In Vitro and In Vivo Evaluation of a Small-Molecule APJ (Apelin Receptor) Agonist, BMS-986224, as a Potential Treatment for Heart Failure

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BACKGROUND: New heart failure therapies that safely augment cardiac contractility and output are needed. Previous apelin peptide studies have highlighted the potential for APJ (apelin receptor) agonism to enhance cardiac function in heart failure. However, apelin’s short half-life limits its therapeutic utility. Here, we describe the preclinical characterization of a novel, orally bioavailable APJ agonist, BMS-986224.

METHODS: BMS-986224 pharmacology was compared with (Pyr1) apelin-13 using radio ligand binding and signaling pathway assays downstream of APJ (cAMP, phosphorylated ERK [extracellular signal-regulated kinase], bioluminescence resonance energy transfer–based G-protein assays, β-arrestin recruitment, and receptor internalization). Acute effects on cardiac function were studied in anesthetized instrumented rats. Chronic effects of BMS-986224 were assessed echocardiographically in the RHR (renal hypertensive rat) model of cardiac hypertrophy and decreased cardiac output.

RESULTS: BMS-986224 was a potent (Kd=0.3 nmol/L) and selective APJ agonist, exhibiting similar receptor binding and signaling profile to (Pyr1) apelin-13. G-protein signaling assays in human embryonic kidney 293 cells and human cardiomyocytes confirmed this and demonstrated a lack of signaling bias relative to (Pyr1) apelin-13. In anesthetized instrumented rats, short-term BMS-986224 infusion increased cardiac output (10%–15%) without affecting heart rate, which was similar to (Pyr1) apelin-13 but differentiated from dobutamine. Subcutaneous and oral BMS-986224 administration in the RHR model increased stroke volume and cardiac output to levels seen in healthy animals but without preventing cardiac hypertrophy and fibrosis, effects differentiated from enalapril.

CONCLUSIONS: We identify a novel, potent, and orally bioavailable nonpeptidic APJ agonist that closely recapitulates the signaling properties of (Pyr1) apelin-13. We show that oral APJ agonist administration induces a sustained increase in cardiac output in the cardiac disease setting and exhibits a differentiated profile from the renin-angiotensin system inhibitor enalapril, supporting further clinical evaluation of BMS-986224 in heart failure.

Key Words: apelin ■ heart failure ■ hemodynamics ■ ligands ■ signal transduction

The long-term prognosis for patients diagnosed with heart failure (HF) remains poor despite available treatment options. More than half of patients with HF have reduced ejection fraction (EF) and exhibit significant impairment in ventricular contractility. In those patients, there is a high unmet need for novel therapies to safely augment cardiac contractility and output given the poor long-term outcomes associated with established inotropic agents.

The APJ (apelin receptor) and its endogenous peptidic ligands constitute an emerging hormonal system...
WHAT IS NEW?

- Apelin is a natural hormone that improves cardiac function in humans when given by intravenous infusion and represents a potentially promising therapeutic agent in heart failure. However, due to high instability, apelin is not suitable for chronic oral administration, which limits its further evaluation in patients with heart failure.
- In this study, we have identified molecule BMS-986224 whose biological and cardiovascular effects closely resemble those of apelin peptide in cells and animal models.
- Unlike apelin, BMS-986224 has drug-like properties, and when administered orally to rats with heart failure, it produces sustained increase in cardiac output.

WHAT ARE THE CLINICAL IMPLICATIONS?

- Our studies show that BMS-986224 is a novel, orally bioavailable molecule whose biological effects closely resemble those of apelin peptide, making it a suitable therapeutic candidate for further evaluation in the clinical setting.

