Sustained Cardiovascular Actions of APJ Agonism During Renin–Angiotensin System Activation and in Patients With Heart Failure

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Background—To assess cardiovascular actions of APJ agonism during prolonged (Pyr1)apelin-13 infusion and renin–angiotensin system activation.

Methods and Results—Forty-eight volunteers and 12 patients with chronic stable heart failure attended a series of randomized placebo–controlled studies. Forearm blood flow, cardiac index, left ventricular dimensions, and mean arterial pressure were measured using bilateral venous occlusion plethysmography, bioimpedance cardiography, transthoracic echocardiography, and sphygmomanometry, respectively, during brief local (0.3–3.0 nmol/min) and systemic (30–300 nmol/min) or prolonged systemic (30 nmol/min) (Pyr1)apelin-13 infusions in the presence or absence of renin–angiotensin system activation with sodium depletion or angiotensin II coinfusion. During sodium depletion and angiotensin II coinfusion, (Pyr1)apelin-13–induced vasodilatation was preserved (P<0.02 for both). Systemic intravenous (Pyr1)apelin-13 infusion increased cardiac index, whereas reducing mean arterial pressure and peripheral vascular resistance index (P<0.001 for all) irrespective of sodium depletion or angiotensin II (0.5 ng/kg per minute) coinfusion (P>0.05 for all). Prolonged 6-hour (Pyr1)apelin-13 infusion caused a sustained increase in cardiac index with increased left ventricular ejection fraction in patients with chronic heart failure (ANOVA; P<0.001 for all).

Conclusions—APJ agonism has sustained cardiovascular effects that are preserved in the presence of renin–angiotensin system activation or heart failure. APJ agonism may hold major promise to complement current optimal medical therapy in patients with chronic heart failure.


Key Words: APJ • apelin • heart failure • human • vasodilatation

Identified in 1993,1 the APJ receptor is widely expressed throughout the body and, in particular, on the endothelium, vascular smooth muscle cells and cardiomyocytes.2 It is a G-protein–coupled receptor, remaining orphaned until its endogenous ligand, apelin, was discovered in 1998.3 Although various apelin peptide fragments exist, the pyroglutamated 13-amino acid form of apelin, (Pyr1)apelin-13, is the most potent3,4 and abundant form in cardiac tissue,5 and stimulates the APJ receptor to cause vasodilatation6,7 and positive inotropism.8–11

Clinical Perspective on p 491

A key emerging aspect of the apelin–APJ system is its interaction with the renin–angiotensin system.12 The APJ and angiotensin II type I receptors tend to colocalize and share similar sequence homology; ≈50% in the transmembrane domains. These systems mediate opposing actions on vascular tone,11 fluid regulation,14 and inflammation,15 suggesting a reciprocal relationship. Transcription of apelin is reduced during angiotensin II elevation,16 whereas inhibition of angiotensin II type-1 receptor transcription increases APJ expression.17 The 2 receptors also have the potential to heterodimerize, altering their cellular activation pathways.15 The effects of APJ agonism may therefore be attenuated by renin–angiotensin system activation.

The apelin–APJ system is required to maintain cardiac function. Apelin-deficient mice exhibit reduced cardiac contractility with aging and severe cardiac failure in response to increased afterload.18 Concordant with a role in maintaining normal cardiac function, ventricular APJ expression is
reduced in heart failure, and this may theoretically limit its utility as a therapeutic target. Furthermore, in keeping with many G-protein–coupled receptors, the potential for receptor desensitization and tachyphylaxis would limit the clinical utility of any potential therapeutic strategy targeting chronic APJ receptor agonism. Exogenous apelin increases cardiac output in preclinical models of the failing heart and patients with chronic stable heart failure. There are limited data assessing prolonged APJ agonism, and presently clinical studies in heart failure are restricted to acute infusions.

The aims of this study were to assess the cardiovascular actions of (Pyr1)apelin-13 in a range of physiological and pathophysiological settings and, specifically, to determine the effect of renin–angiotensin system activation on the vascular and systemic actions of (Pyr1)apelin-13, and to define the cardiovascular actions of prolonged systemic (Pyr1)apelin-13 administration in healthy volunteers and patients with heart failure.

