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The apelin–APJ system in heart failure Pathophysiologic relevance and therapeutic potential

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ABSTRACT

Apelin is the endogenous ligand for the previously orphaned G protein-coupled receptor, APJ. This novel peptidic signalling pathway is widely represented in the heart and vasculature, and is emerging as an important regulator of cardiovascular homeostasis. In preclinical models, apelin causes nitric oxide-dependent vasodilatation, reduces ventricular preload and afterload, and increases cardiac contractility in rats with normal and failing hearts. Apelin–APJ signalling also attenuates ischemic myocardial injury and maintains cardiac performance in ageing and chronic pressure overload. Downregulation of apelin and APJ expression coincides with declining cardiac performance raising the possibility that diminished apelin–APJ activity may have pathophysiologic implications. At present, data from human studies is limited but changes in apelin and APJ expression in patients with chronic heart failure parallel those seen in preclinical models. Detailed clinical investigation is now required to establish the role of apelin in human cardiovascular physiology and pathophysiology, and to determine the therapeutic potential of augmenting apelin signalling in patients with heart failure.

1. Introduction

Heart failure constitutes a major and growing health burden in developed nations. Despite considerable treatment advances over the past two decades, it has a prognosis worse than that of many cancers and results in severe morbidity with impaired quality of life and recurrent hospitalisation [1,2]. The development of novel treatments for patients with heart failure therefore remains a major priority. G protein-coupled receptors (GPCRs) play an essential role in the physiological control of the cardiovascular system and represent a major target for existing pharmacological treatments [3,4]. Many of the recent pharmacological advances in the treatment of heart failure, including angiotensin II type 1 (AT1) and beta-adrenergic receptor blockers, have arisen through the specific targeting of GPCR systems, and have provided additive incremental morbidity and mortality benefits [5,6].

In 1993 a novel GPCR called APJ was identified through the Human Genome Project [7]. Despite sharing significant sequence homology with AT1, APJ did not display specific binding for angiotensin II and remained orphaned until 1998 when its endogenous ligand was identified from bovine stomach extracts and named apelin (APJ endogenous ligand) [8]. Since its discovery, the apelin–APJ system has emerged as an important regulator of cardiovascular homeostasis that may play a role in the pathophysiology of heart failure and represents an exciting target for the development of new therapies [9–11].

In this article we will review the biology of the apelin–APJ system and its role in cardiovascular homeostasis. We will then discuss the evidence for altered apelin–APJ regulation in the setting of heart failure and consider how attenuated apelin signalling may contribute to the pathophysiology of this condition. Finally we will explore the therapeutic potential of

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targeting the apelin–APJ system in heart failure and, in particular, the rationale for augmenting apelin–APJ activity as a means of preserving and restoring cardiac performance.

2. The apelin–APJ system

2.1. Apelin

The apelin gene, located on the long arm of the human X chromosome, encodes a 77 amino acid preproprotein that is then cleaved to shorter active peptides (Fig. 1) [8,12,13]. The full-length mature peptide comprises 36 amino acids (apelin-36) and was originally isolated from bovine stomach extracts. Gel filtration chromatography of bovine colostrum confirmed the presence of apelin-36 and revealed a second peak of activity corresponding to a 13 amino acid peptide (apelin-13), which has subsequently been identified in several other tissues. The 13 amino acid peptide possesses a pyroglutamate substitution at the N-terminus; a common post-translational modification that preserves biological activity by rendering the peptide more resistant to enzymatic cleavage. Although not yet identified *in vivo*, the existence of other endogenous apelin isoforms is predicted by several potential proteolytic cleavage sites on apelin-36. Accordingly, synthetic C-terminal fragments of apelin-36 including apelin-19, apelin-17, apelin-16 and apelin-12 also activate the APJ receptor [8,13–18] though fragments shorter than 12 amino acids are biologically inert. The shorter apelin isoforms exhibit greater binding affinity and biological potency than the full-length peptide, the most potent being the pyroglutamated form of apelin-13 that may represent the principally active biological ligand [13,15,16].

Expression of the apelin gene is increased in response to hypoxia under the regulation of hypoxia-inducible factor-1 [19]. In breast tissue there is up-regulation of apelin synthesis during lactation that is mediated by upstream stimulatory factor-1 [20], a fairly ubiquitous transcription factor involved primarily in energy metabolism and cellular proliferation [21]. In adipocytes, apelin gene expression is inhibited in the fasting state and stimulated by refeeding possibly through changes in the plasma concentrations of insulin and counter-regulatory hormones [22,23]. Finally, in magnocellular neurons of the hypothalamus, apelin is upregulated by dehydration, through a mechanism that may involve arginine vasopressin [24].

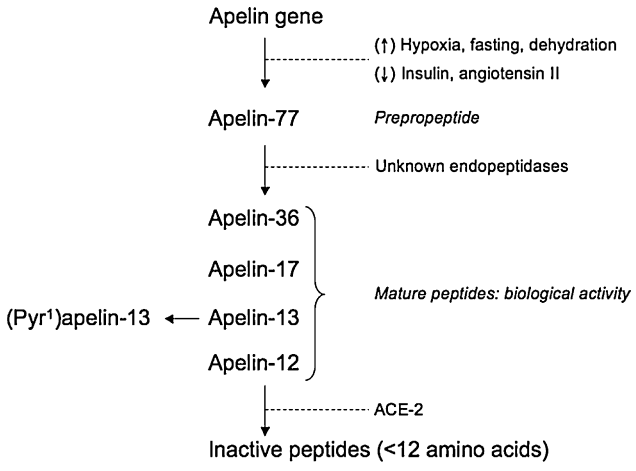

Fig. 1 – Apelin synthesis, post-translational processing and metabolism.