### Nonstandard Abbreviations and Acronyms

| Abbreviation | Description |
|--------------|-------------|
| APJ          | apelin receptor |
| BNP          | brain natriuretic peptide |
| CO           | cardiac output |
| EC50         | half maximal effective concentration |
| EF           | ejection fraction |
| ERK          | extracellular signal-regulated kinase |
| GPCR         | G-protein–coupled receptor |
| HD           | high dose |
| HEK293       | human embryonic kidney 293 |
| HF           | heart failure |
| HR           | heart rate |
| LV           | left ventricle |
| NT-proBNP    | N-terminal pro-B-type natriuretic peptide |
| RAS          | renin-angiotensin system |
| RHR          | renal hypertensive rat |
| SHR          | spontaneously hypertensive rat |
| SV           | stroke volume |

implicated in cardiovascular homeostasis. In a series of human studies, Barnes et al highlighted the promising cardiovascular effects of apelin in the HF setting. For instance, infusion of apelin peptides increased cardiac contractility, cardiac output (CO), and left ventricular (LV) EF, while lowering peripheral vascular resistance, with minimal changes in mean arterial pressure or heart rate (HR). These effects were preserved in patients with chronic HF on background therapy. This makes the apelin-ARJ system an attractive target for therapeutic intervention in the setting of HF, potentially augmenting CO while mitigating hypotension or tachycardia. Apelin is secreted as a 77-amino acid preproprotein that is progressively cleaved to shorter fragments, of which the 13-amino acid (Pyr1) apelin-13 fragment represents the most abundant and active form in vivo. The utility of (Pyr1) apelin-13 as a therapeutic agent in chronic HF has been significantly hindered by its short half-life and instability in circulation (≈5 minutes), necessitating intravenous dosing. Therefore, there is a need for an apelin-like oral agent with an improved pharmacokinetic profile to further assess the therapeutic potential of APJ agonism in a chronic setting.

In this article, we report characterization of a novel, orally bioavailable APJ nonpeptide agonist molecule and describe its preclinical characterization in vitro and in vivo. We demonstrate that BMS-986224 is a potent, selective APJ agonist that closely mimics the (Pyr1) apelin-13 signaling profile. Importantly, we show that BMS-986224 also recapitulates (Pyr1) apelin-13 in vivo pharmacology, with the added benefit of sustained efficacy with oral dosing.

### METHODS

#### Data Sharing

BMS policy on data sharing may be found at https://www.bms.com/researchers-and-partners/independent-research/data-sharing-request-process.html. The available data and methods supporting the findings of this study are available from the corresponding author upon request.

#### In Vitro Characterization Studies

(Pyr1) apelin-13 (GenScript) and BMS-986224 (synthesized by Bristol Myers Squibb) were used in the in vitro experiments.

#### [F] Apelin-13 Radioligand Binding Assays

Stable human embryonic kidney 293 (HEK293) cell lines were cultured to express human, monkey, dog, or rat APJ for purposes of cell membrane extract isolation and radioligand binding experiments. For complete methodology, see Methods in the Data Supplement.

#### GPCR Selectivity Assays

GPCR (G-protein–coupled receptor) competitive radioligand binding selectivity assays were conducted to assess the potential of BMS-986224 for off-target interaction. The assays were performed as described previously (Methods in the Data Supplement).

#### cAMP Inhibition Assays

To assess BMS-986224 suppression of cAMP production, we utilized stable HEK293 (HEK293 ZF) cell lines expressing human, monkey, dog, rat, or mouse APJ proteins, as described in the Methods in the Data Supplement.
β-Arrestin Recruitment
Chinese hamster ovary-K1 PathHunter cells were engineered to stably coexpress the enzyme acceptor-tagged β-arrestin2 and human APJ containing a ProLink tag at the C terminus (Methods in the Data Supplement).

ERK Phosphorylation
HEK293 ZF cells expressing human APJ were incubated with serial dilutions of test compounds, and chemiluminescent signals were measured using a commercial ERK (extracellular signal-regulated kinase) assay kit (Methods in the Data Supplement).

β-Arrestin Recruitment
Chinese hamster ovary-K1 PathHunter cells were engineered to stably coexpress the enzyme acceptor-tagged β-arrestin2 and human APJ containing a ProLink tag at the C terminus (Methods in the Data Supplement).

APJ Internalization Assays
HEK293 ZF cells expressing enhanced green fluorescent protein–tagged human APJ were incubated with serial dilutions of test compounds (Methods in the Data Supplement).

