Apelin Has In Vivo Inotropic Effects on Normal and Failing Hearts

Mark F. Berry, MD; Timothy J. Pirolli; Vasant Jayasankar, MD; Jeffrey Burdick, BS; Kevin J. Morine, BA; Timothy J. Gardner, MD; Y. Joseph Woo, MD

Background—Apelin has been shown ex vivo to be a potent cardiac inotrope. This study was undertaken to evaluate the in vivo effects of apelin on cardiac function in native and ischemic cardiomyopathic rat hearts using a novel combination of a perivascular flow probe and a conductance catheter.

Methods and Results—Native rats (n = 32) and rats in heart failure 6 weeks after left anterior descending coronary artery ligation (n = 22) underwent median sternotomy with placement of a perivascular flow probe around the ascending aorta and a pressure volume conductance catheter into the left ventricle. Compared with sham-operated rats, the ligated rats had significantly decreased baseline Pmax and max dP/dt. Continuous infusion of apelin at a rate of 0.01 μg/min for 20 minutes significantly increased Pmax and max dP/dt compared with infusion of vehicle alone in both native and failing hearts. Apelin infusion increased cardiac contractility, indicated by a significant increase in stroke volume (SV) without a change in left ventricular end diastolic volume (102±16% change from initial SV versus 26±20% for native animals, and 110±30% versus 26±11% for ligated animals), as well as an increase in preload recruitable stroke work (180±24 mm Hg versus 107±9 mm Hg for native animals).

Conclusions—The present study is the first to show that apelin has positive inotropic effects in vivo in both normal rat hearts and rat hearts in failure after myocardial infarction. Apelin may have use as an acute inotropic agent in patients with ischemic heart failure. (Circulation. 2004;110[suppl II]:II-187–II-193.)

Key Words: apelin ■ cardiac output ■ heart failure ■ hemodynamics ■ inotropic agents

Apelin was found in 1998 to be an endogenous ligand for the APJ receptor, which previously had been identified by the Human Genome Project in 1993 as a member of the superfamily of 7-transmembrane G-protein coupled receptors. Apelin and APJ are widely expressed throughout the body and have functional effects in both the central nervous and the cardiovascular systems. Apelin was recently found to be a potent inotropic agent in isolated rat heart preparations. The apelin–APJ signaling pathway has also recently been identified as a potentially important mediator in the pathophysiology of chronic heart failure, because human chronic heart failure patients have left ventricular levels of apelin and APJ that are different compared with normal controls.

To date, there is no information on the functional effects of apelin in ischemic cardiomyopathy, nor is there any information on the cardiovascular effects of apelin in vivo. This study was undertaken to assess the in vivo functional effects of apelin on both native rats and in rats with heart failure 6 weeks after a large myocardial infarct (MI). This study used a combination of an intraventricular pressure volume conductance catheter with a perivascular flow probe placed around the ascending aorta to give a direct and continuous measurement of cardiac output (CO) to evaluate cardiac function.

Methods

Animal Use
Adult male Wistar rats (n = 32; Charles River Laboratories, Boston, Mass) and adult male Lewis inbred rats (n = 37, Charles River Laboratories) were used for this study. Wistar rats were chosen for initial studies of native heart measurements because of cost and ease of availability and handling. For subsequent heart failure studies, Lewis rats were chosen based on low mortality and predictable development of heart failure after left anterior descending coronary artery ligation. All animals received humane care in compliance with the Guide for the Care And Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, DC, 1996). The study was conducted in accordance with the animal care and use guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania.

Creation of Animals With Heart Failure
The Lewis rats were anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (5 mg/kg), endotracheally intubated with a 14-gauge angiocath, and mechanically ventilated with 0.5% isoflurane. A left thoracotomy was performed through the fourth interspace and the proximal left anterior descending artery was encircled with a suture. In the animals with heart failure (n = 26), the suture was tied to create a large anterior left ventricular MI. In the
sham animals (n = 11), the suture was removed without being tied. The animals were closed in 3 layers and recovered.