Methods

Study Participants

Forty-eight healthy volunteers and 12 patients with chronic stable heart failure were recruited. Healthy volunteers were not taking any regular medication and had no history of clinically significant disease and were recruited by advertisements placed throughout the University of Edinburgh campus. One apparently healthy volunteer was withdrawn having been coincidentally found to have a bicuspid aortic valve on screening. Patients were included if they had stable chronic heart failure with a fractional shortening <20%, left ventricular ejection fraction <30%, and New York Heart Association grade II to IV symptoms. Patients were maintained on maximally tolerated doses of evidenced-based therapies, including renin–angiotensin and β-adrenergic inhibitor therapy, although they abstained from their regular medications on the morning of study. Case notes of patients known to the community heart failure service were reviewed, and 38 patients were contacted as they fulfilled inclusion and exclusion criteria. Of these, 24 patients declined to participate, and 2 patients were withdrawn because of inability to obtain stable bioimpedance recordings. All participants abstained from alcohol and caffeine for 24 hours, and were fasted for 4 hours before the study. Studies were performed in a quiet temperature–controlled room (22–25°C). The study was performed with the informed written consent of all subjects, the approval of local research ethics committee, and in accordance with the Declaration of Helsinki.

Forearm Plethysmography

Healthy volunteers underwent brachial artery cannulation with a 27-standard-wire-gauge steel needle under controlled conditions, and the rate of infusion was kept constant at 1 mL/min. Blood flow was measured at 6–minute intervals throughout the study in the infused and noninfused forearms by bilateral forearm venous occlusion plethysmography using mercury-in-silastic strain gauges as described previously. Heart rate and blood pressure were monitored throughout using a semiautomated noninvasive oscillometric sphygmomanometer (HEM 705CP, Omron, Tokyo, Japan).

Impedance Cardiography and Sphygmomanometry

Cardiac index was measured noninvasively with thoracic bioimpedance (HOTMAN Medical). At each time point, cardiac output and venous return were measured with a semiautomated noninvasive oscillometric sphygmomanometer (HEM 705CP, Omron, Tokyo, Japan).

Echocardiography

Echocardiography was performed using a Philips IE33 ultrasound scanner with a 3 MHz transducer. Left ventricular dimensions were assessed by 2-dimensional and M-mode echocardiography in studies of patients with chronic heart failure. All studies were performed and analyzed by the British Society of Echocardiography-accredited sonographers (S.A., A.W.).

Sampling and Assays

Venous cannulae (17-gauge) were inserted into large antecubital veins of both arms to allow drug infusion and sampling of venous blood. Blood samples were drawn into ethylene diamine tetraacetic acid and centrifuged to obtain plasma that was stored at −80°C before analysis. Both healthy volunteers and patients voided their bladder before commencing all studies, and urine was collected throughout the systemic studies. Urinary sodium concentration was determined using an ion selective electrode.

Drugs

The effects of APJ agonism were assessed with synthetic good manufacturing practice-grade (Pyr1)apelin-13 (Genscript, California). (Pyr1)apelin-13, acetylcholine (Novartis AG, Basel, Switzerland), angiotensin II (Bachem, Switzerland), and sodium nitroprusside (Mayne Pharma Plc, Warwickshire, UK) were administered after dissolution in 0.9% saline (Baxter Healthcare Ltd, Thetford, Norfolk, UK) under aseptic conditions.

Study Design

Protocol 1: Sodium Depletion and Peripheral Resistance Vessels

Twelve healthy volunteers attended on 2 occasions, at least 1 week apart, having been randomized in a single-blind crossover design to sodium-deplete or normal sodium-replete diet as described previously. Briefly, for sodium depletion, healthy volunteers were asked to adhere to a diet containing <12 mmol of sodium per day for 3 days before the study visit that they were not blinded to. To ensure prompt sodium depletion, subjects were given a single oral dose of furosemide (20 mg) on day 1 of the diet. For all sodium depletion studies, urine was collected from each individual 24 hours before each visit to assess sodium excretion.

After a 30-minute baseline 0.9% saline infusion, forearm blood flow was assessed after intravenous infusion of (Pyr1)apelin-13 (0.3, 1.0, and 3.0 nmol/min), acetylcholine (5, 10, and 20 µg/min), and sodium nitroprusside (0.5, 1.0, and 2.0 µg/min) given in a double-blind randomized manner, for 6 minutes at each dose. The sequence of vasodilator administration was randomized between subjects but was kept constant for each individual subject to maintain a consistent order for both study visits. Because sodium nitroprusside degrades if exposed to light, all infusions were covered to ensure that blinding was maintained.