Bioluminescence Resonance Energy Transfer–Based Biosensor Assays
The signaling profiles of (Pyr1) apelin-13 and BMS-986224 were assessed in HEK293 cells and cardiomyocytes derived from induced pluripotent stem cells overexpressing human APJ. β-arrestin1 and 2 recruitment and G-protein activation were evaluated using bioluminescence resonance energy transfer–based biosensor assays. The methodology has been described elsewhere13–15 (Methods in the Data Supplement).

In Vivo Characterization Studies
Male Sprague-Dawley rats (≥250 g; Harlan, Charles River, or Envigo) were group-housed on a 12-hour light/dark cycle and habituated for ≥7 days before experimentation. Food and water were provided ad libitum. All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the regulations of the Institutional Animal Care and Use Committee of Bristol Myers Squibb (Methods in the Data Supplement).

Acute Hemodynamic Studies in Normal, Instrumented Rats
For details on surgical preparation procedures and evaluation of (Pyr1) apelin-13 and BMS-986224 in the instrumented rat model see Methods in the Data Supplement and Figure IA and IB in the Data Supplement.

Chronic Studies in the RHR Model
Surgical Preparation
See Methods in the Data Supplement for details.

Subcutaneous Administration of BMS-986224. For details on subcutaneous administration and study details, see Methods in the Data Supplement.

Oral Administration of BMS-986224. For dose selection and study details, see Methods in the Data Supplement.

Statistical Analyses and Study Size Calculations
The determination of the number of animals needed for the acute studies in normal rats and subcutaneous RHR (renal hypertensive rat) studies was based on past experience with similar studies in these models. The variability observed in the data from the subcutaneous RHR studies served to guide sample size in the pivotal oral RHR studies. The variability in CO, observed in vehicle and treatment groups, helped the power the study to detect a 25% increase in CO with 80% power and type 1 error probability of P=0.05. For the dobutamine study, Student’s t test for paired sample means was used to determine statistically significant differences. For the remaining in vivo studies, 1-way ANOVA was used with Tukey or Dunnett tests for multiple comparisons (GraphPad Prism, version 5.0 and above). We used the D’Agostino–Pearson normality test to assess normality of data. A P of <0.05 was considered significant.

RESULTS

In Vitro Pharmacological Characterization of BMS-986224 and Comparison With (Pyr1) Apelin-13
The goal of our studies was to identify a small molecule that would mimic (Pyr1) apelin-13 effects in vitro and in vivo. Pharmacological characterization studies focused on interaction of BMS-986224 with the APJ receptor and downstream signaling pathways known to be modulated by (Pyr1) apelin-13.3 BMS-986224 is a small-molecule APJ agonist identified by extensive optimization of a high-throughput screening lead from over 2 million compounds (see Methods in the Data Supplement for compound structure details).11,16

BMS-986224 Is a High-Affinity, Fully Competitive, and Selective Ligand for the APJ Receptor
In initial assessments, (Pyr1) apelin-13 binding affinity for APJ was consistent with previous reports (see Table III in the Data Supplement and associated results in Results in the Data Supplement).17 Evaluation of BMS-986224 showed it to be a fully competitive and high-affinity orthosteric ligand, displacing (Pyr1) apelin-13 from the human APJ receptor (Figure 1A). The Ki binding constant for BMS-986224 was similar between species (Figure 1B) and displayed similar binding kinetics to (Pyr1) apelin-13 (Table IV in the Data Supplement). BMS-986224 was highly selective for the APJ receptor and did not bind other GPCRs when tested up to 30 µmol/L concentration (Table V in the Data Supplement).