**Apelin Infusion and Hemodynamic Measurements**

The native Wistar rats and the Lewis rats 6 weeks after ligation or sham operation were anesthetized, intubated, and mechanically ventilated as described. A 24-gauge angiocath was placed in the femoral vein after exposure of the femoral vessels. A median sternotomy was performed, and a perivascular flow probe (Transonic Systems) was placed around the ascending aorta to provide continuous measurements of mean CO in mL/min. A 2-French pressure-volume conductance catheter (Millar Instruments) was introduced into the left ventricle (LV) retrograde through the apex as previously described. This catheter gives pressure measurements in mm Hg and volume measurements in relative volume units. Hemodynamic measurements were recorded and analyzed using the ARIA 1 Pressure Volume Analysis software (Millar Instruments). The probe and catheter are shown in Figure 1. All measurements were made with the animals under full anesthesia and the chest open.

Baseline hemodynamic measurements from the flow probe and the conductance catheter were recorded in all animals. For the sham-operated rats, no subsequent interventions or measurements were made. Two native rats were subjected to 4 interventions to verify that the flow probe and conductance catheter measurements responded in a predictable fashion. These interventions were inferior vena cava (IVC) occlusion to reduce preload, intravenous normal saline bolus to increase preload, intravenous injection of dobutamine to increase cardiac contractility, and partial occlusion of the thoracic descending aorta to increase afterload. After each intervention, subsequent interventions were not performed until hemodynamics had returned to a steady state for at least 2 minutes.

In 1 set of animals (20 native rats and all ligated rats), a continuous intravenous infusion of either 5% dextrose in water (D5W) alone or DSW containing human apelin-16 (Phoenix Pharmaceuticals) at a concentration of 200 μg/L was then started at a flow rate of 0.05 mL/min with a peristaltic pump (Variable Flow Mini-Pump, Low Flow, model 3385, Control Company) via the femoral catheter. For the animals receiving apelin, this flow rate resulted in delivery of 0.01 μg of apelin per minute. The infusion was continued for 20 minutes in each group, with hemodynamic measurements from the flow probe and the conductance catheter recorded every 5 minutes. Measurements were taken every 5 minutes for an additional 15 minutes after stopping the continuous infusion.

In the remaining native animals (n = 10), baseline pressure-volume loops were recorded with IVC occlusion to allow analysis of load-insensitive measures of LV function. Infusion of D5W with apelin (n = 5) or DSW alone (n = 5) was then performed as described for 15 minutes, after which IVC occlusion measurements were repeated. All animals were euthanized at the conclusion of measurements under full anesthesia via an injection of KCl.

**Statistical Analysis**

All values are expressed as mean±SEM. The unpaired Student t test using a 2-tailed distribution was used to calculate the statistical significance between the means of the baseline hemodynamic parameters of the sham and ligated animals. Repeated measures ANOVA with Bonferroni posttest was performed using GraphPad InStat Version 3.06 32 bit for Windows (GraphPad Software) to calculate the statistical significance between apelin and control groups at time points 0, 5, 10, 15, and 20 minutes after the start of the intravenous infusion. *P*<0.05 was considered to be significant.

**Results**

**Assessment of Hemodynamics**

The measurements yielded by the flow probe and the conductance catheter varied predictably in the 12 native rats who underwent interventions to change preload, contractility, or afterload. Figure 2A shows that a decrease in preload via occlusion of the IVC decreases LV end diastolic volume (EDV), which results in a decrease in LV pressure and CO as measured by the conductance catheter and the flow probe, respectively. Figure 2B shows that an intravenous normal saline bolus increases EDV, which results in corresponding increases in LV pressure and CO. Figure 2C shows that an intravenous injection of the inotropic dobutamine increases stroke volume (SV), as indicated by a decrease in LV end-systolic volume and no change in EDV, with a corresponding increase in SV. Figure 2D shows that a sudden increase in afterload via partial occlusion of the descending thoracic aorta causes a corresponding increase in LV pressure, whereas CO has a slight initial decrease and then returns toward the value observed immediately before the intervention that increased afterload.

**Mortality and Cardiac Function of Heart Failure Animals**

Five of the 37 Lewis rats that underwent planned survival surgery died in the immediate postoperative period; 4 of these had undergone MI and 1 underwent the sham operation. All animals that survived the perioperative period lived until the time of hemodynamic measurements 6 weeks later. Table 1 shows that the rats that had the left anterior descending ligated had significantly decreased systolic function compared with the sham rats, as measured by maximum pressure (Pmax) and maximal change in pressure over time (max dP/dt). The animals also had significantly decreased diastolic function, as measured by minimum change in pressure over time (min dP/dt) and τ, the time constant of relaxation.

