A Single \( \beta \)-Amino Acid Substitution to Angiotensin II Confers \( AT_2 \) Receptor Selectivity and Vascular Function

Emma S. Jones, Mark P. Del Borgo, Julian F. Kirsch, Daniel Clayton, Sanja Bosnyak, Iresha Welungoda, Nicholas Hausler, Sharon Unabia, Patrick Perlmutter, Walter G. Thomas, Marie-Isabel Aguilar, Robert E. Widdop

Abstract—Novel \( AT_2 \)R ligands were designed by substituting individual \( \beta \)-amino acid in the sequence of the native ligand angiotensin II (Ang II). Relative ATR selectivity and functional vascular assays (in vitro \( AT_2 \)R-mediated vasorelaxation and in vivo vasodepressor action) were determined. In competition binding experiments using either \( AT_1 \)R- or \( AT_2 \)R-transfected HEK-293 cells, only \( \beta \)-Asp\(^1\)-Ang II and Ang II fully displaced \([\text{\textsuperscript{125I}}]\)-Ang II from \( AT_1 \)R. In contrast, \( \beta \)-substitutions at each position of Ang II exhibited \( AT_2 \)R affinity, with \( \beta \)-Tyr\(^4\)-Ang II and \( \beta \)-Ile\(^5\)-Ang II exhibiting \( \approx 1000 \)-fold \( AT_1 \)R selectivity. In mouse aortic rings, \( \beta \)-Tyr\(^4\)-Ang II and \( \beta \)-Ile\(^5\)-Ang II evoked vasorelaxation that was sensitive to blockade by the \( AT_2 \)R antagonist PD123319 and the nitric oxide synthase inhibitor L-NAME. When tested with a low level of \( AT_1 \)R blockade, \( \beta \)-Ile\(^5\)-Ang II (15 pmol/kg per minute IV for 4 hours) reduced blood pressure (BP) in conscious spontaneously hypertensive rats (\( \beta \)-Ile\(^5\)-Ang II plus candesartan, \(-24 \pm 4 \textrm{ mm Hg} \)) to a greater extent than candesartan alone (\(-11 \pm 3 \textrm{ mm Hg} \), \( n = 7 \), \( P < 0.05 \)), an effect that was abolished by concomitant PD123319 infusion. However, in an identical experimental protocol, \( \beta \)-Tyr\(^4\)-Ang II had no influence on BP (\( n = 10 \)), and it was less stable than \( \beta \)-Ile\(^5\)-Ang II in plasma stability assays. Thus, this study demonstrated that a single \( \beta \)-amino acid substitution resulted in a compound that demonstrated both in vitro vasorelaxation and in vivo depressor activity via \( AT_2 \)R.

This approach to the design and synthesis of novel \( AT_1 \)R-selective peptidomimetics shows great potential to provide insight into \( AT_2 \)R function. (Hypertension. 2011;57[part 2]:570-576.)

Key Words: angiotensin \( \bullet \beta \)-amino acid substitutions \( \bullet \) \( AT_2 \) receptor \( \bullet \) depressor \( \bullet \) vasorelaxation

Angiotensin II (Ang II; Asp\(^1\)-Arg\(^2\)-Val\(^3\)-Tyr\(^4\)-Ile\(^5\)-His\(^6\)-Pro\(^7\)-Phe\(^8\)) acts at 2 major receptor subtypes, designated as the type 1 (\( AT_1 \)R) and type 2 (\( AT_2 \)R) angiotensin receptors. The binding of Ang II to \( AT_1 \)R mediates vasoconstriction, cell growth, and remodeling leading to increased blood pressure (BP); cardiac, renal, and vascular hypertrophy; and fibrosis, which is the molecular basis for the clinical application of \( AT_1 \)R antagonism. The binding of Ang II to \( AT_2 \)R is thought to oppose the \( AT_1 \)R-mediated effects of Ang II via vasodilator and antiproliferative effects.

