Heart Failure

Acute Cardiovascular Effects of Apelin in Humans
Potential Role in Patients With Chronic Heart Failure

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Background—Apelin, the endogenous ligand for the novel G protein–coupled receptor APJ, has major cardiovascular effects in preclinical models. The study objectives were to establish the effects of acute apelin administration on peripheral, cardiac, and systemic hemodynamic variables in healthy volunteers and patients with heart failure.

Methods and Results—Eighteen patients with New York Heart Association class II to III chronic heart failure, 6 patients undergoing diagnostic coronary angiography, and 26 healthy volunteers participated in a series of randomized, double-blind, placebo-controlled studies. Measurements of forearm blood flow, coronary blood flow, left ventricular pressure, and cardiac output were made by venous occlusion plethysmography, Doppler flow wire and quantitative coronary angiography, pressure wire, and thoracic bioimpedance, respectively. Intrabrachial infusions of (Pyr1)apelin-13, acetylcholine, and sodium nitroprusside caused forearm vasodilatation in patients and control subjects (all P<0.0001). Vasodilatation to acetylcholine (P=0.01) but not apelin (P=0.3) or sodium nitroprusside (P=0.9) was attenuated in patients with heart failure. Intracoronary bolus of apelin-36 increased coronary blood flow and the maximum rate of rise in left ventricular pressure and reduced peak and end-diastolic left ventricular pressures (all P<0.05). Systemic infusions of (Pyr1)apelin-13 (30 to 300 nmol/min) increased cardiac index and lowered mean arterial pressure and peripheral vascular resistance in patients and healthy control subjects (all P<0.01) but increased heart rate only in control subjects (P<0.01).

Conclusions—Acute apelin administration in humans causes peripheral and coronary vasodilatation and increases cardiac output. APJ agonism represents a novel potential therapeutic target for patients with heart failure. (Circulation. 2010;121:1818-1827.)

Key Words: apelin, AGTRL1 ligand, human ■ APJ receptor, human ■ heart failure

Acelin is the endogenous ligand for the previously orphaned G protein–coupled receptor APJ.1,2 The apelin gene encodes a 77–amino acid preproprotein that undergoes proteolytic cleavage to yield a mature 36–amino acid peptide (apelin-36).3 Shorter C-terminal fragments of apelin-36 have also been detected, including the pyroglutamated form of apelin-13, (Pyr1)apelin-13, which exhibits greater affinity for the APJ receptor and may represent the principal endogenous ligand.5

Clinical Perspective on p 1827

APJ receptors are present on endothelial cells, vascular smooth muscle cells, and cardiomyocytes.4 In preclinical models, apelin signaling exerts major effects on both vascular tone and cardiac contractility. In isolated rat hearts, apelin is the most potent endogenous inotrope described so far,2 and in ex vivo myography studies, it causes vasorelaxation in human mesenteric artery that is attenuated by inhibition of nitric oxide but not prostacyclin.6 In rodents, apelin also increases cardiac contractility in vivo7,8 and causes a rapid fall in both arterial blood pressure and systemic venous tone.9–11 with corresponding reductions in left ventricular afterload and preload.7 Apelin-deficient mice develop premature heart failure unless plasma apelin concentrations are restored.12 Reductions in myocardial apelin and APJ expression have also been demonstrated in experimental rodent models of heart failure.13,14 Despite the apparent downregulation of apelin-APJ activity, the hemodynamic effects of exogenous apelin are maintained or even augmented in rodents with heart fail-

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Given that failing human hearts also exhibit altered patterns of apelin and APJ gene expression and that plasma apelin concentrations are markedly reduced in patients with severe chronic heart failure, the apelin-APJ pathway may represent a promising target for therapeutic intervention in this condition.

We recently provided the first evidence that apelin has vasoactive actions in humans. We demonstrated that intrabrachial apelin infusion causes nitric oxide–mediated arterial vasodilatation with no apparent effect on peripheral venous tone. In the present series of studies, we sought to characterize the in vivo cardiovascular profile of apelin in humans by establishing its effects on peripheral, coronary, and systemic vasculature. As a first step in exploring the therapeutic potential of APJ agonism, we also sought to determine the local vascular and systemic hemodynamic effects of apelin in patients with chronic heart failure.

**Methods**

All studies were performed with the written informed consent of the volunteers, with the approval of the local research ethics committee, and in accordance with the Declaration of Helsinki.