Less is known about the mechanisms regulating the post-translational processing of apelin including the proteolytic cleavage of the longer apelin isoforms to shorter C-terminal fragments and the pyroglutamine modification of the apelin-13. One enzyme implicated in the processing of apelin peptides is angiotensin-converting enzyme (ACE) type 2 [25], a carboxypeptidase that negatively regulates the renin–angiotensin–aldosterone system (RAAS) by cleaving angiotensin II to the biologically inactive peptide angiotensin 1–9 or angiotensin 1–7 [26]. ACE-2 has been reported to hydrolyse both apelin-13 and apelin-36 with high catalytic efficiency [25]. To our knowledge this is the only degradation pathway for apelin yet described, although its physiological significance remains unclear [11].

2.2. The APJ receptor

The human APJ gene is located on the long arm of chromosome 11 and encodes a 377 amino acid G protein-coupled receptor with seven transmembrane-spanning domains [7] for which apelin is the only known ligand. The transcriptional regulation of the APJ gene appears to be complex and, at the time of writing, remains poorly understood. Physiological stimuli for APJ synthesis include acute and chronic stress, salt loading and water deprivation. At the molecular level, a TATA-less promoter region within the gene has recently been identified [27]. The transcriptional factor, Sp1, which initiates transcription of several genes whose promoters lack a TATA box [28], also plays a major role in activation of the APJ promoter. Other factors that contribute to promoter activity include CCAAT/enhancer binding protein, estrogen and glucocorticoid protein complexes [27].

3. Biology of the apelin–APJ system

3.1. Anatomy: tissue localisation of APJ and apelin

The apelin–APJ system has wide representation in the central nervous system and a variety of peripheral tissues (Fig. 2; for review see ref. [9]). In some tissues, such as lung, kidney and adrenal gland, APJ expression may be restricted to the vasculature, though out with the cardiovascular system, APJ receptors have been detected in neurons of the cerebral cortex, hippocampus and hypothalamus, pituitary gland cells, enterochromaffin-like gastric cells, pancreatic islet cells, osteoblasts and T-lymphocytes. The expression pattern of apelin is closely related to that of APJ with co-localisation of the receptor and ligand in many tissues, suggesting a possible autocrine or paracrine signalling pathway. However, apelin is also expressed in cell types lacking the APJ receptor such as adipocytes and has been detected in plasma at levels consistent with a circulating hormone.

Within the human vasculature, both APJ receptor-like immunoreactivity (APJ-LI) and apelin-like immunoreactivity (apelin-LI) are detectable in endothelial cells and vascular smooth muscle cells of human large conduit vessels including