Many studies have used substituted analogs of Ang II as well as receptor mutagenesis to understand the pharmacophore responsible for high-affinity Ang II binding and activity at \( AT_1 \)R and \( AT_2 \)R. Many such structure-activity studies using Ang II analogs were performed using radioligand binding assays and functional assays, such as the measurement of vascular contraction at a time prior to the identification of \( AT_2 \)R subtypes. Previous studies identified that Tyr\(^4\), His\(^6\), and Phe\(^8\) were important for agonism in assays that recognize classical Ang II activity via \( AT_1 \)R stimulation.

Subsequent cloning of the angiotensin receptors revealed that the \( AT_1 \)R is the primary mediator of cardiovascular actions of Ang II. To study \( AT_2 \)R, the peptide compound CGP42112\(^\#\) has been used almost exclusively as a selective \( AT_2 \)R agonist, and the nonpeptide compound PD123319 has been used as a selective \( AT_2 \)R antagonist. Thus, research elucidating the relevance of \( AT_2 \)R modulation of cardiovascular function has been severely hampered by a lack of selective compounds, either agonists or antagonists, to probe \( AT_2 \)R function, particularly in chronic in vivo experiments.

The use of \( \beta \)-amino acids is now an emerging area in the field of peptidomimetics. \( \beta \)-Amino acids are similar in structure to \( \alpha \)-carbons except they contain an “extra” carbon atom, ie, \( \beta \)-amino acid substitution results in an amino acid with an identical side chain (R group) but containing an additional methylene group (CH\(_2\)) in the peptide backbone. \( \beta \)-Amino acid substitution can greatly affect the binding properties and stability of the \( \beta \)-analog peptide through changes to backbone conformation, delayed structural effects, or nonstructural changes to the spatial positioning of the side chain.
Table 1. Peptide Sequences for Native Ang II and β-Substituted Ang Peptides*

<table>
<thead>
<tr>
<th>Peptide Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang II</td>
<td>Asp-Arg-Val-Tyr-Ile-His-Pro-Phe</td>
</tr>
<tr>
<td>β-Asp&lt;sup&gt;-&lt;/sup&gt;-Ang II</td>
<td>β-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe</td>
</tr>
<tr>
<td>β-Arg&lt;sup&gt;-&lt;/sup&gt;-Ang II</td>
<td>Asp-β-Arg-Val-Tyr-Ile-His-Pro-Phe</td>
</tr>
<tr>
<td>β-Val&lt;sup&gt;-&lt;/sup&gt;-Ang II</td>
<td>Asp-β-Val-Tyr-Ile-His-Pro-Phe</td>
</tr>
<tr>
<td>β-Tyr&lt;sup&gt;-&lt;/sup&gt;-Ang II</td>
<td>Asp-β-Tyr-Ile-His-Pro-Phe</td>
</tr>
<tr>
<td>β-Ile&lt;sup&gt;-&lt;/sup&gt;-Ang II</td>
<td>Asp-β-Ile-Ile-His-Pro-Phe</td>
</tr>
<tr>
<td>β-Pro&lt;sup&gt;-&lt;/sup&gt;-Ang II</td>
<td>Asp-β-Pro-Ile-Ile-His-Pro-Phe</td>
</tr>
<tr>
<td>β-Phe&lt;sup&gt;-&lt;/sup&gt;-Ang II</td>
<td>Asp-β-Phe-Ile-Ile-His-Pro-Phe</td>
</tr>
</tbody>
</table>

*α-Amino acid residues in bold denote the position of the β-substitution.

Therefore, in the present study, we have synthesized a series of β-substituted Ang II peptide analogs, such that the corresponding β-substituted amino acid has replaced the α-amino acid at each position in the Ang II sequence (except at His<sup>5</sup>), as shown in Table 1. We hypothesized that this series may exhibit altered AT<sub>2</sub>R:AT<sub>1</sub>R selectivity and peptide stability in vitro and in vivo.

**Methods**

Male 16- to 18-week-old spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats, weighing ~300 to 350 g, and male ~25-week-old FVB/N mice, weighing ~25 to 30 g, were obtained from the Animal Resource Center. Animals were maintained on a 12-hour day/night cycle with standard laboratory rat or mouse chow and water available ad libitum. Experiments were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Monash University Standing Committee on Ethics in Animal Experimentation.