Protocol 2: Local Angiotensin II Infusion and Peripheral Resistance Vessels

Twelve healthy volunteers attended on 1 occasion, and local vascular responses to (Pyr1)apelin were assessed during acute intrabrachial angiotensin II infusion (Figure 1). The dose of angiotensin II (5–30 pmol/min) was up titrated until forearm blood flow was reduced by ≥50%. Once sufficient and stable vasoconstriction was achieved, (Pyr1)apelin-13 (0.3, 1.0, and 3.0 nmol/min) and sodium nitroprusside (2, 4, and 8 µg/min) were coinfused with angiotensin II in a double-blind randomized manner, for 6 minutes at each dose, maintained as above.

Protocol 3: Sodium Depletion and Systemic Hemodynamics

After local vascular studies, the same 12 healthy volunteers progressed to systemic vascular studies (Figure 1). In a double-blind randomized crossover manner, subjects received ascending doses of intravenous (Pyr1)apelin-13 (30, 100, and 300 nmol/min) or...
matched saline placebo, administered for 10 minutes at each dose. (Pyr1)apelin-13 is colorless and is indistinguishable from saline in appearance, maintaining blinding. The sequence of apelin and placebo administration was randomized between subjects but was kept constant for each individual subject to maintain a consistent order for both study visits (Figure 1).

Protocol 4: Systemic Angiotensin II Infusion and Systemic Hemodynamics

Twelve healthy volunteers attended on a further 2 occasions, at least 1 week apart (Figure 1). In a double-blind randomized crossover design, subjects received 2-hour infusions of intravenous angiotensin II at a subpressor dose (0.5 ng/kg per minute) or matched 0.9% saline placebo as described previously. Subjects then received ascending doses of intravenous (Pyr1)apelin-13 (30, 100, and 300 nmol/min) or matched saline placebo, administered for 10 minutes at each dose and again given in a double-blind (maintained as above) randomized crossover manner. As before, the sequence of apelin and placebo administration was randomized between subjects but was kept constant for each individual subject to maintain a consistent order for both study visits.

Protocol 5: Prolonged Systemic Apelin Infusion

Twelve healthy volunteers and 12 patients with chronic heart failure attended on 2 occasions separated by at least 1 week having been randomized in a double-blind (maintained as above) crossover design to (Pyr1)apelin-13 or matched saline placebo (Figure 1). Briefly, 6-hour infusions of (Pyr1)apelin-13 or 0.9% saline placebo were commenced after a 30-minute run period when all hemodynamic variables were stable (consecutive readings within 10%). Cardiac index, peripheral vascular resistance index, and heart rate were assessed using thoracic cardiac bioimpedance and mean arterial pressure calculated from blood pressure using a semiautomated sphygmomanometer at 5-minute intervals throughout the first hour of study and in the second half of each subsequent hour for the following 5 hours. Left ventricular end-diastolic and end-systolic diameters were assessed in 15-minute intervals for the first hour in patients with chronic heart failure.

Data and Statistical Analysis

Variables are reported as mean±standard error of mean and analyzed using repeated-measures ANOVA with post hoc Bonferroni corrections and 2-tailed Student t test as appropriate (GraphPad Prism, GraphPad Software, Inc, San Diego, CA). Residual plots from ANOVA and regression models were visually inspected for each data set acquired with no evidence of non-normality or heterogeneity of variance. Forearm blood flow was calculated from plethysmographic data as described previously. Mean arterial pressure was defined as the sum of the diastolic blood pressure and a third of the pulse pressure. Peripheral vascular resistance index was calculated as mean arterial pressure divided by cardiac index. Left ventricular ejection fraction was calculated by the Teichholz method. Statistical significance was taken as 2-sided P<0.05. Based on power calculations derived from previous studies and a significance level of 5%, the sample sizes (n=12) will give 90% power of detecting the clinically meaningful differences of 0.7 mL/100 mL per minute and 0.6 L/min
in forearm blood flow and cardiac output, respectively. We have previously described the influence of a range of factors on regional and systemic vascular beds using sample sizes of 8 to 12 subjects.\textsuperscript{7,8,24} For additional details about statistical and analytic methods, please see the online-only Data Supplement.