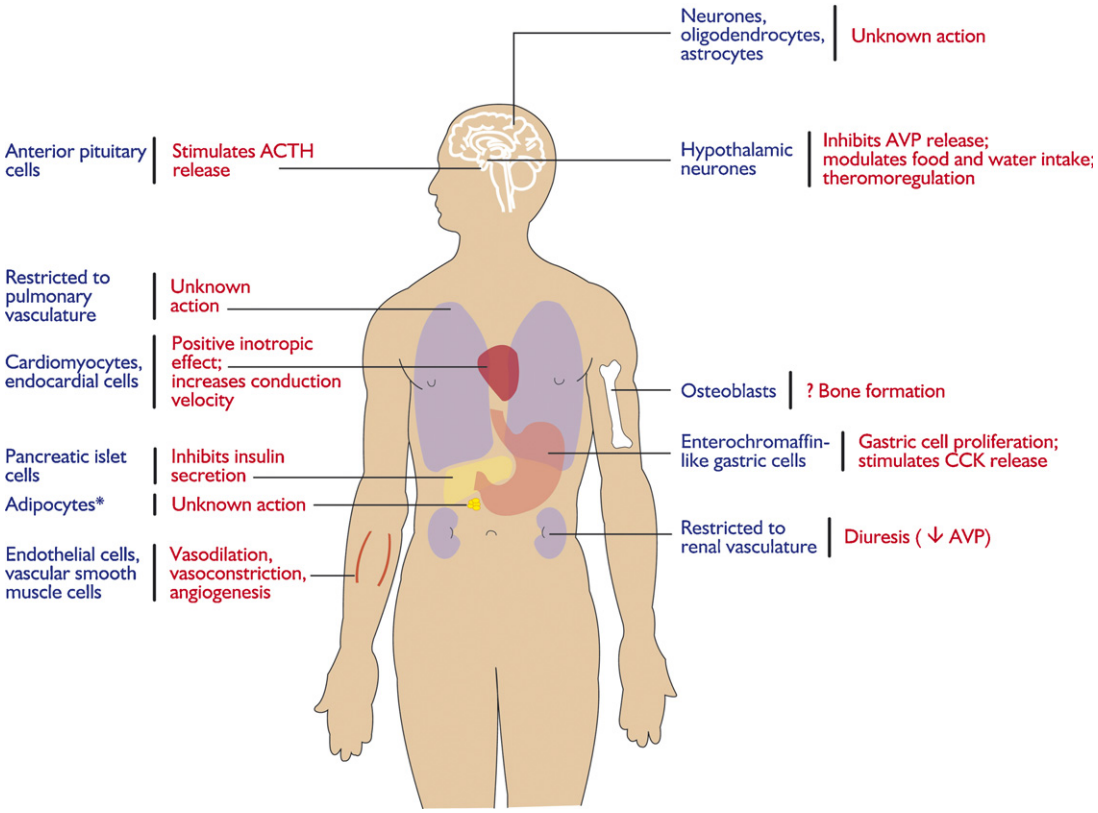

Fig. 2 – Expression and physiological functions of the apelin–APJ system (most physiological effects not yet documented in humans). ACTH, adrenocorticotrophic hormone; AVP, arginine vasopressin; CCK, cholecystokinin. * Site of apelin but not APJ synthesis.

radial artery, left internal mammary artery, coronary artery and saphenous vein, as well as renal, adrenal and pulmonary vessels [29,30]. Intracellular localisation of apelin, in the endoplasmic reticulum, golgi apparatus and secretory vesicles suggests the peptide is synthesised locally in endothelium [30]. Within the human heart, APJ-LI is present in endocardial endothelial cells and, to a lesser extent, in cardiomyocytes [30]. In contrast, apelin-LI expression is 200-fold higher in atrial tissue than ventricular tissue and correlates well with plasma apelin concentrations suggesting it may be the major source of circulating apelin [31]. Staining for apelin-LI is negligible in cardiomyocytes in normal myocardium, but detectable in failing hearts.

3.2. *Biochemistry: intracellular signalling mechanisms*

Apelin causes a concentration-dependent inhibition of forskolin-stimulated production of cyclic AMP in Chinese hamster ovary cells, stably transfected with the APJ receptor suggesting that APJ couples to inhibitory G proteins (G_i) [8]. Apelin peptides also induce Ras-independent activation of extracellular-regulated kinases (ERKs) via protein kinase C [32] as well as activation of p70S6 kinase (an important regulator of translation and cell cycle progression) through ERK- and Akt-dependent phosphorylation pathways [33]. These signalling cascades are inhibited by pertussis toxin implying that they too are mediated by coupling of APJ to G_i. On the other hand, the inotropic effect of apelin (see Section 3.3.3) is only partially suppressed by pertussis toxin [18] and appears to involve activation of phospholipase C and protein kinase C [18], a pathway characteristically activated by G_q proteins [34]. This raises the possibility that the APJ receptor may couple to G_q proteins in addition to G_i proteins. One well-established effect of phospholipase C activation is to increase intracellular production of inositol-1,4,5-trisphosphate (IP3) through hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) [34]. This pathway may account for the observed increase in intracellular Ca^{2+} concentrations in human Nt2.N neurons on exposure to apelin [35]. Of note, several apelin-mediated signalling cascades have been shown to exhibit desensitisation [36] which probably occurs due to internalisation of the APJ receptor following activation [37]. Interestingly differently sized apelin fragments induce a variable duration of receptor internalization that correlates with a differential pattern of desensitisation [36,37]. Finally, the APJ receptor also exhibits nuclear localization [38] suggesting the possibility that its effects may extend beyond the activation of intracellular cascades to transcriptional regulation, similar to other GPCRs such as AT1 [39].

3.3. *Physiology: cardiovascular effects of apelin*

In keeping with its widespread pattern of expression, the apelin–APJ pathway has been implicated in a variety of

physiological functions including glucose metabolism, thermoregulation and fluid balance (Fig. 2; for review see ref. [10]). However, the clearest evidence to date for a physiological role is in the regulation of cardiovascular homeostasis.

3.3.1. *Vascular effects of apelin*

Apelin was originally shown to cause a rapid and transient fall in mean arterial pressure following bolus injection in rats [40]. This finding has been replicated in numerous subsequent studies [14,16,17,41–44] but is absent in mice with a targeted APJ gene knockout confirming that the response is mediated via the APJ receptor [43]. The depressor response is also abolished by co-administration of L-NAME, a nitric oxide synthase inhibitor, suggesting it occurs through nitric oxide-mediated arterial vasodilatation [16]. In keeping with this, apelin can activate endothelial nitric oxide synthase (eNOS) in cultured human umbilical vein endothelial cells [43], possibly via Akt phosphorylation, and stimulates nitric oxide production in rat aorta [45].