**Effect of Apelin on Native Heart Function**

Apelin administration significantly increased Pmax and maximum dP/dt in the native rats compared with control (Figure 3A and Table 2, *P*<0.05 at time points 15 and 20 minutes). Apelin also increased CO, although the results approached but did not reach statistical significance (Figure 4A and Table...
Figure 2. The effects of various interventions on the left ventricular (LV) volume and pressure measured by the conductance catheter and the cardiac output (CO) measured by the flow probe. Each intervention was performed at time zero, when animal hemodynamics were at steady-state. Volume is measured in relative volume units (RVUs). A, Decrease in preload via inferior vena cava (IVC) occlusion. B, Increase in preload via 1 mL intravenous saline bolus. C, Increase in contractility via intravenous dobutamine bolus. D, Increase in afterload via partial occlusion of the descending thoracic aorta.
2. Contractility, assessed by change in SV over time, was also significantly increased (Figure 5A and Table 2, \( P < 0.05 \) for time points 10, 15, and 20 minutes). SV was calculated from the CO measured by the flow probe and the heart rate measured by the conductance catheter. To a lesser degree, apelin also improved diastolic function, as measured by minimum dP/dt (Table 2 and Figure 3A), although apelin had no effect on \( \tau \), the relaxation time constant (data not shown). These effects were not caused by increased preload and LVEDV from the volume infusion (Figure 5A). These effects were also not caused by decreased afterload, because LV end systolic pressure did not decrease with apelin administration (Figure 4A) or increased chronotropy, because heart rate did not increase with apelin infusion and changes in heart rate were not significantly different between control and treated animals (Figure 4A). Of note, heart rate did decrease significantly over time in both groups, probably as a result of cumulative anesthesia and ambient temperature loss during surgery. This decrease in heart rate is likely the reason for the decrease in CO observed in control animals.

Hemodynamic parameters obtained during IVC occlusion both at baseline and after 15 minutes of apelin or control infusion also show that apelin increases cardiac contractility (Figure 6). Representative pressure-volume loops obtained during IVC occlusion show that the loops are unchanged by control infusion but increased significantly upward by apelin infusion (Figure 6A). Analysis of the relationship between stroke work (SW) and EDV, which was determined from pressure-volume loops obtained during IVC occlusion, also demonstrates that apelin increases cardiac contractility. The slope of this relationship, which is preload recruitable stroke work (PRSW), was unchanged by control infusion and increased significantly by apelin infusion (Figure 6B, 6C).

Effect of Apelin on Function of Hearts in Postinfarction Failure

Apelin administration to the rats in failure 6 weeks after MI also significantly increased Pmax and max dP/dt, as well as CO compared with control (Figures 3B and 4B and Table 2, \( P < 0.05 \) at time points 5, 10, 15, and 20 minutes). Contractility was also significantly increased (Figure 5B and Table 2, \( P < 0.05 \) for time points 15 and 20 minutes). Apelin also improved diastolic function, as measured by minimum dP/dt (Figure 3B and Table 2), although again apelin had no effect on \( \tau \), the relaxation time constant (data not shown). These effects were not caused by increased heart rate (Figure 4B) or increased preload and LVEDV from the volume infusion (Figure 5B). These effects were also not caused by decreased afterload, because LV end systolic pressure did not decrease with apelin administration (Figure 4B).

**TABLE 1. Baseline Hemodynamic Data for Sham-Operated and Ligated Rats 6 Weeks After Initial Surgery**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham Animals</th>
<th>Ligated Animals</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum LV pressure, mm Hg</td>
<td>91±4</td>
<td>53±2</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Maximum LV dP/dt, mm Hg/sec</td>
<td>4530±360</td>
<td>1530±100</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Minimum LV dP/dt, mm Hg/sec</td>
<td>-4650±400</td>
<td>-1020±80</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Time constant of relaxation, msec</td>
<td>13±1</td>
<td>26±2</td>
<td>&lt;0.002</td>
</tr>
</tbody>
</table>