**Peptide Synthesis**

Peptides were synthesized on a 100-mmol scale using standard Fmoc chemistry and Wang resin. The resin was washed (3 x 30 s) with N,N,N',N'-tetramethyl-uronium hexafluoro-phosphate (3 eq. to resin loading), 1,1,3,3-tetramethyl urea, and diethyl ether. The TFA was evaporated under a stream of N<sub>2</sub>, and the peptide was precipitated by the addition of diethyl ether. The precipitate was filtered and reconstituted in H<sub>2</sub>O/acetonitrile (1:1) for lyophilization. Peptides were then purified to homogeneity by preparative reverse-phase high-performance liquid chromatography (HPLC) using an Agilent HP1200 system fitted with a Vydac C<sub>18</sub> (250 x 22 mm) reverse-phase column. The eluents were 0.1% aqueous TFA and 0.1% TFA in acetonitrile. The identities and purities of purified peptides were assessed by analytic HPLC and mass spectrometry using an Agilent 1100 MSD SL ion trap mass spectrometer.

**Binding Assays**

The generation of plasminid expressing hemagglutinin-tagged versions of the AT<sub>1</sub>R and AT<sub>2</sub>R have been described previously. HEK-293 cells in 12-well plates were transfected with either AT<sub>1</sub>R or AT<sub>2</sub>R plasmids (0-6 μg/well) using lipofectAMINE (4-8 μL/well), as described previously, and stably expressing clones obtained by selection with G418 (1 mg/mL) and dilution limiting. HEK cell clones expressing either AT<sub>1</sub>R or AT<sub>2</sub>R were plated in 12-well plates for whole-cell competition binding assays using the nonselective Ang II ligand [L<sup>2</sup>T]-Ang II and various Ang II peptides. Nonlinear regression of the data was achieved using GraphPad Prism (GraphPad Software Inc.), and IC<sub>50</sub> values, representing the concentration at which each ligand displaced 50% binding of [L<sup>2</sup>T]-Ang II in either AT<sub>1</sub>R- or AT<sub>2</sub>R-transfected HEK-293 cells, were calculated as affinity estimates for each ligand. Log ratios of IC<sub>50</sub> values for each ligand at AT<sub>1</sub>R:AT<sub>2</sub>R were determined as a measure of AT<sub>2</sub>R selectivity.

**In Vitro Reactivity**

Thoracic aortic rings obtained from male FVB/N mice (~25 weeks old) were set up for AT<sub>1</sub>R relaxation assays as described previously. Tissues were precontracted to 40% to 50% of a maximum U46619 (~3 x 10<sup>-7</sup> mol/L)-evoked contraction, and concentration-response curves to CGP42142 or selected β-Ang II peptidomimetic were performed in tissues that were preincubated with the AT<sub>1</sub>R antagonist losartan (100 μmol/L). Analogous studies were performed with peptides either alone or in the presence of PD123319 (1 μmol/L) or L-NNAME (1 μmol/L), performed in parallel. One tissue served as a time control (precontraction only).

**In Vivo BP Assays**

Experiments were performed in male SHR, aged 16 to 20 weeks and weighing between 300 and 385 g. Rats were anesthetized (75 mg/kg ketamine and 10 mg/kg xylazine, both intraperitoneally, supplemented as required). Two catheters were inserted into the right jugular for intravenous drug administration, one implanted into the right carotid artery for direct BP measurement as described previously. Rats were housed in individual cages and allowed free access to food and water while maintained on 12-hour day/night cycle. The arterial catheter was infused overnight with heparinized saline using an infusion pump. Twenty-four hours after the surgery, the arterial catheter was attached to a pressure transducer, and mean arterial pressure (MAP) and heart rate were computed from the phasic BP signal via a MacLab-8 data acquisition system (ADInstruments).