Bolus intravenous apelin injection reduces mean circulatory filling pressure [41], an accurate reflection of systemic venous tone [46] implying that apelin can function as both an arterial and venous dilator. Indeed the concurrent reduction in both mean arterial pressure and mean circulatory filling pressure is remarkable as most depressor agents actually increase mean circulatory filling pressure via reflex sympathetic activation and suggests apelin is a more efficacious venodilator than either nitrates or hydralazine. These changes in vascular tone are matched by corresponding alterations in ventricular loading conditions: acute apelin administration reduces left ventricular end-diastolic area and left ventricular end-systolic pressure [47]. Although mice deficient in the APJ gene have normal basal blood pressure, they exhibit an exaggerated pressor response to angiotensin II, and those with a deletion of both the AT1 receptor and APJ receptor have less of a reduction in baseline blood pressure than isolated AT1 receptor deficient mice suggesting a contribution to basal vascular dilator tone [43]. However, the vascular actions of apelin may be more complex, extending beyond activation of eNOS. Consistent with the presence of APJ receptors on vascular smooth muscle, apelin causes constriction *ex vivo* in isolated segments of human saphenous vein denuded of endothelium [48]. Apelin also stimulates myosin-light chain phosphorylation in rat and mouse vascular smooth muscle [49], a key step in contraction. Collectively these data suggest that apelin can act directly on APJ receptors within vascular smooth muscle to induce contraction but that in the presence of functioning endothelium, this effect is outweighed by stimulation of local nitric oxide production via endothelial APJ receptors (Fig. 3).

Paralleling the vascular effects in rodents, apelin mediates constriction in *ex vivo* human saphenous vein segments stripped of endothelium [48], but causes vasorelaxation in *ex vivo* human mesenteric arteries with intact endothelium. The latter effect is attenuated by nitric oxide synthase but not cyclooxygenase inhibition [50]. At the time of writing there are no reports of the *in vivo* effects of apelin in man. However, the only *in vivo* study in large mammals to date has raised the possibility that cardiovascular responses to apelin may exhibit interspecies differences. Acute bolus administration of apelin in an ovine model at equivalent doses to rodent studies elicited a biphasic haemodynamic response with an initial transient reduction in arterial pressure and rise in heart rate followed rapidly by an increase in blood pressure and concomitant fall in heart rate [51]. Cardiac output was not measured early in this study but fell during the hypertensive phase, parallelling the reduction in heart rate and coinciding with rises in peripheral vascular resistance and right atrial pressure. Importantly, four out of ten sheep in the study exhibited electrocardiographic abnormalities following

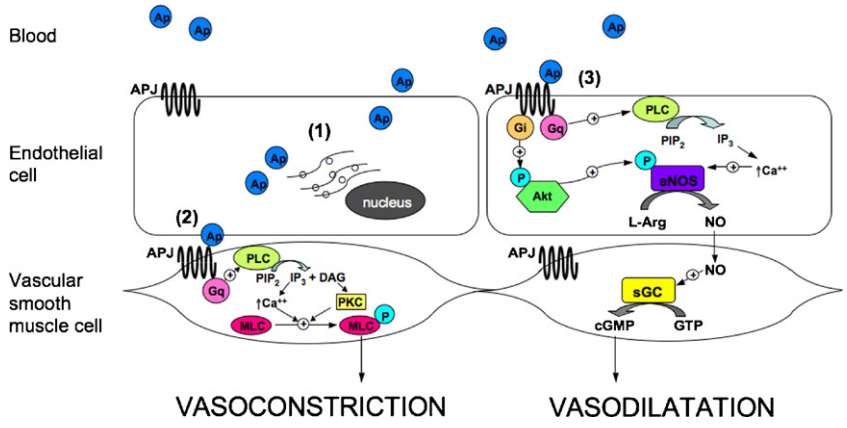

Fig. 3 – Proposed model of vascular effects of apelin–APJ system. (1) Apelin is synthesised locally in endothelial cells then transported to luminal and basolateral cell membranes. (2) Apelin peptides directly activate APJ receptors on vascular smooth muscle cells leading to vasoconstriction, possibly through coupling to a Gq protein. (3) Locally released and circulating apelin molecules activate endothelial APJ receptors generating NO release; NO diffuses into smooth muscle cells producing vasodilatation. In the presence of intact endothelium the net effect is vasodilatation. Ap, apelin; P, phosphate; Gi, inhibitory G protein; Gq, G_q protein; PLC, phospholipase C; PKC, protein kinase C; IP3, inositol-3,4,5-trisphosphate; DAG, diacylglycerol; NO, nitric oxide; sGC, soluble guanylate cyclase; eNOS, endothelial nitric oxide synthase; PIP2, phosphatidylinositol bisphosphate; MLC, myosin light chain; L-Arg, L-Arginine; cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate.

administration of high dose apelin including varying degrees of atrioventricular block. One sheep also developed ST elevation in the context of profound hypotension.

3.3.2. *Cardiac effects of apelin*

In keeping with the localization of APJ receptors within the heart, apelin exhibits direct myocardial effects. In isolated rat hearts, apelin increases contractility at sub-nanomolar concentrations and augments the preload-induced increase in dP/dt_{max} [18]. Apelin also induces sacromere shortening in individual cardiomyocytes obtained from both normal and failing myocardium [52], and increases contractility in isolated right ventricular trabeculae from failing rat hearts [53]. Although these studies all support a positive inotropic role for apelin, there are discrepancies in the reported findings. While the reported EC_{50} value for apelin in normal intact rat hearts was 33 pM, making apelin the most potent inotrope yet studied, concentrations more than 1000-fold higher than this failed to elicit an increase in contractility in normal rat trabeculae, and produced only a very modest response in failing trabeculae. In addition the slow-onset sustained increase in contractility over a period of 30 min observed in the intact rat heart model, contrasts sharply with the transient response of only 1–2 min seen in single cardiomyocytes. Such discrepancies may be attributable to the different methodologies employed or regional variation in the density of APJ receptors within the heart. In isolated cardiomyocytes, the absence of mechanical load may have contributed to a more limited response, especially as apelin only increased preload-induced dP/dt_{max} in intact hearts, at higher loading conditions. Given the preferential localization of apelin and APJ receptors in endocardial cells over cardiomyocytes, it is also tempting to speculate that signal transduction from activated endocardial APJ receptors might play a predominant role in mediating the inotropic effects of apelin. If so, preservation of this signalling pathway in intact hearts might account for the greater responses to apelin seen in this model.