BMS-986224 Suppressed cAMP Production and Stimulated β-arrestin Recruitment, ERK Phosphorylation, and API Internalization
APJ activation has been shown to inhibit cAMP production, stimulate β-arrestin recruitment, increase ERK phosphorylation, and induce receptor internalization.18–20 Both BMS-986224 and (Pyr1) apelin-13 fully inhibited forskolin-mediated cAMP production, with similar mean half maximal effective concentration (EC50) values for human APJ of 0.02±0.02 and 0.05±0.07 nmol/L, respectively (Figure 2A; Table VI in the Data Supplement). Mean EC50 values for monkey, dog, rat, and mouse APJ were similar for the two agonists across species (Table VI in the Data Supplement).
Relative to (Pyr1) apelin-13, BMS-986224 fully stimulated β-arrestin recruitment, ERK phosphorylation, and APJ internalization in Chinese hamster ovary-K1 or HEK293 ZF cells (Figure 2B through 2D). Although the overall concentration-response curves were similar, there was a tendency for BMS-986224 to be more potent than (Pyr1) apelin-13 (Figure 2B and 2D).

**BMS-986224 and (Pyr1) Apelin-13 Exhibited Similar Signaling Profiles in Bioluminescence Resonance Energy Transfer-Based Assays**

To assess the potential for biased signaling with respect to (Pyr1) apelin-13, BMS-986224 was evaluated in a suite of bioluminescence resonance energy transfer-based biosensor assays. As shown in Figure 3A through 3I, both ligands were able to fully activate the different Gi family members. Across the assays, the EC50 values for BMS-986224 and (Pyr1) apelin-13 were similar, with a tendency for BMS-986224 to be more potent than (Pyr1) apelin-13 (Figure 2B and 2D).

|                  | Human APJ  | Monkey APJ | Dog APJ  | Rat APJ  |
|------------------|------------|------------|----------|----------|
|                  | Ki (nM),   | Ki (nM),   | Ki (nM), | Ki (nM), |
| **mean ± SD**    | mean ± SD  | mean ± SD  | mean ± SD| mean ± SD|
| BMS-986224       | 0.074 ± 0.055 | 0.056 ± 0.016 | 0.037 ± 0.026 | 0.049 ± 0.023 |
| (Pyr1) apelin-13 | 0.169 ± 0.039 | 0.074 ± 0.027 | 0.027 ± 0.004 | 0.064 ± 0.032 |

**Figure 1. Characterization of BMS-986224 binding properties in the APJ (apelin receptor) binding assay.* A, Representative experiment illustrating decreased binding of [3H] apelin-13 to cell membranes expressing the human APJ with increasing concentrations of unlabeled BMS-986224 or (Pyr1) apelin-13. B, Summary of inhibitory constant (K) for BMS-986224 and (Pyr1) apelin-13 binding to APJ from multiple species. Pyr indicates pyroglutamyl. *(Pyr1) apelin-13 was used as a positive control in these experiments. †n=4 for human and rat assays, and ‡n=3 for monkey and dog assays.**

Acute Hemodynamic Studies in Normal, Instrumented Rats

Similar to the favorable in vivo vasoactive action of (Pyr1) apelin-13 observed in humans, we hypothesized that BMS-986224 would also produce an increase in CO when administered acutely. We tested this hypothesis in an instrumented anesthetized rat model. (Pyr1) Apelin-13 was first tested to verify that
the CO response seen in human studies was recapitulated in rats. Acute administration of (Pyr1) apelin-13 in anesthetized rats produced dose-dependent increases in stroke volume (SV), CO, and maximum rate of ventricular pressure rise (Table VIII in the Data Supplement; Figure IV in the Data Supplement) that were similar to those observed in humans. Interestingly, the plasma levels of (Pyr1) apelin-13 required to produce pharmacodynamic response in rats and humans were also similar (≈10 nmol/L total concentration, data not shown),21 suggesting that results in this model may be translatable to humans.