### Results

#### Study Participants

Healthy volunteers were men, aged 21±0 years with a body mass index of 22±1 kg/m\(^2\). Patients were elderly and mostly men with heart failure predominantly of ischemic origin and maintained on maximally tolerated optimal medical therapy (Table). Infusions were well tolerated with no serious adverse events. Studies were stopped prematurely in 2 patients with heart failure: 1 patient because of persistent hypertension (systolic blood pressure >200 mm Hg; not present at baseline) and the other because of acute exacerbation of long-standing back pain.

#### Renin–Angiotensin System Activation

##### Sodium Depletion

Healthy volunteers adhered to the sodium-deplete diet with a marked reduction in urinary sodium excretion (34±6 versus 175±21 mmol/d; \(P<0.0001\)) and increases in both plasma renin activity (4.2±0.9 versus 0.8±0.2 ng/mL per hour; \(P<0.01\)) and plasma angiotensin II concentrations (11.6±1.9 versus 5.1±1.0 pg/mL; \(P<0.01\)) in comparison with the sodium-replete diet.

##### Sodium Depletion and Peripheral Resistance Vessels

There were no changes in heart rate, blood pressure, or noninfused forearm blood flow throughout all studies. Baseline forearm blood flow was unchanged by sodium depletion (1.9±0.2 versus 1.9±0.2 mL/100 mL per minute; \(P>0.05\)). Both (Pyr\(^1\))apelin-13 and sodium nitroprusside caused vasodilatation in the infused arm (\(P<0.001\) for all), and these responses were similar under both dietary conditions (\(P<0.05\) for sodium-deplete versus sodium-replete diet; Figure 2A). Acetylcholine caused dose-dependent vasodilation under both dietary conditions (\(P<0.01\), sodium-deplete versus sodium-replete diet; Figure 2A).

##### Local Angiotensin II Infusion and Peripheral Resistance Vessels

Angiotensin II was infused at a mean rate of 29±4 ng/min with resultant increase in local angiotensin II concentrations (95.6±16.0 versus 4.5±1.0 pg/mL; \(P<0.0001\)) and reduced blood flow (from 2.5±0.4–1.3±0.3 mL/100 mL per minute; \(P=0.001\)) in the infused forearm. In the setting of local vasoconstriction, both (Pyr\(^1\))apelin-13 (\(P=0.02\)) and sodium nitroprusside (\(P<0.0001\)) caused vasodilatation (Figure 2B).

#### Sodium Depletion and Systemic Hemodynamics

Compared with matched placebo, intravenous (Pyr\(^1\))apelin-13 infusion increased cardiac index (\(P<0.001\) for both), reduced mean arterial pressure (\(P<0.02\) for both), and decreased peripheral vascular resistance index (\(P<0.001\) for both) with no differences between the sodium-deplete and sodium-replete diets (\(P>0.05\) for all; Figure 3). A trend to increased heart rate was evident after the sodium-replete diet (\(P=0.06\)) that was not present during sodium depletion. In keeping with our previous work,\textsuperscript{8} (Pyr\(^1\))apelin-13 rapidly achieved its peak systemic hemodynamic effects.

### Table. Baseline Characteristics of Patients With Chronic Heart Failure

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64±3</td>
</tr>
<tr>
<td>Sex, men/women</td>
<td>8(75%)/4(25%)</td>
</tr>
<tr>
<td>Pathogenesis, ischemic/idiopathic</td>
<td>8(75%)/4(25%)</td>
</tr>
<tr>
<td>NYHA</td>
<td>II/III/IV (75%/42%/0%)</td>
</tr>
<tr>
<td>Left ventricular function</td>
<td>9/12 (75%)/3/12 (25%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Echocardiographic measures</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular end-diastolic diameter/cm</td>
<td>6.3±0.3</td>
</tr>
<tr>
<td>Left ventricular end-systolic diameter/cm</td>
<td>5.7±0.3</td>
</tr>
<tr>
<td>Fractional shortening</td>
<td>0.13±0.02</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>19±2%</td>
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</table>