The inotropic effects of the apelin–APJ system in rodents extend to the *in vivo* setting where acute apelin infusion increases dP/dt_{max} and cardiac output [44,54,55] as well as load-independent measures of myocardial contractility such as ventricular elastance and preload recruitable stroke work [47]. Significantly, chronic administration also leads to an increase in cardiac output without inducing left ventricular hypertrophy (LVH) [47].

3.3.3. *Mechanisms of apelin-mediated inotropy*

The mechanisms by which apelin exerts its inotropic effects have been only partially elucidated and remain the subject of debate. The effects are independent of angiotensin II, endothelin-1, catecholamines and nitric oxide release [18] but appear to involve activation of the sacrolemmal Na^+/H^+ exchanger (NHE), probably through phospholipase C and protein kinase C-dependent pathways (Fig. 4) [18,52]. In single cardiomyocytes, NHE activity increases following exposure to apelin while, in intact rat hearts, the inotropic response to apelin is markedly attenuated by a specific inhibitor of NHE. Stimulation of NHE can lead to intracellular alkalinization and sensitization of cardiac myofilaments to intracellular Ca^{2+} [56]. In keeping with this, the increased NHE activity is accompanied by an increase in intracellular pH [52]. Moreover, cytoplasmic Ca^{2+} transients are unchanged and perforated patch clamp recordings show that apelin does not alter voltage-gated Ca^{2+} channels in cardiomyocytes. These data suggest that apelin may increase myocardial contractility by enhancing the sensitivity of myofilaments to activator Ca^{2+} rather than increasing intracellular Ca^{2+} concentrations. However, activation of NHE can also indirectly increase intracellular Ca^{2+} as the resulting accumulation of Na^+ within cells stimulates the reverse mode Na^+/Ca^{2+} exchanger (NCX) [57]. In intact rat hearts, inhibition of NCX also suppresses the apelin-induced inotropic response indicating that this mechanism may contribute to apelin-mediated inotropic activity. Furthermore, in failing rat trabeculae, apelin failed to alter steady-state force–$[Ca^{2+}]$i relations but increased the amplitude of the intracellular Ca^{2+} transient [53]. Thus the inotropic effects of apelin may involve increased intracellular Ca^{2+} availability in addition to enhanced myofilament responsiveness to Ca^{2+} ions.

4. The apelin–APJ system in heart failure

4.1. *Altered patterns of expression and synthesis*

4.1.1. *Animal models of heart failure*

Expression of apelin and APJ is increased or maintained in animals with left ventricular hypertrophy and compensated heart failure but downregulated in those with severe, decompensated heart failure. This downregulation may be related to increased activity of the renin–angiotensin–aldosterone system (RAAS). In one heart failure model, several pharmacological treatments retarded the progression to CHF, but only AT1 receptor antagonism prevented the downregulation of apelin–APJ expression. Furthermore, infusion of angiotensin II over 24 h, even at sub-pressor doses, reduced both apelin and APJ expression, and this effect was also

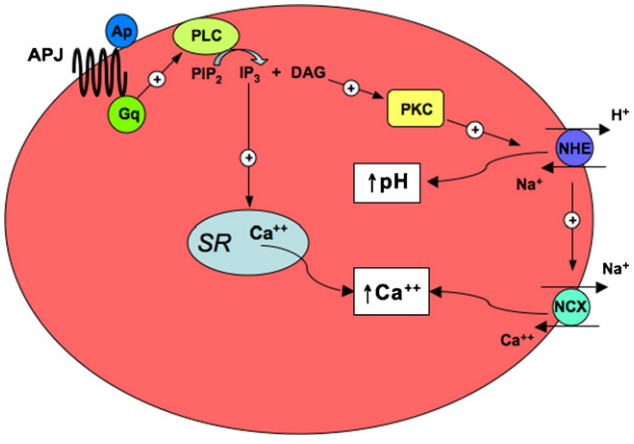

Fig. 4 – Possible intracellular mechanisms of apelin-mediated positive inotropic effects. Ap, apelin; Gq, G_q protein; PLC, phospholipase C; SR, sacroplasmic reticulum; NHE, Na^+/H^+ exchanger; NCX, reverse Na^+/Ca^{2+} exchanger; APJ, APJ receptor; PKC, protein kinase C; PIP2, phosphatidylinositol bisphosphate; IP3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol.

abolished by concurrent AT1 receptor blockade. Cardiac dilatation in advanced heart failure may contribute to downregulation of the apelin–APJ system since cardiomyocytes subjected to mechanical stretch *in vitro* exhibit markedly reduced expression of both apelin and the APJ receptor [18].