LV indicates left ventricle.
Discussion

Apelin was found to be the endogenous ligand for the orphan receptor APJ in 1998. The apelin gene, located on band q25–26.1 of the X chromosome, encodes a 77-amino acid preproprotein. The 41 N-terminal amino acid residues represent secretory signal sequences, and the 36 C-terminal residues comprise apelin-36, which is the protein that binds APJ. Both apelin and APJ have a wide distribution throughout the body, with apelin plasma concentrations much lower than tissue concentrations, suggesting that apelin works in an autocrine or paracrine manner. In the rat central nervous system, apelin controls respiration and regulates arterial blood pressure and water intake. After its discovery, APJ was also anticipated to play a role in cardiovascular homeostasis because its gene, located on band q12 of chromosome 11, is 40% to 50% identical with the gene for the angiotensin receptor (AT1) in the hydrophobic trans-membrane regions of these receptors, and APJ and AT1 have a similar tissue distribution. Even though angiotensin II does not bind APJ and apelin has no sequence homology with angiotensin II, apelin has been found to have cardiovascular effects.

Apelin and APJ are both expressed in the heart. Human coronary artery endothelial cells and human coronary artery smooth muscle cells express apelin, and APJ is located in vascular smooth muscle cells at very low density in both diseased and nondiseased human epicardial coronary arteries. Cardiomyocytes express less, but still detectable, levels of apelin and APJ. Apelin is also located in the endothelium of the small arteries of other viscera and lowers blood pressure via a nitric oxide-dependent mechanism. Apelin has recently been demonstrated to be a potent inotrope. Administration of apelin-16 induced a dose-dependent gradual increase in developed tension in isolated rat heart preparations, with a significant increase in contractility observed 2 minutes after the start of apelin infusion and maximal increase seen after 24 minutes. Apelin signal transduction from the membrane receptor to its nuclear targets involves activation of the mitogen-activated protein

| TABLE 2. Hemodynamic Changes After 20 Minutes of Apelin Infusion for Native Rats and Rats in Heart Failure |
|---------------------------------|---------------------------------|---------------------------------|
|                                | Native Rats                     | Heart Failure Rats              |
|                                | Baseline (n=20)                 | Baseline (n=22)                 |
|                                | D5W Infusion (n=10)             | D5W Infusion (n=11)             |
|                                | Apelin Infusion (n=10)          | Apelin Infusion (n=11)          |
| CO, mL/min                     | 34±3                           | 22±2                           |
|                               | 31±3                           | 20±2                           |
|                               | 45±7                           | 32±2†                          |
| Pmax, mm Hg                    | 77±3                           | 53±2                           |
|                               | 80±3                           | 59±4                           |
|                               | 99±7†                          | 73±2†                          |
| Max dP/dt, mm Hg/sec           | 3320±190                       | 1530±100                       |
|                               | 3740±130                       | 1600±150                       |
|                               | 5320±520‡                      | 2680±180‡                      |
| ∆SV, %                         | 0                              | 150±100                        |
|                               | 20±2                           | 160±150                        |
|                               | 102±16‡                        | 2680±180‡                      |
| Min dP/dt, mm Hg/sec           | -2790±190                      | -1020±80                       |
|                               | -2310±270                      | -1100±120                      |
|                               | -3560±500†                     | -1420±80‡                      |

CO indicates cardiac output; Pmax, maximum left ventricular pressure; Max dP/dt, maximum change in pressure over time; ∆SV, change in stroke volume from baseline; Min dP/dt, minimum change in pressure over time.

*P<0.05 compared to D5W.
†P<0.01 compared to D5W.
‡P<0.001 compared to D5W.

Figure 4. The effects of apelin infusion on CO, heart rate (HR), and left ventricular end systolic pressure (LVESP) in (A) native rats and (B) rats in heart failure. Heart rate is measured in beats per minute (BPM). Infusion was from time 0 to 20 minutes. *P<0.05, †P<0.01, ‡P<0.001.
kinase cascade. Stimulation of APJ by apelin is via the α-subunit of a Pertussis toxin-sensitive G-protein coupled to APJ in a Ras-independent but protein kinase C-dependent pathway. Apelin does not work via adrenergic pathways, because β-adrenergic receptor and α-receptor antagonism, as well as inhibition of myocardial nitric oxide synthase and endothelin receptor antagonism, had no effect on the apelin-induced increase in developed tension. Inhibition of phospholipase C, protein kinase C, the sarcolemmal Na/H+ exchanger, and the reverse mode sarcolemmal Na+/Ca2+ exchanger all significantly suppress the effects of apelin. The inotropic effects of apelin may be caused by Na+/H+ exchanger-mediated intracellular alkalinization and sensitization of cardiac myofilaments to intracellular Ca2+, as well as to Na+/H+ exchanger-mediated accumulation of intracellular Na+ that indirectly increases intracellular Ca2+ via the reverse mode Na+/Ca2+ exchanger.