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**Protocol Design**

**Protocol 1: Peripheral Arterial Studies**

Twelve patients with heart failure and 12 age- and sex-matched healthy control subjects attended on a single occasion and had blood drawn for baseline measurement of brain natriuretic peptide (BNP) and plasma renin activity (Figure 1). In a double-blinded randomized manner, subjects then received intrabrachial infusions of (Pyr1)apelin-13 (0.3, 1, or 3 nmol/min), acetylcholine (5, 10, or 20 µg/min) and sodium nitroprusside (SNP; 1, 2, 4 µg/min) for 6 minutes at each dose with drugs separated by a 20-minute saline infusion. Blood flow was measured at 6-minute intervals throughout the study in the infused and noninfused forearms by venous occlusion plethysmography.

**Protocol 2: Coronary Artery and Myocardial Contractility Studies**

In a double-blind randomized manner, intracoronary boluses of apelin-36 (20 and 200 nmol in 2 mL) and 0.9% saline (2 mL), followed by a single-blinded nonrandomized bolus of glyceryl trinitrate (GTN; 100 µg in 2 mL), were administered in 6 subjects via a coronary guide catheter. Doses were selected on the basis of our previous study, and coronary blood flow was assumed to be 5 times that of the forearm. All drug boluses were followed by a 2-mL 0.9% saline flush; 5-minute washout intervals were allowed between drugs. Coronary blood flow, left ventricular pressures, and the maximal rise in left ventricular pressure (dP/dtmax) were calculated at baseline and before and after each drug bolus as described below.
Protocol 3: Systemic Hemodynamics Studies in Healthy Volunteers

Eight subjects attended on 2 occasions at least 1 week apart. In a double-blind randomized manner, subjects received three 5-minute intravenous infusions of either apelin-36 or (Pyr⁴)apelin-13 (30, 100, or 300 nmol/min) and three 5-minute infusions of saline placebo, with the apelin and saline placebo infusions separated by a 30-minute saline washout. Doses of apelin were determined from our previous study in which 30 nmol/min caused a rise in systemic plasma apelin concentrations. Hemodynamic recordings were made at 10-minute intervals before the beginning of drug infusion and at 5-minute intervals thereafter. Venous blood samples were obtained at baseline, at peak drug infusion, and at 5 and 30 minutes after drug infusion for measurement of plasma apelin-36 concentration.

Protocol 4: Systemic Hemodynamics Studies in Patients With Heart Failure

Eight patients with heart failure and 8 healthy matched control subjects attended on a single occasion. In a double-blind randomized manner, intravenous (Pyr⁴)apelin-13 (30, 100, and 300 nmol/min) and matched saline placebo were infused for 5 minutes at each dose. Apelin and saline infusions were separated by a 30-minute saline infusion. Hemodynamic recordings were made at 10-minute intervals before the beginning of drug infusion and at 5-minute intervals thereafter. Venous blood samples were obtained at baseline and at 5, 15, and 30 minutes after drug infusion for measurement of BNP, arginine vasopressin, and plasma renin activity.

Subjects

Healthy and control volunteers had no history of any clinically significant medical condition or symptoms of recent illness and avoided vasoactive and nonsteroidal antiinflammatory drugs for 7 days before the studies. Patients attending for diagnostic angiography were excluded if they had left main stem or severe coronary artery stenosis, previous coronary intervention, or clinical or echo-cardiographic evidence of cardiac failure. Patients with heart failure were eligible for inclusion if they had stable New York Heart Association class II to IV symptoms, were on maximally tolerated doses of heart failure medication for at least 3 months, and had objective evidence of left ventricular impairment (left ventricular end-diastolic diameter >5.5 cm and left ventricular ejection fraction <40% or shortening fraction <20%). Patients were excluded if they had hemodynamically significant valvular heart disease, renal or hepatic failure, or previous malignant ventricular arrhythmias. Patients withheld their usual medications on the study day until completion of the study protocol, and all participants abstained from alcohol for 24 hours and from food and caffeine-containing drinks for at least 4 hours before each study.

Drugs

The effects of APJ agonism were assessed with synthetic pharmacological-grade apelin-36 and (Pyr⁴)apelin-13 (Cinalfa AG, Laüfelfingen, Switzerland). Acetylcholine (Novartis AG, Basel, Switzerland), SNP (Mayne Pharma Plc, Warrickshire, UK), and GTN (Nitrocine, UCB Pharma, Brussels, Belgium) were administered as control vasodilators. All drugs were administered after dissolution in 0.9% physiological saline (Baxter Healthcare Ltd, Thetford, Norfolk, UK) under aseptic conditions on the study day.