Regulation of the apelin–APJ pathway is altered by acute ischemic injury. Apelin gene expression and secretion in isolated cardiomyocytes is increased by acute hypoxia through the hypoxia inducible factor pathway [19]. Accordingly, apelin expression is upregulated *in vivo* within 24 h of myocardial infarction. Endogenous cardiac apelin and APJ are increased in rats with ischemic heart failure 6 weeks post-myocardial infarction [55]. It is not clear whether the stimulus for this upregulation is ischemia or the early onset of heart failure. In contrast, both apelin and APJ expression fall in a further rodent model of ischemic myocardial injury caused by repeated isoprotenerol administration [44]. However, it must be noted that this model produced extensive myocardial injury and very severe heart failure associated with hypotension and grossly elevated left ventricular end-diastolic pressure (LVEDP). Interestingly, while cardiac APJ mRNA levels were markedly downregulated in these rats, both tissue levels and overall apelin-binding capacity of APJ within the heart were increased. This might reflect either more efficient post-transcriptional processing of APJ or diminished breakdown of existing APJ receptors, with or without a contribution from enhanced receptor recycling.

4.1.2. *Patients with chronic heart failure*

In keeping with the findings from preclinical models, apelin–APJ expression is altered in patients with chronic heart failure (CHF). Initial reports suggested that plasma apelin concentrations were mildly elevated in the early stages of heart failure but fell with more advanced disease [31,58]. In support of this, there are now several reports of depressed plasma apelin concentrations in patients with advanced CHF [31,59–61]. Furthermore, in patients with severe CHF, the improvement in New York Heart Association (NYHA) symptoms class and left ventricular ejection fraction seen following cardiac resynchronisation therapy is paralleled by restoration of normal plasma apelin concentration [61]. Although some recent studies have produced conflicting findings, careful scrutiny suggests that apparent inconsistencies may be largely attributable to differences in study populations (Table 1). For example, Chong et al. reported that plasma apelin concentrations were lower in patients with CHF than in normal controls irrespective of NYHA symptom class or left ventricular ejection fraction [59]. However, these investigators studied a cohort of patients with disproportionately severe heart failure: 73% of patients were NYHA class III or IV and only 3% were NYHA class I. Moreover mean ejection fraction was 15% and, even in NYHA class II patients, it was a mere 18%. In contrast two subsequent studies have since claimed that plasma apelin concentrations are unaltered in patients with CHF compared with age-matched controls [62,63]. However, in both of these studies, more than 95% of patients were in NYHA functional class I or II, and the average left ventricular ejection fraction was 40 and 42%, respectively. Therefore, taken together, current data strongly suggest that apelin expression is at least maintained and possibly augmented in mild, compensated heart failure but declines with advancing disease.

Two studies to date have attempted to characterise alterations in cardiac expression of apelin and APJ in patients with heart failure. APJ mRNA levels within the left ventricle are reduced by 44% in patients with advanced heart failure due to idiopathic dilated cardiomyopathy (IDC) but were unaltered in patients with ischemic cardiomyopathy [31]. Although apelin expression increases within the left ventricle irrespective of aetiology, mRNA levels within the atria do not rise and, in fact, protein levels fall. As the atria are likely to represent the major source of apelin production, this may account for the reduced levels within the circulation in severe heart failure. Interestingly, myocardial expression of the APJ gene and cardiac apelin tissue concentrations were markedly increased following offloading of the ventricle by implantation of a left ventricular assist device [58]. Indeed of all genes, APJ was the most consistently and significantly upregulated.

In summary, the collective data from human and animal studies suggest that apelin–APJ expression (a) is upregulated in response to hypoxia/ischemia, (b) is maintained or even augmented in conditions of chronic pressure overload and the

Table 1 – Plasma concentration of apelin in patients with CHF

Authors	NYHA class	Mean LVEF	Aetiology	Plasma apelin concentration
Chong et al. [59]	III/IV (73%) I/II (27%)	15.6%	Mixed	Decreased
Goetze et al. [60]	Not stated	20%	Mixed	Decreased
Francia et al. [61]	III/IV (100%)	25%	Mixed	Decreased
Foldes et al. [31]	III (100%)	Not stated	Coronary artery disease	Decreased
Chen et al. [58]	III/IV (51%)	>25% (50%)	Mixed	Increased in early stages; lower in severe disease
	I/II (49%)	<25% (50%)		
Miettinen et al. [62]	IV (0%) III (3%) II (48%) I (49%)	40%	Idiopathic dilated cardiomyopathy	Normal
Codognotto et al. [63]	I (100%)	42% (median)	Idiopathic dilated cardiomyopathy	Normal

early stages of heart failure, but (c) is substantially down-regulated in severe heart failure. Possible factors contributing to the decline of apelinergic expression in severe heart failure include increased RAAS activation and myocardial stretch. These changes may be reversible as cardiac apelin and APJ are upregulated after mechanical offloading of the left ventricle.

4.2. *Functional effects of apelin signalling in myocardial stress and heart failure*

The cardiovascular profile of the apelin–APJ system and its altered regulation in heart failure suggest a possible role in the pathophysiology of heart failure as well as a potential therapeutic application. In support of this, there is now increasing evidence that apelin signalling influences the progression of heart failure and improves cardiac performance in the failing heart.