The apelin–APJ signaling pathway has also recently been identified as a potentially important mediator in the pathophysiology of chronic heart failure, which affects >4 million patients in the United States and occurs because of coronary artery disease in 70% of patients. The left ventricles of human heart failure patients have increased levels of apelin mRNA with similar levels of APJ mRNA compared with healthy controls.

Figure 5. The effects of apelin infusion on stroke volume (SV), percent change in SV from baseline, and left ventricular end diastolic volume (EDV) in (A) native rats and (B) rats in heart failure. Volume is measured in relative volume units (RVUs). Infusion was from time 0 to 20 minutes. *P<0.05, †P<0.01, ‡P<0.001.

Figure 6. A, Representative pressure-volume loops for a single animal from the control and apelin groups at baseline and after 15 minutes of infusion. Control infusion does not change the loops, whereas apelin infusion shifts the loops upward. B, Apelin causes an increase in PRSW, the slope of the SW–EDV relationship, indicating increased contractility. Control infusion does not change PRSW. C, Representative SW–EDV relationships obtained during preload reduction for a single animal in the control (top graph) and apelin (bottom graph) groups at baseline and after 15 minutes of infusion show that apelin increases the slope, which is PRSW. The slope is unchanged by control infusion. *P<0.01 compared with apelin baseline, †P<0.05 compared with control infusion.
Mechanically offloading failing human hearts with left ventricular assist device placement significantly increases left ventricular APJ gene expression and apelin levels. Although one recent study showed that plasma apelin levels were decreased in human heart failure patients, another showed that plasma apelin levels increase in early heart failure before decreasing in late disease, suggesting that the apelin–APJ system is recruited to support the failing heart in mild to moderate left ventricular dysfunction.

The ex vivo effects of apelin on normal hearts were confirmed in vivo in this study. In addition, the effects of apelin were maintained in ischemic cardiomyopathic hearts. This study is the first to show that apelin maintains its inotropic effects in hearts in failure. Apelin thus holds promise for use as a novel inotropic agent for patients with ischemic cardiomyopathy.

This study used a novel hemodynamic monitoring system. A pressure-volume conductance catheter is valuable in measuring small animal cardiovascular dynamics because it gives simultaneous cardiac chamber pressure and volume measurements, which previously have been validated using echocardiography and requires catheter placement exactly along the longitudinal axis of the ventricle. Cardiac contractility can be assessed with the conductance catheter by examining pressure-volume relationships while changing cardiac load conditions, such as reducing preload with IVC occlusion. The use of a perivascular flow probe around the ascending aorta in this study in conjunction with the conductance catheter allowed us to assess cardiac contractility in our study without changing load conditions. The flow probe measures volume directly and allows direct measurement of CO, which can be coupled with the heart rate measurement from the conductance catheter to give an exact measurement of SV. In our study, in which a minimal amount of fluid was infused and in which the pressure-volume conductance catheter showed that LVEDV (used as an index for preload) did not increase and LV end systolic pressure (used as an index for afterload in the absence of aortic stenosis) did not decrease, changes in SV can be used as a surrogate measurement of change in ejection fraction and, therefore, as a surrogate measurement of cardiac contractility. This system therefore allowed identification of cardiac-specific alterations in an intact animal without requiring any animal manipulation, which could have perturbed the position of the catheter within the left ventricle and potentially made the comparison of volume measurements between various time points difficult.

The effects of apelin-16, which is 100% identical between humans and rats and has previously been shown to have functional effects in rat hearts, were assessed in this study. Investigations into the effects of using apelin of both larger and smaller fragments are needed. Although apelin with shorter C-terminal fragments than apelin-36 have higher activity, these fragments also have more transient effects than apelin-36. The sustained effects seen with the longer fragment are probably because the N-terminal is thought to modulate dissociation from the receptor, whereas the C-terminal is thought to bind to the APJ receptor. A longer apelin fragment may result in a more sustained inotropic effect than that seen in this study. Studies examining the effects of chronic apelin administration on long-term cardiac function will also be useful in assessing apelin treatment of chronic heart failure.

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References
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