Measurements

All studies were carried out in a quiet, temperature-controlled room (23°C to 25°C) with subjects in the supine position.

Peripheral Arterial Studies

Subjects 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 in the infused and noninfused forearms by bilateral forearm venous occlusion plethysmography using mercury-in-silastic strain gauges as described previously.

Coronary Artery and Myocardial Contractility Studies

After diagnostic coronary angiography, a coronary guide catheter was engaged in the ostium of the left coronary artery, and a 0.014-in 12.5-MHz Doppler wire (FloWire, Cardiometrics, Endosonics, Rancho Cordova, Calif) was positioned in the coronary artery to measure blood flow velocity. Coronary angiography was performed immediately before and 30 seconds after each drug bolus. Coronary luminal diameter was measured by quantitative computerized analysis with an automated edge contour detection analysis system from end-diastolic frames of each angiogram, and cross-sectional area was calculated with circular geometry assumed. Blood flow velocity was determined from the average peak velocity of the Doppler signal (FloMap, Cardiometrics). Coronary blood flow was defined as half the product of the average peak velocity and the cross-sectional area of the coronary artery.

Another catheter was inserted into the left ventricle, and a 0.014-in pressure wire (RA Diagnostics, Upplands, Sweden) was placed in the left ventricular cavity to measure left ventricular pressures continuously. Ten-second samples of pressure recordings within the left ventricle were taken before and at 60 seconds after each drug bolus and used to calculate dp/dtmax and peak left ventricular systolic and end-diastolic pressures.

Systemic Hemodynamic Studies

Venous cannulas (17 gauge) were inserted into large antecubital veins of both arms to allow drug infusion and sampling of venous blood. Blood pressure and heart rate were recorded with a semiautomated noninvasive oscillometric sphygmomanometer (HEM 705CP, Omron, Tokyo, Japan); mean arterial pressure was defined as the sum of the diastolic blood pressure and a third of the pulse pressure. Cardiac output was measured noninvasively with thoracic bioimpedance (NCCOM, BoMed Medical Manufacturing Ltd, Irvine, Calif) and corrected for body surface area to give cardiac index. At each time point, cardiac output was taken as the mean of 3 recordings, each recording representing the average of 15 consecutive heartbeats. Peripheral vascular resistance index was calculated as mean arterial pressure divided by cardiac index (expressed in dyne·s·m⁻²·m²). ECG was monitored continuously during each study.

Assays

Blood samples (10 mL) were drawn into tubes containing either EDTA or lithium heparin and kept on ice before centrifugation at 2000g for 30 minutes at 4°C. Platelet-free plasma was decanted and stored at −80°C before assay. Plasma apelin-36 concentration was measured with the apelin-12 microplate ELISA assay kit (Phoenix Pharmaceuticals Ltd, Burlingame, Calif). This antibody has 100% cross-reaction with apelin-12, apelin-13, and apelin-36, but cross-reaction with (Pyr⁴)apelin-13 is unknown. Plasma renin activity was measured under standard conditions through the generation of angiotensin I as determined by radioimmunoassay (DiaSorin Ltd, Wokingham, UK). Plasma BNP and arginine vasopressin concentrations were measured by immunoradiometric assay (Shionogi and Co Ltd, Osaka, Japan) and direct radioimmunoassay (Buhlmann Laboratories AG, Schonebach, Switzerland), respectively.

Data and Statistical Analyses

For 80% power at 2-sided P < 0.05 and based on our previously published data, 6, 8, 12, 14, and 24 patients were selected to detect differences in the primary end points of forearm blood flow of 0.83 mL·100 mL⁻¹·min⁻¹, coronary blood flow of 19 mL/min, cardiac output of 0.70 L/min, and cardiac output of 0.56 L/min in protocols 1 through 4, respectively. Forearm20 and coronary blood flow20 and systemic hemodynamic variables18,25 were analyzed in a blinded fashion as described previously. Plasma renin activity and BNP concentrations were not normally distributed and were log transformed before statistical analysis. Variables are reported as mean±SEM and analyzed with repeated-measures ANOVA with posthoc Bonferroni corrections and 2-tailed Student t
Peripheral Blood Flow