Rats were assigned to receiving a randomized within-animal treatment regime over 4 days, as previously reported to determine in vivo AT<sub>2</sub>R-mediated depressor function. For this protocol, animals received β-substituted Ang II ligand alone, AT<sub>1</sub>R antagonist candesartan (0.01 mg/kg IV) alone, β-substituted Ang II ligand plus candesartan, or the previous combination plus the AT2R antagonist PD123319 (50 μg/kg/min for 2 hours). We tested 2 groups of SHR. Group 1 received β-Tyr<sup>-</sup>-Ang II (15 pmol/kg per minute for 4 hours) as the test ligand (n=11), whereas group 2 received β-Ile<sup>-</sup>-Ang II (15 pmol/kg per minute for 4 hours) as the test ligand (n=9). It should be noted that we have repeatedly tested a saline infusion over this time, and it does not alter MAP. In another set of experiments, WKY rats (n=6) were set up for conscious MAP.
Protein kinase C (PKC) activation plays a key role in the development of hypertensive cardiovascular disease. Many PKC isotypes are expressed in the vasculature and play an important role in vascular smooth muscle cell proliferation and growth. The PKC inhibitor, bisindolylmaleimide I (BIM-I) is a specific and selective inhibitor of the 

A 572 Hypertension  March 2011, Part 2

Figure 1. Competition binding from 3 separate experiments (each in triplicate) for β-substituted Ang II peptides, native Ang II, and the AT2R antagonist PD123319 against 125I-Ang II in either AT1R-transfected (A) or AT2R-transfected (B) HEK-293 cells. IC50 values are listed in Table 2.

Statistical Analysis

All data are presented as mean responses±SEM. Differences in vasorelaxation or MAP between treatments were analyzed using a 2-way repeated-measure ANOVA. Nonlinear regression of binding data was also performed. All statistical analyses were performed using GraphPad Prism (version 5.0; GraphPad Software Inc.). Probability values <0.05 were considered statistically significant.

Results

As expected, Ang II displayed low nanomolar affinity at both the AT1R and AT2R expressed in HEK-293 cells. The AT2R antagonist PD123319 fully displaced AT2R binding but not AT1R binding (Figure 1). β-substitutions at each position of Ang II were well tolerated with respect to AT2R binding since all compounds completely displaced 125I-Ang II in AT2R-transfected HEK-293 cells (β-Asp1 Ang II equipotent with native Ang II) (Figure 1B). In contrast, apart from β-Asp1-Ang II and Ang II, all other peptides poorly displaced AT1R binding, with substitutions in positions 4 and 5 being relatively insensitive at 1 μmol/L (Figure 1A). IC50 values for all ligands at AT1R and AT2R are listed in Table 2, from which it was determined that β-Tyr4-Ang II and β-Ile5-Ang II were both ≈1000-fold selective for AT2R over AT1R. These selectivities were conservative estimates because the latter 2 compounds had IC50 values for AT1R arbitrarily set at 10 μmol/L since there was little displacement at 1 μmol/L.

On the basis of these binding data, we have tested functionality of 3 key peptides: a nonselective ligand (β-Asp1-

Table 2. IC50 Values (in nmol/L) and Relative AT2R Selectivity of Peptide Ligands

<table>
<thead>
<tr>
<th>Ligand</th>
<th>AT1R</th>
<th>AT2R</th>
<th>Log IC50 (AT1R: AT2R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang II</td>
<td>3.29</td>
<td>0.65</td>
<td>0.70</td>
</tr>
<tr>
<td>PD123319</td>
<td>&gt;1000</td>
<td>8.32</td>
<td>3.08†</td>
</tr>
<tr>
<td>β-Asp1-Ang II</td>
<td>1.58</td>
<td>0.79</td>
<td>0.30</td>
</tr>
<tr>
<td>β-Arg2-Ang II</td>
<td>275</td>
<td>2.24</td>
<td>2.09</td>
</tr>
<tr>
<td>β-Val3-Ang II</td>
<td>133</td>
<td>2.77</td>
<td>1.68</td>
</tr>
<tr>
<td>β-Tyr4-Ang II</td>
<td>&gt;1000</td>
<td>3.19</td>
<td>3.50†</td>
</tr>
<tr>
<td>β-Ile5-Ang II</td>
<td>&gt;1000</td>
<td>10.6</td>
<td>2.98‡</td>
</tr>
<tr>
<td>β-Pro7-Ang II</td>
<td>1000</td>
<td>2.46</td>
<td>2.61</td>
</tr>
<tr>
<td>β-Phe8-Ang II</td>
<td>420</td>
<td>6.56</td>
<td>1.81</td>
</tr>
</tbody>
</table>