<table>
<thead>
<tr>
<th>Concomitant therapy</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-blockade</td>
<td>10/12 (83.3%)</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitor/angiotensin receptor blocker</td>
<td>11/12 (91.7%)</td>
</tr>
<tr>
<td>Aldosterone receptor antagonist</td>
<td>5/12 (41.7%)</td>
</tr>
<tr>
<td>Implanted cardiodefibrillator</td>
<td>5/12 (41.7%)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>3/12 (25%)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>11/12 (91.7%)</td>
</tr>
<tr>
<td>Statin</td>
<td>6/12 (50%)</td>
</tr>
<tr>
<td>Loop diuretic therapy</td>
<td>4/12 (33.3%)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>3/12 (25%)</td>
</tr>
</tbody>
</table>

NYHA indicates New York Heart Association.
Intravenous angiotensin II infusion (0.5 ng/kg per minute) increased systemic angiotensin II concentrations (from 3.7±0.4–7.3±0.8 pg/mL; \(P=0.001\)). Although it was our intention to administer a subpressor dose of angiotensin II, we observed a small increase in both mean arterial pressure (from 82±1–86±1 mm Hg; \(P=0.0014\)) and peripheral vascular resistance index (from 1420±80–1529±91 dynes·s·cm\(^{-5}\)·m\(^{-2}\); \(P<0.0001\)) with a small reduction in cardiac index (from 4.6±0.2–4.4±0.2 L/min per m\(^2\); \(P=0.0288\)) during angiotensin II infusion. Compared with placebo, angiotensin II infusion had no effect on the increase in cardiac index or the reduction in mean arterial pressure or peripheral vascular resistance index observed during systemic (Pyr1)apelin-13 infusion (ANOVA; \(P>0.05\); Figure 4). There seemed to be an initial increase in heart rate after apelin infusion (ANOVA; \(P<0.02\)) during angiotensin II coinfusion, although this was not significant with the placebo infusion.

**Systemic Angiotensin II Infusion and Systemic Hemodynamics**

Intravenous angiotensin II infusion (0.5 ng/kg per minute) increased systemic angiotensin II concentrations (from 3.7±0.4–7.3±0.8 pg/mL; \(P=0.001\)). Although it was our intention to administer a subpressor dose of angiotensin II, we observed a small increase in both mean arterial pressure (from 82±1–86±1 mm Hg; \(P=0.0014\)) and peripheral vascular resistance index (from 1420±80–1529±91 dynes·s·cm\(^{-5}\)·m\(^{-2}\); \(P<0.0001\)) with a small reduction in cardiac index (from 4.6±0.2–4.4±0.2 L/min per m\(^2\); \(P=0.0288\)) during angiotensin II infusion. Compared with placebo, angiotensin II infusion had no effect on the increase in cardiac index or the reduction in mean arterial pressure or peripheral vascular resistance index observed during systemic (Pyr1)apelin-13 infusion (ANOVA; \(P<0.02\); Figure 4). There seemed to be an initial increase in heart rate after apelin infusion (ANOVA; \(P<0.02\)) during angiotensin II coinfusion, although this was not significant with the placebo infusion.

**Prolonged Systemic Apelin Infusion: Healthy Volunteers**

Compared with placebo, (Pyr1)apelin-13 caused an increase in cardiac index during the first hour (ANOVA; \(P<0.0001\)) that was sustained throughout the 6-hour infusion (ANOVA; \(P<0.0001\)). There was an apparent increase in heart rate during the first hour (ANOVA; \(P=0.11\)) that became statistically significant during the remaining 5 hours of infusion (ANOVA; \(P=0.0002\)). Peripheral vascular resistance index was reduced during the first hour of infusion (ANOVA; \(P<0.0001\)), although this was not sustained by the end of infusion (ANOVA; \(P=0.12\)). Mean arterial pressure was unchanged throughout the (Pyr1)apelin-13 infusion (Figure 5), and there were no changes in urinary volume or sodium excretion (Student \(t\) test; \(P>0.05\)).

**Patients With Chronic Heart Failure**

Intravenous (Pyr1)apelin-13 infusion increased cardiac index during the first hour (ANOVA; \(P=0.003\)), and this was sustained throughout the 6-hour infusion (ANOVA; \(P=0.0003\)). Both mean arterial pressure and peripheral vascular resistance index were reduced during the initial hour of infusion (ANOVA; \(P<0.0008\) and \(P=0.0001\), respectively), and this was maintained during the 6-hour infusion (ANOVA; \(P=0.007\) and \(P=0.002\), respectively). There was an apparent trend for an increase in heart rate during the first hour of infusion (ANOVA; \(P=0.051\)), although this did not persist or reach statistical significance during the 6-hour infusion (ANOVA; \(P=0.42\); Figure 6), and there were no changes in urinary volume or sodium excretion (Students \(t\) test; \(P>0.05\)). Fractional shortening and left ventricular ejection fraction were markedly increased after apelin infusion (ANOVA; \(P<0.0001\); Figure 7).