There was no significant change in heart rate, blood pressure, or noninfused forearm blood flow in all studies. In the infused arm, (Pyr1)apelin-13, acetylcholine, and SNP caused dose-dependent vasodilatation ($P<0.001$) in both healthy volunteers and patients with heart failure (Figure 2). Although vasodilatation to (Pyr1)apelin-13 and SNP was similar between the groups, vasodilatation to acetylcholine was reduced in patients with heart failure. Although basal forearm blood appeared to be lower in patients with heart failure, there was no statistical difference between patients and control subjects. To account for any apparent baseline imbalance in blood flow, we expressed the vasodilatation as a percentage change in forearm blood flow from baseline.26 However, the findings were consistent and the significance was unchanged (data on file).

Coronary Blood Flow and Myocardial Contractility

Patients were 60±4 years of age, and 5 were men. All patients had near-normal coronary arteries with no hemodynamically significant flow-limiting stenoses (all <25% lumen stenosis).

There were no significant changes in coronary blood flow or hemodynamic variables after injection of 20 nmol apelin-36 (data on file). Compared with saline placebo, there was an increase in coronary blood flow after intracoronary administration of 200 nmol apelin-36 and a trend toward an apparent increase with GTN, which did not reach statistical significance (Figure 3). Compared with placebo, both 200 nmol apelin-36 and GTN caused an increase in dP/dtmax and a slight reduction in peak left ventricular systolic and left ventricular end-diastolic pressures. Compared with placebo, GTN caused a fall in mean arterial pressure (94±8 versus 80±9 mm Hg; $P<0.01$) and a rise in heart rate (67±5 versus 69±5 bpm; $P<0.001$), but apelin had no effect on blood pressure (94±8 versus 94±7 mm Hg; $P=0.9$) or heart rate (67±5 versus 68±5 bpm; $P=0.3$).

Systemic Hemodynamic Studies

Healthy Volunteers

Saline placebo infusion had no effect on any of the hemodynamic variables (Figure 4). Both (Pyr1)apelin-13 and apelin-36 increased heart rate and cardiac output while reducing peripheral vascular resistance with no overall effect on blood pressure (Figure 4 and Table 2). The small rise in heart rate was seen early and was not sustained (Table 2). There appeared to be no clear dose-response relationship at the doses used in the present study, and consistent with our previous study,17 apelin-36 had a more sustained offset of action than (Pyr1)apelin-13. Plasma concentration of apelin-36 increased from 0.24±0.07 pg/mL at baseline to 115.86±16.97 pg/mL during maximal infusion ($P<0.0001$) and by 5 minutes after infusion had fallen to 39.95±7.82 pg/mL ($P=0.002$ versus peak infusion), suggesting a plasma half-life of <5 minutes. By 30 minutes after infusion, plasma apelin-36 concentrations had returned to near baseline concentrations.

Results

All studies were well tolerated with no serious adverse events or ECG changes during apelin administration. Patients with heart failure were predominantly male and middle-aged with the heart failure attributable to ischemic heart disease or dilated cardiomyopathy (Table 1). They were well treated with optimal or maximally tolerated heart failure therapy and had mild to moderate symptoms with a low ejection fraction and elevated BNP concentrations and plasma renin activity (Table 1).

| Table 1. Baseline Characteristics of Patients With Heart Failure and Healthy Control Subjects |
|-------------------------------------------------|---------------------------------|-----------------|-----------------|
| Peripheral Arterial Studies                      | Systemic Hemodynamic Studies    |
| Control Subjects (n=12)                          | Control Subjects (n=8)           | Patients (n=12) | Patients (n=8)  |
| Age, y                                          | 60±2                            | 57±3            | 55±4            |
| Sex, M/F                                        | 3/9                             | 2/10            | 8/0             |
| BMI, kg/m²                                      | 27±1                            | 27±1            | 25±1            |
| LVEDD, cm                                       | 7.5±0.6                         | 6.5±0.5         |
| EF, %                                           | 27±3                            | 28±4            |
| NYHA class II/III, n                            | 10/2                            | 4/4             |
| Origin (IHD/DDCM), n                            | 6/6                             | 5/3             |
| Medication, n                                   | ACEI/ARB                        | 0/12            | 12/12           |
|                                                    | β-blocker                       | 0/12            | 8/12            |
|                                                    | Diuretic                        | 0/12            | 12/12           |
|                                                    | Aldosterone antagonist          | 0/12            | 6/12            |
|Baseline                                         | Heart rate, bpm                 | 66±3            | 68±2            |
|                                                  | MAP, mm Hg                      | 98±3            | 96±2            |
|                                                  | Infused FBF                     | 3.2±0.4         | 2.6±0.3         |
|                                                  | Noninfused FBF                  | 3.0±0.4         | 2.4±0.3         |
|                                                  | CI, L·min⁻¹·m⁻²                 | ...             | 3.3±0.5         | 2.7±0.6 |
|                                                  | PVRI, dynes·s·cm⁻²              | ...             | 2423±290        |
|                                                  | Log BNP, pg/mL                  | 0.9±0.1         | 2.1±0.2         |
|                                                  | Log PRA, ng/mL                  | 0.1±0.1         | 1.1±0.2         |
|                                                  | AVP, pg/mL                      | ...             | 3.0±1.9         |