Following validation of the model with (Pyr1) apelin-13, BMS-986224 was tested at doses of 1, 10, and 100 μg/kg per minute, which produced dose-dependent increases in SV, CO, and maximum rate of ventricular pressure rise compared with vehicle-treated animals (Figure 4A through 4C; Figure VA through VC in the Data Supplement; see Table IX in the Data Supplement). These doses promoted increase in SV by an average of 6.0%, 12.6%, and 12.3% from baseline, respectively. CO increased by 8.2% from baseline at the 1 μg/kg per minute dose, 15.4% from baseline with the 10 μg/kg per minute dose, and a similar 15.9% increase at the highest dose. Based on the increases in CO, 1 μg/kg per minute was considered the minimum effective dose and 10 μg/kg per minute the maximum effective dose. Plasma exposure of BMS-986224 was dose dependent, ranging from 95±5 nmol/L (1 μg/kg per minute) to 150±21±220 nmol/L (100 μg/kg per minute; Table X in the Data Supplement). When adjusted for protein binding, free BMS-986224 plasma concentration at the minimum effective dose equaled 2 nmol/L, which was ≈100-fold above the cAMP EC50 and 10-fold above compound Kᵢ, corroborating our findings with (Pyr1) apelin-13.

Although there appeared to be a slight initial increase in HR at the onset of infusion with BMS-986224, this difference was transient and did not persist for the duration of infusion at any dose (Figure 4D; Figure VD in the Data Supplement). A small decrease in mean arterial pressure from baseline was noted, primarily at the beginning of infusion at 10 μg/kg per minute (1.9%; P<0.001 versus vehicle) and 100 μg/kg per minute (2.1%; P<0.001 versus vehicle; Figure 4E; Figure VE in the Data Supplement).
Figure 3. Representative concentration-response curves for G-protein activation and β-arrestin recruitment with (Pyr1) apelin-13 and BMS-986224 using bioluminescence resonance energy transfer (BRET)-based biosensor assays in human embryonic kidney 293 cells overexpressing APJ (apelin receptor) together with respective G proteins and β-arrestins. BRET signal values were converted into percentage of activation using the nonstimulated control as 0% and (Pyr1) apelin-13 maximal response as 100%. EC50 indicates half maximal effective concentration; PDZ-RhoGEF, rho guanine nucleotide exchange factor; and Pyr, pyroglutamyl.
Figure 4. BMS-986224 acute hemodynamics parameters and comparison with dobutamine in instrumented anesthetized rats.

Percentage change in (A) stroke volume, (B) cardiac output (CO), (C) maximum rate of ventricular pressure rise (dP/dt\(_{max}\)), (D) heart rate (HR), and (E) mean arterial pressure during 15-min intravenous (IV) infusion and 15-min washout of BMS-986224 (1, 10, 100 μg/kg per min) in instrumented anesthetized rats. Comparison of percentage change in (F) CO and (G) HR during and after 15-min IV infusion of BMS-986224 at a maximum efficacious dose or dobutamine at an equivalent efficacious dose.
Dobutamine is an established inotropic agent known to increase CO in large part by increasing HR. Our studies recapitulated these findings, with dobutamine-treated rats showing a marked, dose-dependent increase in HR in addition to increases in SV and CO when infused over 2 minutes (Table XI in the Data Supplement). When infused for 15 minutes at 1 μg/kg per minute, the mean increase in CO with dobutamine (11.2% from baseline) was similar to the maximum increase in CO achieved with BMS-986224 (≈15%), but unlike BMS-986224, dobutamine resulted in a substantial increase in HR (Figure 4F and 4G; Figure VI in the Data Supplement). Taken together, the cardiovascular effects of BMS-986224 in anesthetized rats closely resembled effects seen with (Pyr1) apelin-13 and were clearly differentiated from dobutamine.

**Chronic Studies in the RHR Model**

To develop BMS-986224 as a chronic therapy, it was important to determine whether chronic administration of BMS-986224 would increase CO in an experimental model of human heart disease. We selected the RHR model for chronic studies because it exhibits some of the common phenotypes of human hypertension-induced HF, including cardiac hypertrophy and fibrosis, diastolic dysfunction (increased isovolumic relaxation time), and increased BNP (brain natriuretic peptide) and NT-proBNP (N-terminal pro-B-type natriuretic peptide), as well as decreased SV and CO (data not shown and Wong et al).