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**Figure 2.** A. Sodium depletion and peripheral resistance vessels. Infused forearm blood flow with normal (open symbols) or sodium-deplete diet (closed symbols) during intrabrachial (Pyr1)apelin (top, circles; 2-way ANOVA, \(P>0.05\)), acetylcholine (middle, triangles; 2-way ANOVA, \(P<0.001\)), and sodium nitroprusside (bottom, squares; 2-way ANOVA, \(P>0.05\)). Bonferroni post test: *\(P<0.05\), **\(P<0.01\), ***\(P<0.0001\). B. Local angiotensin II infusion and peripheral resistance vessels. Infused forearm blood flow after vasoconstriction with angiotensin II during intrabrachial infusion of (Pyr1)apelin (circles; ANOVA, \(P=0.02\)) or sodium nitroprusside (squares; ANOVA, \(P<0.0001\)). Bonferroni post test: *\(P<0.05\), **\(P<0.01\), ***\(P<0.0001\).
Discussion

In these randomized clinical studies, the in vivo cardiovascular effects of APJ agonism with (Pyr1)apelin-13 have been assessed in a range of complementary physiological and pathophysiologic contexts. We have demonstrated that APJ agonism induces peripheral vasodilatation and increased cardiac output in both healthy volunteers and patients with chronic heart failure that is unaltered by renin–angiotensin system activity. In patients with heart failure, we present novel data showing that apelin causes sustained hemodynamic benefits associated with improved left ventricular performance. These studies suggest that therapeutic strategies targeting APJ agonism hold major promise to complement current optimal medical therapy in patients with chronic heart failure.

Increasing evidence suggests interaction between the APJ–apelin and renin–angiotensin systems. Transcription of apelin is reduced during angiotensin II elevation, whereas inhibition of angiotensin II type-1 receptor transcription results in increased APJ expression. In APJ-deficient animal models, there is an exaggerated pressor response to angiotensin II, whereas double angiotensin II type I and APJ knock out animals have elevated blood pressure relative to angiotensin II type I–deficient mice. Moreover, angiotensin II–induced myocardial fibrosis can be prevented by apelin through inhibition of plasminogen activator inhibitor type-1 production. Finally, there is in vitro evidence of heterodimer formation by the APJ and angiotensin II type-1 receptors with consequent alteration of downstream signaling from each receptor. These findings suggest an important interaction between these hormonal systems. However, we have demonstrated that the hemodynamic cardiovascular effects of apelin are preserved despite achieving a range of plasma angiotensin II concentrations using different methods of local and systemic renin–angiotensin system activation. This suggests that there is no major functional interaction in vivo, although we cannot exclude interactions in other settings.

We have used different methods to activate the renin–angiotensin system. The application of sodium-deplete diet increased plasma renin activity, with corresponding fall in urinary sodium excretion, which more than doubled plasma angiotensin II concentrations. Equally, angiotensin II infusions resulted in 21-fold and 2-fold increases in local and systemic plasma angiotensin II concentrations, respectively, that resulted in effects on both local and systemic hemodynamic variables. Thus, in all phases of the study, we successfully achieved a range of renin–angiotensin system activation and do not think that the lack of effect is attributable to failure to augment plasma angiotensin II concentrations.

We acknowledge that dietary modification will impact other hormone systems, such as vasopressin, especially given its reciprocal relationship with apelin. Equally, infusion of angiotensin II may impact other hormone systems that could possibly confound our findings. However, in vivo, the ability to assess elevated angiotensin II in isolation is limited, and alterations in the renin–angiotensin system will inevitably activate other neurohumoral systems. Furthermore, diseases states that have increased renin–angiotensin system activity, such as heart failure, will have corresponding alterations in other neurohumoral systems.