BMI indicates body mass index; LVEDD, left ventricular end-diastolic diameter; EF, ejection fraction; NYHA, New York Heart Association; IHD, ischemic heart disease; DCM, dilated cardiomyopathy; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; MAP, mean arterial pressure; FBF, forearm blood flow; CI, cardiac index; PVRI, peripheral vascular resistance index; PRA, plasma renin activity; and AVP, arginine vasopressin. Data are expressed as mean±SEM when appropriate. $^{*}P<0.05$, $^{†}P<0.01$ versus control subjects.

data test as appropriate (Graph-Pad Prism, GraphPad Software Inc, San Diego, Calif). Statistical significance was taken at the 5% level.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
and did not differ from baseline values \((P > 0.05)\).

**Patients With Heart Failure**

Compared with saline placebo, intravenous administration of (Pyr1)apelin-13 caused an increase in cardiac output and reductions in blood pressure and peripheral vascular resistance in both patients and control subjects and an increase in heart rate in control subjects \((P < 0.01)\) for all; Figure 5, Table 2, and Figure I of the online-only Data Supplement). Perhaps reflecting the higher resting heart rate and use of β-blockade, there was no change in heart rate in patients with heart failure. There were no differences in the responses to apelin between patients with heart failure and control subjects except for heart rate, for which a greater response to apelin was observed in control subjects \((P < 0.05)\; \text{Figure 5})

Plasma BNP concentration \((P < 0.01)\) and renin activity \((P < 0.001)\) were higher at all time points in patients compared with control subjects but were not altered in either group by apelin infusion \((2\text{-way ANOVA with posthoc Bonferroni tests}; \text{Figure II of the online-only Data Supplement})\). Plasma arginine vasopressin concentration did not differ between groups \((P = 0.21)\) and was unaltered by apelin infusion in both groups \((2\text{-way ANOVA}; \text{Figure II of the online-only Data Supplement})\).

**Discussion**

For the first time, we have demonstrated that short-term apelin infusion causes peripheral and coronary vasodilatation, reduces cardiac preload and afterload, and increases cardiac output in vivo in humans. These important hemodynamic effects appear to be preserved in patients with heart failure. We conclude that APJ agonism has potential therapeutic benefits in patients with heart failure maintained on optimal contemporary medical therapy.

We have previously shown, in keeping with data from animal9 and ex vivo6 models, that apelin causes vasodilatation in vivo in the human forearm circulation through predominantly nitric oxide–dependent mechanisms.17 We have extended this work here to demonstrate that apelin-induced vasodilatation is preserved in patients with heart failure. Although the study may have been underpowered to detect small intergroup differences, we saw no evidence of a major attenuation of vasodilatation to apelin in patients with heart failure. This is perhaps surprising because the same patients demonstrated reduced vasodilatation to acetylcholine, which,
like apelin, relies predominantly on endothelium-dependent pathways. One possible explanation is that alterations in endogenous plasma apelin concentrations\textsuperscript{16} or APJ expression\textsuperscript{15} in the setting of heart failure may augment responses to exogenous apelin and thereby offset any diminution of endothelial function. On the other hand, it is controversial whether patients with stable heart failure have evidence of major endothelial dysfunction,\textsuperscript{27} especially when maintained on optimal medical therapy. Given the rapidity of its metabolism, the reduced vasodilatation to acetylcholine in patients with heart failure may be due to lower basal blood flow rather than endothelial dysfunction per se.\textsuperscript{28}