**Subcutaneous Administration Studies With BMS-986224**

To establish an exposure–response relationship, BMS-986224 was given as a subcutaneous infusion via osmotic minipumps. The low dose targeted a steady-state concentration of ≈100 nmol/L (based on the minimum effective plasma levels of BMS-986224 observed in the instrumented rat model), and the high dose (HD) targeted a steady-state concentration that was ≈10-fold greater than the low dose.

The study design and results of the subcutaneous study of BMS-986224 at low doses and HDs in the RHR model are described in detail in the Data Supplement. Briefly, the achieved steady-state plasma concentrations during 10-day infusion were 102 and 2686 nmol/L at low dose and HD, respectively. At the low dose, BMS-986224 increased SV and CO without affecting other measured parameters, including the measured diastolic parameters, cardiac fibrosis, and heart weight in RHR. However, the increases in SV and CO were not noted with HD BMS-986224. The loss of effect at HD was further confirmed in a repeat study (Figures VII and VIII in the Data Supplement). These findings indicate that acute effects on CO seen in normal rat can be recapitulated in a disease model, with a potential loss of effect with high and continuous exposures to APJ agonism that may result from desensitization/tachyphylaxis.

**Oral Administration Studies With BMS-986224**

To further study the effects of repeated exposure to BMS-986224 and to guide human dose projection, we evaluated BMS-986224 following oral administration (Figure 5; Figures IX through XII in the Data Supplement). BID doses were selected to target plasma concentrations of ≈200 nmol/L at peak (0.1 mg/kg) and trough (1 mg/kg). The 200 nmol/L concentration was 2-fold greater than the minimum effective concentration in the acute instrumented anesthetized rat model to account for pharmacokinetic variability. Following BID oral administration of BMS-986224 at 0.1 and 1 mg/kg, peak plasma concentrations averaged 205±11 and 2095±161 nmol/L, respectively, and trough concentrations averaged 25±4 and 302±29 nmol/L, respectively. Exposures for the oral 0.1 and 1 mg/kg BID doses were area under the plasma drug concentration–time curve (0–24)=3421 nmol/L·h and area under the plasma drug concentration–time curve (0–24)=34280 nmol/L·h, respectively.

After 10 days of BMS-986224 administration, body weight was significantly decreased in the vehicle- and BMS-986224–treated animals versus sham-operated animals (this effect was attributed to the stress of BID dosing). Similar to the subcutaneous infusion studies, renal hypertension led to increased heart weight in the vehicle–treated versus sham-operated animals, and a significant reduction in heart weight was observed in the enalapril–treated versus vehicle–treated animals (Figure XH in the Data Supplement).

The cardiovascular parameters were measured by echocardiography at trough (Figure 5A through 5F) and peak (Figure IX through IXF in the Data Supplement) plasma concentrations. At trough, SV and CO were significantly increased relative to vehicle (P<0.05) for both the 0.1 mg/kg (SV, 62%; CO, 53%) and 1 mg/kg (SV, 56%; CO, 52%) doses. Additionally, a significant increase in LV end-diastolic volume versus vehicle was observed at 1 mg/kg. A small reduction in HR that reached statistical significance (P<0.05) was observed with the lower BMS-986224 dose. There were no significant differences in LV mass or EF between BMS-986224–treated and vehicle–treated animals. Overall, the changes measured at peak were similar and consistent with those observed at trough plasma levels.

In contrast to the response seen with BMS-986224, subcutaneous administration of the angiotensin-converting enzyme inhibitor enalapril reduced LV mass, heart weight, and EF and increased LV end-diastolic volume without significant effect on SV, CO, and HR when compared with vehicle–treated animals (Figure XA through XH in the Data Supplement). Overall, our findings from
the oral BID dosing studies indicated that increased SV and CO could be achieved with oral administration and sustained with prolonged dosing. Further, intermittent administration may allow for a wider dose range that is not subject to the tachyphylaxis observed with prolonged HD subcutaneous administration. In addition, the cardiovascular effects of BMS-986224 were clearly different from renin-angiotensin system (RAS) inhibitor enalapril.

Finally, we wanted to ascertain whether BMS-986224 would display similar sustained efficacy when administered QD. After 10 days of BMS-986224 QD administration, body weight was significantly decreased in the vehicle- and BMS-986224-treated animals versus sham-operated animals; this effect was attributed to the stress of QD dosing.