†IC50 value of 10 μmol/L at AT1R used for calculation.
AngII and 0.01 mg/kg Candesartan

**Results from liquid chromatography mass spectrometry indicated that β-Tyr<sup>4</sup>-Ang II was rapidly degraded, compared to β-Ile<sup>5</sup>-Ang II. Noticeably, the formation of 2 main metabolites, des-Asp<sup>1</sup>-β-Tyr<sup>4</sup>-Ang II and des-Asp<sup>1</sup>, Phe<sup>8</sup> β-Tyr<sup>4</sup>-Ang II also were degraded quickly. The main metabolites of β-Ile<sup>5</sup>-Ang II were identified as β-Ile<sup>5</sup>-Ang III and des-Asp<sup>1</sup>, Phe<sup>8</sup> β-Ile<sup>5</sup>-Ang II. These metabolites also showed greater proteolytic stability. This was determined by the analysis of the accumulation of Ang III (des-Asp<sup>1</sup>-Ang II) or the β-substituted Ang III metabolite over time (measured by LC/MS) from the respective Ang II parent compounds in spiked plasma that were compared against synthesized standards of β-substituted Ang III peptides (Figure 7).

**Figure 2.** A, In vivo AT<sub>1</sub>R-mediated pressor responses in WKY rats (n=6) in response to Ang II (5 ng), β-Asp<sup>1</sup>-Ang II (5 ng), β-Tyr<sup>4</sup>-Ang II (40 ng), and β-Ile<sup>5</sup>-Ang II (40 ng). **P<0.001, ***P<0.01 vs native Ang II. B, In vitro AT<sub>1</sub>R-mediated vasorelaxation in mouse aorta (in the presence of 100 nmol/L losartan) in response to Ang II (5 ng), β-Tyr<sup>4</sup>-Ang II (40 ng), and β-Ile<sup>5</sup>-Ang II (40 ng). ***P<0.01 vs peptide alone or with PD123319 or L-NAME.

**Figure 3.** In vitro AT<sub>1</sub>R-mediated vasorelaxation in mouse aorta (in the absence of losartan) in response to β-Ile<sup>5</sup>-Ang II alone or in the presence of the AT<sub>1</sub>R antagonist PD123319 or the NOS inhibitor L-NAME (n=5 to 7). **P<0.01 for peptide alone vs with PD123319, L-NAME, or time control.

**Figure 4.** Effect of β-Tyr<sup>4</sup>-Ang II (15 pmol/kg per minute for 4 hours) on MAP in spontaneously hypertensive rats (n=11) in the absence and presence of candesartan (0.01 mg/kg IV) or PD123319 (50 μg/kg/min for 2 hours).

**Figure 5.** Effect of β-Ile<sup>5</sup>-Ang II infusion (15 pmol/kg per minute for 4 hours) on MAP in SHR (n=9) in the absence and presence of candesartan (0.01 mg/kg IV) or PD123319 (50 μg/kg/min for 2 hours). **P<0.01 for β-Ile<sup>5</sup>-Ang II plus candesartan combination vs all other treatments.
Figure 6. Plasma stability of native Ang II, compared with β-Tyr\(^4\)-Ang II or β-Ile\(^5\)-Ang II, assessed by determining the percentage peptide remaining following incubations in plasma over 24 hours.

**Discussion**

We have made the discovery that a single β-substitution in Ang II at the tyrosine (Y) or isoleucine (I) residue can markedly increase AT\(_2\)R selectivity and function, together with prolonging plasma stability. As such, these relatively simple peptide modifications represent a novel strategy to design AT\(_2\)R-selective peptidomimetic ligands for subsequent functional testing.