We have demonstrated for the first time that apelin is a direct coronary vasodilator that increases myocardial contractility in humans. In addition, it causes a reduction in both peak and end-diastolic left ventricular pressures. It is notable that a very similar hemodynamic profile of effects occurred after GTN administration. Although we did not measure peripheral plasma apelin concentrations during these studies, we believe it is likely that there was overspill of both apelin and GTN into the peripheral circulation, leading to alterations in peripheral vascular tone. Thus, some of the apparent inotropic effect of apelin may be attributable to a reduction in afterload secondary to peripheral vasodilatation. This interpretation is supported by both the reduction in maximal left ventricular pressure and the apparent absence of any direct cardiac effects after injection of the lower dose of apelin and would be in keeping

![Graphs showing coronary blood flow, maximum LV dp/dt, LV end-diastolic pressure, and percentage change in cardiac index and peripheral vascular resistance index during infusion of apelin-36 and placebo.](http://circ.ahajournals.org/)

Figure 3. Coronary blood flow (n=6) and left ventricular (LV) pressures (n=5) after bolus injection of apelin-36 (200 nmol), GTN (100 μg), and 0.9% saline. *P<0.05, †P=0.07, paired Student t test vs 0.9% saline.

Figure 4. Percentage change from baseline in cardiac index and peripheral vascular resistance index during infusion of (Pyr\textsuperscript{1})apelin-13 or matched saline placebo and apelin-36 or matched saline placebo in healthy subjects. *P<0.05, **P<0.01, ***P<0.001, 2-way ANOVA (apelin vs placebo) with posthoc Bonferroni tests.
with the clear evidence of peripheral arterial vasodilatation in our forearm and systemic infusion studies. However, in contrast to GTN, which caused a marked fall in systemic blood pressure and corresponding rise in heart rate, the higher dose of apelin had no effect on blood pressure or heart rate, raising the possibility of an additional direct inotropic effect. Determining whether apelin causes load-independent increases in myocardial contractility is challenging in a clinical study and requires the use of a conductance catheter with manipulation of cardiac filling pressures.

Assuming that the observed reductions in filling pressures reflect alterations in peripheral vascular tone, the reduction in left ventricular end-diastolic pressure would provide the first in vivo evidence of apelin-induced vasodilatation in humans. Although we have previously reported that apelin has no apparent effect on venous tone in superficial hand veins, these veins are not representative of other venous beds and are of less hemodynamic importance than capacitance veins. The finding that apelin reduces left ventricular end-diastolic pressure in humans is in keeping with the evidence of a powerful venodilator effect in rodents and provides further important rationale for its potential therapeutic application in heart failure.

Systemic apelin administration causes a reduction in peripheral vascular resistance accompanied by an increase in cardiac output and heart rate. In young healthy subjects, blood pressure is maintained, whereas in older subjects and patients with heart failure, there is a small reduction. The changes in systemic hemodynamic variables did not demonstrate a clear dose-response relationship and appeared to demonstrate a flat dose response. This was also seen in our previous studies in the peripheral forearm circulation and may reflect our selection of doses or an “all-or-nothing” response.

The hemodynamic changes observed in patients with heart failure during acute apelin infusion were not accompanied by a fall in plasma BNP concentrations. A likely explanation is the brief infusion protocols used here because BNP responds to alterations in filling pressures over a period of several hours and is relatively insensitive to short-term hemodynamic changes.

The reduction in peripheral vascular resistance during systemic apelin infusion demonstrates the hemodynamic significance of the local vasodilatation observed in our regional infusion studies and is in strong agreement with data from animal studies. In addition, preclinical data demonstrated powerful direct effects of apelin on cardiac contractility both in vitro and in vivo. We therefore believe that there is likely to be a dual mechanism underlying the rise in cardiac output: a reflex increase caused by peripheral vasodilatation and an increase in myocardial contractility through a direct inotropic action. This is consistent with our findings from the intracoronary infusion of apelin.
In considering apelin as a potential therapeutic target for patients with heart failure, the above profile of action is broadly favorable. Some established inotropic therapies have been associated with adverse outcomes in this patient group. In particular, agents that act by increasing intracellular calcium concentrations tend to increase myocardial oxygen consumption, exert proarrhythmic effects, and promote left ventricular hypertrophy. However, apelin appears to exert its inotropic effects at least in part through increasing myofilament sensitivity to calcium and, in animal models, does not induce left ventricular hypertrophy, even during long-term administration. In addition, its ability to reduce cardiac loading conditions may serve to limit cardiac work and myocardial oxygen consumption. Recent data indicate an inverse relationship between blood pressure and mortality in patients with chronic heart failure.31 However, this association appears to reflect low ejection fraction and cardiac output rather than the blood pressure–lowering effects of pharmacological therapies. Indeed, several agents that improve prognosis in heart failure, including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and β-blockers, also lower blood pressure. Importantly, the anti-hypertensive effect of apelin is achieved through a reduction in peripheral vascular resistance that is associated with an increase in cardiac output.