The 0.06 mg/kg QD dose was targeted to achieve a peak concentration of 100 nmol/L and the 0.2 mg/kg dose to provide an area under the plasma drug concentration–time curve (0–24) of 3421 nmol/L*h, which was observed following low-dose oral BID. The experimental study design is shown in Figure IF in the Data Supplement. Results of the RHR low-dose and HD QD study with BMS-986224 are described in detail in the Data Supplement. Briefly, BMS-986224 at both doses increased both SV and CO, reaching significance at peak concentration with HD BMS-986224 (Figure XI in the Data Supplement); subcutaneous enalapril behaved as expected (Figure XII in the Data Supplement). Thus, BMS-986224 was equally efficacious in the RHR model when dosed QD or BID.

**DISCUSSION**

The goal of this work was to identify a small-molecule APJ receptor agonist that would recapitulate the potency and pharmacology of (Pyr^1) apelin-13 but provide an acceptable pharmacokinetic profile for oral dosing.

Though apelin and APJ receptor expression and signaling have been extensively studied in cellular assay systems,^24^ the signaling pathways that mediate apelin effects in vivo are not well understood due to rapid down-regulation of receptor in explanted tissue cultures.^^25^
Therefore, in our evaluation of BMS-986224, we utilized (Pyr\(^1\)) apelin-13 peptide as a benchmark and showed that BMS-986224 potency, receptor pharmacology, and downstream signaling were similar. Furthermore, BMS-986224 was found to be highly selective for APJ, without affinity for many GPCRs commonly present in cardiovascular tissues. Previous studies have hypothesized a potential link between G-protein biased APJ agonism and improved efficacy in vivo.\(^{29-30}\) In our studies, BMS-986224 closely recapitulated (Pyr\(^1\)) apelin-13 signaling and did not exhibit meaningful bias. Future studies are needed to assess G-protein signaling pathways modulating cardiovascular activity in vivo and whether biased agonism could further potentiate the cardiovascular activity of BMS-986224.

The acute cardiovascular response of BMS-986224 in normal anesthetized rats was consistent with human apelin studies. BMS-986224 enhanced cardiac contractility and CO without affecting HR and with minimal changes in mean arterial pressure. Additional studies with pressure-volume loops are needed to determine the relative contribution of cardiac contractility to CO increase. The strikingly similar hemodynamic response seen in BMS-986224 and (Pyr\(^1\)) apelin-13 in preclinical models is consistent with similar compound potency across various APJ receptor assays and species. Of note, previous studies with (Pyr\(^1\)) apelin-13 in dogs with advanced HF have also shown improvements in CO and SV without affecting HR.\(^{30}\) Further, the plasma levels of (Pyr\(^1\)) apelin-13 required to produce pharmacodynamic response in rats and humans were also similar (≈10 nmol/L total concentration; Brash et al\(^{31}\) and data not shown). This further suggests that our results in rats could be translatable to humans. Importantly, (Pyr\(^1\)) apelin-13 and BMS-986224 enhanced CO without raising HR, suggesting potentially safer mechanism of action compared with the legacy inotrope dobutamine.

Despite its promising therapeutic potential, it has been difficult to study APJ agonism in the setting of chronic HF. Apelin peptides are highly unstable in plasma, difficult to measure, and lack oral bioavailability.\(^9\) Our studies with BMS-986224 in the RHR model have shown that APJ agonism can result in sustained efficacy with either parenteral or oral dosing. Most importantly, improvement in CO and SV was achieved with oral dosing (BID and QD). Unlike subcutaneous dosing, oral administration of BMS-986224 did not lead to tachyphylaxis, even at the highest dose tested (maximum plasma concentration, >300 nmol/L), potentially indicating that oral administration provides a wider dose window to achieve sustained efficacy while avoiding tachyphylaxis and receptor desensitization.\(^{31}\)