There is intense interest in the development of selective AT\(_2\)R agonists, particularly since PD123319 (AT\(_2\)R antagonist) and AT\(_2\)R knockout mice only indirectly inform on AT\(_2\)R function but do not address the importance of direct AT\(_2\)R activation. Compound 21 was recently developed, modeled on the C-terminal pentapeptide structure of Ang II. Compound 21 evoked a cardioprotective effect following myocardial infarction in rats, and we have shown that Compound 21 acutely lowers BP (during AT1R blockade) in conscious hypertensive animals. Clearly, there is an unmet need to develop a range of AT\(_2\)R agonists to examine class effects of AT\(_2\)R agonists directly in a number of cardiovascular diseases including heart failure and stroke.

The majority of previous studies that have reported on novel Ang II peptides have examined binding profiles in a range of tissues with varying proportions of AT\(_1\)R and AT\(_2\)R, often with no functional correlate to assess functionality. In the present study, using homogenous AT\(_1\)R-transfected cell lines, we have shown that individual β-amino acid substitutions were well tolerated for AT\(_2\)R binding but not for AT\(_1\)R binding, suggesting the constraints on AT\(_1\)R binding are more strict than AT\(_2\)R. Consistent with these findings, alanine or glycine scans of Ang II caused only minor changes in AT\(_2\)R binding affinity, whereas there was sometimes marked reduction in AT\(_1\)R binding. In the current study, on the basis of differential displacement of AT\(_2\)R and AT\(_1\)R binding, β-Tyr\(^4\)-Ang II and β-Ile\(^5\)-Ang II exhibited negligible AT\(_1\)R binding, similar to the AT\(_2\)R antagonist PD123319, thus identifying these peptides, which are modified at positions 4 and 5, as highly selective (>1000-fold) AT\(_2\)R ligands. These findings are consistent with other studies highlighting the importance of modification to the central portion of Ang II (at positions 4 and 5) for AT\(_2\)R binding. Substitution of His\(^6\) by 4-NH\(_2\)-Phe\(^6\) in Ang II produced a peptide with high AT\(_2\)R-to-AT\(_1\)R selectivity in binding assays. Hallberg and colleagues have also performed a number of modifications to Ang II, principally around the Tyr\(^4\)-Ile\(^5\) residues, resulting in a number of highly AT\(_2\)R-selective peptidomimetics. For example, γ-turn scaffolds have been introduced around residues 4 and 5 to produce AT\(_2\)R-selective compounds. In other studies, analogs with tyrosine-functionalized bicyclic dipeptides replacing the Tyr\(^4\)-Ile\(^5\) residues presented an extended backbone conformation with heightened AT\(_2\)R-to-AT\(_1\)R selectivity. The majority of the aforementioned studies relied on binding affinity estimates derived from displacement of iodinated Ang II from AT\(_2\)R in rat liver membranes and AT\(_2\)R in pig uterine membranes. However, functional assays on Ang II peptidomimetics that would indicate agonist or antagonist cardiovascular activity are lacking.

The Ang II peptidomimetics with β-Y and β-I substitutions both caused relaxation of isolated mouse aortic rings in a PD123319-sensitive manner, as also seen with CGP42112 used in parallel tissues. Thus, both binding and functional data would suggest that simple modification to the central portion of Ang II (at positions 4 and 5) yielded highly selective AT\(_2\)R agonists, signaling via nitric oxide, whereas β-Asp\(^1\)-Ang II was a nonselective agonist causing AT\(_2\)R-mediated relaxation and AT\(_1\)R-mediated in vivo pressor effects. Despite both β-Tyr\(^4\)-Ang II and β-Ile\(^5\)-Ang II causing vasorelaxation in vitro, only the latter was an effective vasodilator in vivo in SHR, during AT\(_1\)R block. This effect was confirmed to be mediated by the AT\(_2\)R as it could be reversed by concomitant PD123319 infusion.

The magnitude of β-Ile-Ang II–induced hypotension was similar to that observed in response to CGP42112, Ang (1–7), and Compound 21 in analogous studies.