In contrast to apelin, acetylcholine-induced vasodilatation was impaired after sodium depletion and renin–angiotensin system activation, suggesting the presence of endothelial dysfunction. In vitro angiotensin II promotes superoxide anion generation when incubated with vascular smooth muscle

Figure 3. Sodium depletion and systemic hemodynamics. Systemic hemodynamic response to (Pyr1)apelin-13 (closed) or placebo (open) during normal diet (circles) or sodium-deplete diet (squares). Two-way ANOVA with repeated measures. Bonferroni post test: *P<0.05, **P<0.01, ***P<0.0001.
Furthermore, in whole animals treated with angiotensin II, superoxide generation is increased, and vasorelaxation to acetylcholine is impaired. The effect of dietary sodium on endothelial function has previously been assessed in both healthy individuals and patient populations, leading to diverse findings. Some report the induction of vascular dysfunction with sodium loading and no effect of sodium depletion, most likely reflecting different cohorts and protocols.

Apelin is the most potent in vitro inotrope, being effective at subnanomolar concentrations. Although the cellular
mechanisms mediating its inotropic effects are not fully characterized, sodium-hydrogen channel activation, with resultant increase in pH and increase in myofilament calcium sensitivity, is important in addition to increased intracellular calcium concentrations. Apelin–APJ expression and plasma apelin concentrations are reduced in heart failure. Animal models demonstrate reduced ventricular APJ receptor expression in heart failure, and this is consistent with expression studies in human myocardium. There are concerns that myocardial APJ receptor density in heart failure will be insufficient to evoke a response that does not readily saturate. Furthermore, preclinical data demonstrate that surface APJ receptor

Figure 6. Prolonged systemic apelin infusion, heart failure. Systemic hemodynamic response to (Pyr1)apelin-13 (closed) and placebo (open) presented during first hour and subsequent 6 hours of infusion in patients with chronic stable heart failure. Two-way ANOVA with repeat measures. Bonferroni post test: *P<0.05, **P<0.01, ***P<0.0001.

Figure 7. Echocardiography data for left ventricular dimensions, fractional shortening, and ejection fraction during the first hour of (Pyr1) apelin-13 infusion (closed) and placebo infusion (open) in patients with heart failure. Two-way ANOVA with repeat measures. Bonferroni post test: *P<0.05, **P<0.01, ***P<0.0001.
activation leads to its rapid internalization to perinuclear compartments. Additionally, acute (Pyr4)apelin infusion exhibits a prompt plateau in the dose–response relationship. Taken together, this could suggest rapid receptor desensitization and tachyphylaxis: a common feature of other G-protein–coupled receptor signaling pathways. Our study demonstrated persistent hemodynamic effects throughout a 6-hour infusion, suggesting sustained cardiovascular effects without evidence of major tachyphylaxis. Indeed, the response in patients with heart failure is of comparable magnitude with that observed in healthy volunteers. Moreover, we demonstrate marked improvements in echocardiographic-derived measures of left ventricular performance, suggesting a direct effect on myocardial contractility. However, it is difficult to delineate whether this resulted from direct isotropic effects or a response to peripheral vasodilatation. Our previous data from studies of acute peripheral, systemic, and intracoronary apelin infusions suggest that both effects are likely to be significant. Whatever the mechanism, the observed improvement in indices of left ventricular function with apelin infusion in the context of left ventricular systolic impairment are extremely encouraging. To address load-independent effects on contractility would be challenging and require highly invasive techniques, including insertion of left ventricular conductance catheters and inferior vena cava obstruction.

Although there are well-established effective medications in heart failure that improve patient outcome, mortality and economic burden remain unacceptably high. As such, there is a need to improve current therapy further, and we think that apelin may have such a role. The emerging data from preclinical studies support a causative role in the pathogenesis of heart failure, and APJ agonism can restore activity of a system that directly contributes to ventricular function. Current pharmacological interventions largely target maladaptive neurohumoral responses, whereas apelin may present an exciting opportunity to optimize endogenous adaptive responses further. The combination of increased cardiac output with minimal changes in mean arterial pressure (peak reduction of 4 mm Hg in our studies) or heart rate would be a highly attractive addition to the current armamentarium of therapy for chronic heart failure. For many clinicians, the up titration of current therapies, or the addition of new therapies, is commonly hindered by concerns about hypotension and further vasodilatation. The cardiovascular profile of apelin makes it a very attractive potential novel candidate that could be added to current optimal therapy without concerns over severe hypotension or reflex tachycardia. We would like to explore the effects of more prolonged APJ agonism about several days, weeks, or months. However, there are currently no available oral or parental preparations that can easily deliver prolonged APJ agonism to assess the potential sustained therapeutic benefits of this intervention in chronic heart failure.