In the setting of hypertension-induced cardiac dysfunction, the beneficial effects of BMS-986224 were selective for functional cardiac improvements in hemodynamics, SV, and CO, without affecting cardiac structure (LV mass and cardiac fibrosis). This was in clear contrast to enalapril—a RAS inhibitor—which prevented hypertension, cardiac hypertrophy, cardiac fibrosis, and diastolic dysfunction without impacting SV and CO. This suggests that the APJ pathway modulates cardiac function via a mechanism independent of the RAS, also corroborated by Barnes et al.\(^4\) Furthermore, the BMS-986224-mediated increase in CO was not associated with a reduction in blood pressure, suggesting a direct impact on cardiac myocyte function, in line with previous findings using apelin peptides.\(^{30,32,33}\) Overall, our studies suggest that prolonged APJ agonism could represent a novel add-on approach to current HF therapies to selectively improve hemodynamics and increase CO. Future clinical studies are needed to assess the safety and efficacy of such an approach when combined with other currently approved agents with effects on cardiac remodeling. It should be pointed out that the effects of enalapril observed in RHR were expected. Similar findings were also reported with delapril—a congeners of enalapril—in SHRs (spontaneously hypertensive rats), which is another model of hypertensive heart disease. Delapril decreased LV EF, increased LV internal diameter, lowered BNP, decreased LV posterior wall thickness, and decreased heart weight compared with vehicle in SHR.\(^{34}\)

During manuscript preparation, a study by Ason et al\(^{35}\) described characterization of nonpeptidic APJ agonist molecules AM-8123 and AMG 986 in preclinical models. The compounds described exhibited similarities to our findings but also important differences. Similarly, Ason et al confirmed that it is feasible to identify nonpeptidic orally bioavailable molecules that closely resemble the potency, efficacy, and receptor signaling profile of (Pyr\(^1\)) apelin-13. Compounds described by Ason et al, much like BMS-986224, did not demonstrate G-protein selective bias relative to (Pyr\(^1\)) apelin-13. While Ason et al utilized different preclinical models, their studies were consistent with our overall conclusion that acute and chronic activation of APJ pathway leads to selective improvement in CO and stroke volume, without significantly altering HR and mean arterial pressure or worsening cardiac maladaptive remodeling and hypertrophy. The main difference in our findings relative to Ason et al is the demonstration of differential mechanism of action relative to the RAS pathway. Ason et al compared their compounds to losartan—a RAS antagonist—in the rat myocardial infarction model, to show comparable positive effects of APJ agonism and RAS blockade on systolic and diastolic function and reduction in cardiac hypertrophy and postmyocardial infarction collagen deposition. In contrast, our studies utilized the RHR model, where cardiovascular pathology is caused by renal injury–driven RAS pathway activation. Unlike Ason et al, we observed that BMS-986224 actions in RHR were highly selective to SV and CO parameters alone, without appreciable effects on diastolic function or cardiac remodeling, in clear contrast to enalapril—a RAS pathway inhibitor. It is not clear whether these differences are model
driven, but they do support mounting evidence suggesting the pleiotropic effects of APJ receptor are likely dependent on disease setting.

Our studies have several limitations. Although BMS-986224 was designed to closely resemble the signaling profile and receptor interaction characteristics of (Pyr)¹ apelin-13, it is possible that unknown differences between these compounds could limit extrapolation of preclinical findings with BMS-986224 to those seen with apelin peptides in humans. It should also be noted that only male rats were used in our studies. While the sustained effect on CO seen in the RHR model is promising, the mechanism responsible for these changes is not clear, and more studies are needed to assess their relevance to human disease.

In conclusion, we identified a novel, potent, selective, and orally bioavailable small-molecule APJ receptor agonist that recapitulated APJ receptor signaling properties and in vivo cardiovascular effects of endogenous (Pyr)¹ apelin-13. We showed that increased cardiac function could be sustained in a disease model with prolonged oral administration, supporting further evaluation of BMS-986224 in the clinical setting. Whether the favorable effects of BMS-986224 observed in preclinical models can be translated to human HF remains to be determined.

ARTICLE INFORMATION
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