4.2.1. *Endogenous apelin*

Mice with targeted knockout of the apelin gene exhibit normal cardiac development and baseline haemodynamic parameters, but develop progressive left ventricular dysfunction and dilatation from around 6 months of age [64]. Importantly, exogenous replacement of apelin in adult life prevents the decline in cardiac performance, suggesting it is attributable to contemporary loss of apelin activity and not the manifestation of a latent developmental defect. Similarly, when apelin-deficient mice are subjected to chronic pressure overload by surgical constriction of the aorta, they develop severe and progressive heart failure, in contrast to wild-type mice that exhibit only slight impairment of cardiac contractility. Interestingly, genetic analysis reveals differential gene expression within the hearts of mutant and wild-type mice during pressure overload with the former displaying marked upregulation in defined sets of genes including those involved in extracellular matrix remodelling, fibrosis and the regulation of muscle contraction. These genetic data imply an important role for endogenous apelin signalling in maintaining cardiac performance under conditions of chronic cardiac stress.

The first evidence that disturbed endogenous apelin–APJ signalling may have functional relevance in human heart failure was recently provided in a cohort of patients with dilated cardiomyopathy [65]. In these patients, possession of a polymorphism of the APJ receptor, 212A (the biological significance of which is unknown) was associated with slower progression of heart failure. Notably, no difference in the frequency of this polymorphism was noted between DCM patients and controls, suggesting that the pathophysiological significance of apelin signalling in heart failure may not relate to causation of heart failure *per se*, but rather in modulating the progression of myocardial dysfunction.

4.2.2. *Exogenous apelin*

The ability of apelin peptides to enhance contractility in healthy myocardium while simultaneously reducing loading conditions suggests potential therapeutic application in heart failure. Crucially, these effects are maintained and possibly even amplified in the failing heart. Apelin increases contractility *in vitro* to the same or even greater extent in failing myocardium as it does in normal myocardium [52,53]. *In vivo*, in rats with established heart failure, post-myocardial infarction, apelin infusion restores ejection fraction, increases cardiac output and reduces LVEDP [54,55]. Importantly, these effects occurred despite an up-regulation of endogenous apelin and APJ within the heart [55]. Furthermore, in a rat model of severe heart failure that exhibits features of cardiogenic shock, apelin improved cardiac contractility, loading conditions and blood pressure despite a reduction in myocardial APJ receptor expression of over 50% [44]. These findings suggest that the signalling capacity of cardiac APJ receptors is not exhausted even when endogenous apelin levels are increased or when APJ receptor expression is diminished.

The beneficial effects of exogenous apelin administration may extend beyond improving cardiac performance in established heart failure to affording cardioprotection during myocardial injury. In the isoprotenerol heart failure model outlined above, concurrent administration of apelin preserved cardiac function and reduced indices of myocardial injury, preventing the development of heart failure [44]. Moreover, in rat models of myocardial infarction, bolus infusion of apelin at the time of reperfusion, reduced infarct size *in vitro* and *in vivo* by 39 and 43%, respectively [66]. The reduction in infarct size was associated with activation of components of the reperfusion injury salvage kinase pathway, a key cell-signalling system in protection against ischemia-reperfusion injury [67]. In light of this, the aforementioned upregulation of apelin expression in response to ischemia may serve as an endogenous cardioprotective mechanism to limit myocardial injury during ischemia.

Thus apelin signalling protects and preserves myocardial function in the face of both acute ischemic injury and chronic stress in the form of ageing and pressure overload. Moreover, acute apelin administration improves cardiac performance in models of established heart failure irrespective of alterations in endogenous expression.

5. Clinical perspective

5.1. *A novel neurohormonal target in heart failure?*

The success of neurohormonal blockade in heart failure [68] has prompted hopes that the pharmacological manipulation of other biological molecules active in disease progression may yield further advances in treatment. A related strategy is to enhance the activity of endogenous mechanisms such as the natriuretic peptide system [69] that oppose the unfavourable haemodynamic changes and pathological cardiac remodelling characteristic of CHF. Nesiritide, an intravenous form of human B-type natriuretic peptide was recently approved by the US Food and Drug Administration (FDA) for the treatment of acutely decompensated heart failure based on its ability to reduce symptoms of dyspnoea, decrease preload and afterload, and increase cardiac output [70]. The vasodilatory, diuretic and cardioprotective effects of apelin raise the possibility that the apelin–APJ pathway might represent a novel 'compensatory' endogenous system in heart failure. Whereas the natriuretic peptides are over expressed in heart failure [69], apelin expression is reduced. Restoring or

augmenting apelinergic activity in patients with CHF might therefore offer greater potential for improving cardiac performance and retarding disease progression. It is worth noting however that despite encouraging effects on symptoms and haemodynamic indices, there is no evidence to date that treatment with nesiritide improves clinical outcomes in heart failure and concerns have been raised regarding its safety [70]. Furthermore, disappointing results from several recent clinical trials have suggested a possible ceiling of benefit from targeting neurohormonal systems in heart failure [71].