Although we think that the hemodynamic profile of apelin would be beneficial, there are obvious concerns with any positive inotrope in the setting of chronic heart failure. Previous agents have caused significant increases in mortality that have been attributed to sudden arrhythmic death. In our limited early studies, we have not seen any proarrhythmic effect, but there remains the possibility that long-term APJ agonism will have this undesired effect on the myocardium.

In conclusion, we have shown that the local and systemic cardiovascular effects of apelin are sustained and not altered by renin–angiotensin activation or heart failure. We think that this system warrants further assessment as a novel therapy in this important area that continues to be associated with major morbidity and mortality.

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Disclosures

None.

References

Heart failure represents a significant healthcare burden that is expected to grow. Morbidity and mortality remains unacceptably high, and there is a need to improve current medicinal therapy. The apelin–APJ system is an emerging hormone system that is implicated in cardiovascular homeostasis, principally as a vasodilator and positive inotrope. Of particular interest, myocyte cardiac APJ expression is downregulated in heart failure, suggesting a direct role in ventricular dysfunction. Additionally, the APJ receptor is a therapeutic target that should be fully explored.
Sustained Cardiovascular Actions of APJ Agonism During Renin–Angiotensin System Activation and in Patients With Heart Failure


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Supplemental Material

Additional detail regarding methods; randomization, blinding and recruitment

A suitably trained researcher within the department, competent in Good Clinical Practice, performed randomization for each protocol. This blinding code was held securely in the Welcome Trust Clinical Research Facility and investigators had no access to this code. A protocol was in place to ensure that emergency unbinding could be undertaken if required. This was not necessary throughout this clinical trial.

For those participating in the salt restriction protocol, each of the local and systemic studies was performed on the same day and as such under the same dietary conditions. The order of drug infusion in the local and systemic infusion was independent of each other. The volunteers we responsible for their own dietary modification, following specific guidelines that were provided, and therefore were not blinded to the salt restriction designed in this protocol. All investigators remained blinded during this protocol.

All infusions are colorless and as such there is no way visual way of knowing what medication is infused. Sodium nitroprusside is colorless, however is sensitive to ultra-violet light that requires the syringe to be covered. For each of the forearm plethysmography studies, all infusions were covered in order to maintain blinding. For systemic hemodynamic studies, blinding was maintained by virtue of (Pyr1)apelin-13 being colorless and indistinguishable from saline.

Each of the protocols was completed in series and participants recruited for each study after completion of the preceding protocol. For healthy volunteer recruitment participants responding to advertisements displayed on campus at the University of Edinburgh, as agreed under the Ethics Committee approving these trials. In order for enrolment all inclusion and exclusion criteria were fulfilled and informed consent obtained.

Patients with chronic stable heart failure known to the clinical cardiology service at the Royal Infirmary of Edinburgh and community heart failure service were contacted and provided with information relevant to the trial. A screening visit proceeded entry
to the trial to ensure inclusion and exclusion criteria were fulfilled and to obtain informed consent.

**Additional detail regarding statistical and analytic methods**

The ANOVA model used to analyze these data from each protocol was a mixed model ANOVA with both time and dose as fixed effects and subject as a random effect.

None of the terms is nested. All patients have been recruited from the same site and in each of the study protocols used a cross-over design such that individuals have acted as their own controls. These studies were not powered to assess the effects of differing term that may have been present within the populations.

Interactions of treatment were assessed by time and were non-significant. Given the small samples sizes, we did not anticipate identifying any interactions.

For analysis an arbitrary dose was assigned rather than a true numerical value. No specific modeling of dose-response was performed. The aim of these studies was to assess the effects of APJ agonism compared to placebo or under varying levels of renin angiotensin activation rather that to construct a dose response curve. The repeated measures ANOVA models assume compound symmetry (constant variance and covariance over time).

There are no individual contrasts between specific time points or dose levels and this was not pre-specified in our analysis. We have used Bonferroni corrections to provide adjustment of p-values and assess differences that may be present.