One possibility for the discrepancy between in vitro (mouse) and in vivo (rat) functional data may relate to a species difference in AT\(_1\)R abundance, although this seems unlikely since we have reported identical in vivo AT\(_2\)R-mediated vasorelaxation evoked by Compound 21 in aortae from mice and aortae and mesenteric arteries from SHR, and β-Ile\(^5\)-Ang II was effective in both mouse and rat tissues in the current study. Rather, the discrepancy between the in
vitro and in vivo effects of β-Tyr⁴-Ang II may relate to the fact that, in plasma stability assays, β-Ile⁵-Ang II had a half-life ~10 times longer than native Ang II, while the half-life of β-Tyr⁴-Ang II was only double that of Ang II. Thus, β-substitution at position 5 conferred greater protease resistance to the molecule. However, it is acknowledged that, in vitro, plasma stability measurement does not account for tissue metabolism or cellular uptake, which will be addressed in future studies by determining plasma concentrations following in vivo peptide infusion. Nevertheless, it would appear that the major metabolite formed from β-Ile⁵-Ang II degradation, at least in plasma, was β-Ile⁵-Ang III, which accumulated to 3-times–greater levels than the corresponding metabolites, β-Tyr⁴-Ang III or Ang III, from their respective Ang II parent molecules. In this context, Ang III can act as an endogenous AT₂R ligand to lower BP and evoke natriuresis in a number of settings.32,33

Thus, the in vivo vasodepressor effect seen by β-Ile⁵-Ang II, but not β-Tyr⁴-Ang II, may be due to slowed metabolism of Ile⁵-Ang II acting at AT₂-R. However, at this stage, we cannot totally discount additional effects of other metabolites such as β-Ile⁵-Ang III, or even an Ile-modified-Ang (1–7) or Ang (2–7), contributing to β-Ile⁵-Ang II depressor activity.

In summary, our relatively simple chemical strategy of β-amino acid substitution to Ang II has resulted in Ang peptidomimetics with substantial AT₂-R selectivity, which translated into functional agonist activity. As such, this type of peptidomimetic design shows great potential for the production of research tools to provide insight into AT₂-R function. In particular, our study has revealed that β-Ile⁵-Ang II acted as a novel AT₂-R-selective agonist with in vivo vasodepressor activity.

Perspective

Research elucidating the effects of AT₂-R modulation of cardiovascular function has been severely hampered by a lack of selective agonists or antagonists to probe AT₂-R function, particularly in chronic in vivo experiments. In the current study, we have performed simple modifications to the peptide Ang II by incorporating an additional methylene group in the peptide backbone, resulting in a series of peptides with preferential AT₂-R selectivity. In particular, β-substitutions at positions 4 and 5 conferred marked selectivity for agonism at AT₂-R over AT₁-R. This strategy could be used to investigate a number of related peptides and will allow more detailed comparison with other AT₂-R agonists on cardiovascular function, thus providing greater information for class effects of AT₂-R.

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Disclosures

None.

References


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CHBPR 164301: A SINGLE BETA-AMINO ACID SUBSTITUTION TO ANGIOTENSIN II CONFFERS AT2 RECEPTOR SELECTIVITY AND VASCULAR FUNCTION

Emma S Jones¹, Mark P Del Borgo², Julian F Kirsch¹, Daniel Clayton², Sanja Bosnyak¹, Iresha Welungoda¹, Nicholas Hausler³, Sharon Unabia², Patrick Perlmutter³, Walter G Thomas⁴, Marie-Isabel Aguilar², Robert E Widdop¹*

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HPLC traces of plasma samples following incubation of a 1mg/mL solution of Angiotensin II at A) 0 mins B) 60 mins and C) 24 hours. The molecular weight of each peak is also shown and peptides identified.
HPLC traces of plasma samples following incubation of a 1mg/mL solution of β-Tyr-Angiotensin II at A) 0 mins B) 60 mins and C) 24 hours. The molecular weight of each peak is also shown and peptides identified.
HPLC traces of plasma samples following incubation of a 1mg/mL solution of β-Ile-Angiotensin II at A) 0 mins B) 60 mins and C) 24 hours. The molecular weight of each peak is also shown and peptides identified.