5.2. *Inotropic therapies in heart failure*

There is currently a lack of safe and effective inotropic therapies in patients with heart failure. Presently available positively inotropic treatments, such as β1 agonists and phosphodiesterase inhibitors, have adverse short and long-term outcomes that include an increase in mortality [72]. In contrast to these agents, chronic apelin administration increases cardiac output without inducing left ventricular hypertrophy. Indeed, the ability of apelin to reduce cardiac loading conditions in addition to enhancing contractility may serve to limit cardiac work and myocardial oxygen demand, thus avoiding the deleterious effects associated with established inotropic therapies. In this respect the actions of apelin closely resemble those of the novel Ca^{2+} sensitizing agent levosimendan. This compound also exerts an inotropic effect by increasing the sensitivity of contractile apparatus to Ca^{2+} while simultaneously reducing ventricular loading conditions [73]. However, it is noteworthy that, despite the theoretical advantages of levosimendan, a recent large-scale randomized trial in patients with acute decompensated heart failure failed to demonstrate any improvement in clinical outcomes with this agent compared with dobutamine [74].

6. Future directions

6.1. *Preclinical studies*

The cardiovascular effects of acute apelin administration in rodents are now relatively well characterised but the impact of chronic administration requires further attention. One of the most encouraging findings to date with apelin is the enhancement of cardiac performance with chronic administration that occurred without inducing left ventricular hypertrophy. Further studies are required to confirm this finding over longer treatment periods and to establish the effects of chronic dosing in rodent models of heart failure. The suggestion that apelin may enhance myocardial contractility by improving myofilament sensitivity to Ca^{2+} may have important implications for its therapeutic potential in heart failure but current data are conflicting. Further work is therefore required to clarify the mechanisms underlying the inotropic effects of apelin and to reconcile the striking differences in response seen in different experimental models. Greater understanding is also needed of the molecular mechanisms governing apelin and APJ gene expression as well as the processes involved in post-translational processing and metabolism of apelin peptides. Novel insights into these mechanisms might provide alternative strategies for augmenting apelin signalling such as measures to enhance endogenous apelin synthesis and secretion or preserve biological activity by inhibiting breakdown. In addition they may also provide an explanation for the apparent downregulation of apelin–APJ expression in chronic heart failure and suggest strategies to reverse this decline. Finally, the development of mice with targeted knockout of the APJ or apelin gene has already offered insights into the role of endogenous apelin signalling and provides an ideal model to determine the importance of endogenous apelin in cardioprotection during acute myocardial injury.

6.2. *Clinical studies: cardiovascular physiology*

Although the cardiovascular profile of apelin in rodents suggests potential therapeutic application, the relevance of the apelin–APJ pathway in human cardiovascular physiology and pathophysiology has yet to be established. Recent unexpected responses to apelin infusion in sheep [51] and experiences with clinical studies of other peptidic systems [75] underscore the potential for important interspecies variation. Limited data from *ex vivo* myography studies suggests that the vasomotor effects of apelin extend to human blood vessels. However, there are currently no data on the functional effects of apelin or its APJ receptor *in vivo* in man and its therapeutic potential cannot be ascertained without detailed clinical investigation.

Given the combination of vasodilatation and positive inotropy induced by apelin in animal models, initial *in vivo* studies in man will need to determine the direct actions of apelin on both the heart and vasculature through regional apelin infusions. Systemic apelin infusion will then reveal the net effect of these actions on haemodynamic variables such as heart rate, blood pressure and cardiac output. These *in vivo* investigations should be complimented by further *ex vivo* myography studies in human vessels from a range of vascular beds and *in vitro* assessment of the inotropic effects of apelin in human myocardial tissue.

6.3. *Clinical studies: cardiovascular pathophysiology*

Despite several existing reports of altered plasma apelin concentrations in patients with chronic heart failure, there is a need for further appropriately sized longitudinal studies to clarify changes in apelin expression at different stages of the disease and to determine whether a decline in levels accompanies or even predicts cardiac decompensation. Such studies would determine the utility of apelin as a potential novel biomarker in CHF and help to clarify whether its downregulation contributes to the progression of heart failure.

Once the cardiovascular profile of apelin in health has been established, its physiological effects in patients with heart failure can be compared with appropriately matched controls. This should hopefully pave the way for clinical trials to establish whether modulation and enhancement of the apelin–APJ system has therapeutic benefit in patients with heart failure. One of the most promising areas is in the treatment of acute decompensated heart failure where the effects of intravenous apelin infusion can be examined.

However, the therapeutic potential of long-term APJ agonism in patients with chronic heart failure will require development of long-acting apelin agonists or effective strategies to enhance endogenous apelin activity.

7. Conclusion

The cardiovascular profile of the apelin–APJ system makes it an attractive therapeutic prospect for patients with heart failure. Emerging evidence from preclinical models suggests that, as well as improving cardiac performance in established heart failure apelin may preserve myocardial function in the face of chronic cardiac stress and afford protection during acute myocardial injury. Detailed clinical investigation is now required to characterise the *in vivo* cardiovascular effects of apelin in man and to establish the safety of systemic apelin administration, particularly in view of the electrocardiographic abnormalities observed in an ovine model. Such studies may prepare the ground for clinical trials to determine the therapeutic efficacy of augmenting apelin–APJ activity in patients with heart failure.

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