There are now several reports of reduced plasma apelin concentrations in patients with heart failure3 suggesting scope for augmentation of apelin-APJ activity. By demonstrating preserved local and systemic cardiovascular responses to exogenous apelin in patients with heart failure, we confirm here that APJ signaling capacity is not exhausted by endogenous apelin in this patient cohort, an essential prerequisite for therapeutic strategies using APJ agonism.

The clinical relevance of our findings is enhanced by the inclusion of patients maintained on existing evidence-based pharmacological therapies. This is particularly important given the recent emergence of direct interactions between the renin-angiotensin and apelin-APJ systems, including direct antagonism of angiotensin II by apelin.13,32 Because all but one of our patients with heart failure were receiving treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, our findings imply a role for apelin in cardiovascular regulation that is independent of angiotensin II signaling pathways and suggest potential for pharmacological synergism through combined APJ agonism and renin-angiotensin system inhibition.

We acknowledge that this study has assessed only the short-term effects of apelin administration. We cannot reliably infer the consequences of long-term APJ agonism in patients with chronic heart failure. However, our study suggests that apelin infusion might be of benefit in patients with acute decompensated heart failure in whom these hemodynamic effects of vasodilatation, increased cardiac output, reduced preload, and potential inotropy...
would be beneficial. Our data on the direct cardiac effects of apelin are not definitive; further work is needed to assess the load-independent effects of apelin on myocardial contractility.

Conclusions

We have described here the local and systemic hemodynamic effects of apelin infusion in humans. We have demonstrated, for the first time, direct vascular, coronary, and systemic effects that are preserved in patients with heart failure. We conclude that APJ agonism has important cardiovascular effects in humans that have therapeutic potential in the treatment of acute and chronic heart failure.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Heart failure constitutes a major and growing health burden in developed nations. Despite considerable treatment advances, it has a prognosis worse than that of many cancers and results in severe morbidity with impaired quality of life and recurrent hospitalization. The development of novel treatments for patients with heart failure therefore remains a major research priority. Apelin was recently identified as the endogenous ligand for the novel G protein–coupled receptor APJ. In preclinical models, apelin enhanced myocardial contractility and cardiac output, reduced ventricular preload and afterload, and retarded progression of heart failure. We have recently shown for the first time that apelin is vasoactive in humans, causing reproducible nitric oxide–mediated vasodilatation in the human forearm. In the present series of studies, we demonstrate that systemic apelin infusion in humans increases cardiac output while lowering peripheral vascular resistance. In addition, we demonstrate that apelin is a coronary vasodilator and increases cardiac contractility in humans. Finally, we show that both the local vascular and systemic hemodynamic effects of apelin are preserved in patients with stable chronic heart failure maintained on optimal contemporary pharmacological therapy. We therefore believe that APJ agonism represents an exciting novel therapeutic target in patients with both acute and chronic heart failure.
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SUPPLEMENTAL MATERIAL
Figure 2

Log plasma BNP concentration (pg/mL)

Plasma AVP concentration (pg/mL)

Log plasma Renin activity (ng/mL)
Supplementary Figure Legends

**Figure 1.** Percentage change from baseline in mean arterial pressure, heart rate, cardiac index and peripheral vascular resistance index during infusion of (Pyr₁)apelin-13 (■) and 0.9% saline placebo ( ●) in patients with chronic heart failure (left hand panel) and matched controls (right hand panel). Values are mean±SEM. 2-way ANOVA (apelin v placebo).

**Figure 2.** Plasma BNP and AVP concentrations and plasma renin activity in venous blood samples from heart failure patients (■) and matched controls ( ■) before and after infusion of (Pyr₁)apelin-13. Values are mean±SEM. **P<0.01, ***P<0.001, 2 way ANOVA (patients v controls) with post-hoc Bonferroni tests.