A, Cruden NL, Huson L, Japp AG, Newby DE (2013) Sustained cardiovascular actions of APJ agonism during renin-angiotensin system activation and in patients with heart failure. *Circulation Heart failure* 6 (3):482-491. doi:10.1161/CIRCHEARTFAILURE.111.000077
117. Sorli SC, Le Gonidec S, Knibiehler B, Audigier Y (2007) Apelin is a potent activator of tumour neoangiogenesis. *Oncogene* 26 (55):7692-7699. doi:10.1038/sj.onc.1210573
118. Yang L, Su T, Lv D, Xie F, Liu W, Cao J, Sheikh IA, Qin X, Li L, Chen L (2014) ERK1/2 mediates lung adenocarcinoma cell proliferation and autophagy induced by apelin-13. *Acta biochimica et biophysica Sinica* 46 (2):100-111. doi:10.1093/abbs/gmt140

Figure captions

Fig 1 Antagonism between Apelin/APJ system and renin-angiotensin system. ACE angiotensin converting enzyme; ACE 2 angiotensin converting enzyme 2; Ang Angiotensin

Fig 2 Apelin-induced increased contractility via calcium-dependent mechanism. PLC phospholipase C; PIP₂ phosphoinositol biphosphate; DAG diacylglycerol; IP₃ inositol triphosphate; PKC protein kinase C; NHE sodium-hydrogen exchanger; NCE sodium-calcium exchanger; IP₃R inositol triphosphate receptor; RyR Ryanodine receptor; SR sarcoplasmic reticulum

Fig 3 Apelin-induced increased contractility via calcium-independent mechanisms. PLC phospholipase C; PIP₂ phosphoinositol biphosphate; DAG diacylglycerol; IP₃ inositol triphosphate; PKC protein kinase C; NHE sodium-hydrogen exchanger; MEK 1/2 mitogen-activated protein kinase kinase; ERK 1/2 extracellular signal-regulated kinases 1/2; MLCK myosin light chain kinase; RMLC regulatory myosin light chain;





