Elucidation of the Structure-Activity Relationships of Apelin: Influence of Unnatural Amino Acids on Binding, Signaling and Plasma Stability

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Introduction

Apelin is the endogenous ligand of APJ receptor, a member of the G protein-coupled receptor superfamily. There is currently little information on the structure/activity relationship (SAR) of apelin (Scheme 1). In an effort to better delineate SAR, we synthesized analogs of apelin-13 modified at selected positions with unnatural amino acids, with a particular emphasis on the C-terminal portion and Pro\textsuperscript{12}. Analogs were then tested in binding and functional assays by evaluating G\textsubscript{i/o} mediated reduction in cAMP levels and by assessing β-arrestin2 recruitment to the receptor. The plasma stability of new analogs was also assessed. Several were found to possess increased binding, biased β-arrestin2 signaling and higher stability compared to the parent peptide.

Scheme 1
\begin{align*}
\text{\texttt{<Glu}}^{1}\text{-R}^{2}\text{-P}^{3}\text{-L}^{5}\text{-S}^{6}\text{-H}^{7}\text{-K}^{8}\text{-G}^{9}\text{-P}^{10}\text{-M}^{11}\text{-P}^{12}\text{-F}^{13} & \\
\text{Important for binding [1]} & \text{Important for binding and APJ internalization [1]} \\
\text{ACE-2 cleavage, Half-life in vitro < 1min [2]} & \\
\end{align*}

Results and Discussion

The C-terminal Phe\textsuperscript{13} of apelin-13 was replaced by unnatural amino acids (R1, Table 1). This set of modifications was performed on the Met11Nle analog which possesses a similar profile in terms of affinity, coupling to second messenger cascades, and stability to that of apelin-13 (IC\textsubscript{50} 5.7 nM ; EC\textsubscript{50} cAMP 1.9 nM ; EC\textsubscript{50} β-arr2 91 nM). Analogs Phe13Dip and Phe13Bip displayed a 10-fold difference in affinity suggesting that the C-terminal binding site is deep rather than wide. Interestingly, Phe13Cha exhibited an affinity comparable to that of apelin-13, indicating that hydrophobic interactions are necessary for binding, but aromatic, π -stacking type interactions are not essential. Phe13-1Nal and Phe13-2Nal showed an interesting trend in the β-arrestin2 pathway. Replacement of Pro\textsuperscript{12} by Aib provided a very potent analog, and Pro12Aminoindane exhibited a biased signaling in β-arrestin2 pathway (R2, Table 1). Finally, C-terminally modified analogs showed significant improvements in plasma stability over apelin-13, whereas modification of Pro12 displayed more variable results (Scheme 2).
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Dip</th>
<th>Bip</th>
<th>1Nal</th>
<th>2Nal</th>
<th>Cha</th>
<th>(2,4,5-trifluoro)F</th>
<th>Aib</th>
<th>Aminoidane</th>
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<tbody>
<tr>
<td>IC_{50} (nM)</td>
<td>88 ± 6</td>
<td>7.8 ± 0.4</td>
<td>14 ± 0.9</td>
<td>1.2 ± 0.1</td>
<td>2.3 ± 0.6</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>20 ± 1</td>
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<tr>
<td>EC_{50} cAMP (nM)</td>
<td>ND</td>
<td>10 ± 3</td>
<td>28 ± 2</td>
<td>20 ± 6</td>
<td>20 ± 8</td>
<td>20 ± 9</td>
<td>30 ± 13</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>EC_{50} β-arr2 (nM)</td>
<td>630 ± 179</td>
<td>361 ± 64</td>
<td>522 ± 110</td>
<td>70 ± 11</td>
<td>170 ± 32</td>
<td>32 ± 9</td>
<td>46 ± 10</td>
<td>1204 ± 208</td>
</tr>
</tbody>
</table>

Scheme 2

% of peptide remaining after 1h, 2h and 3h of incubation in rat plasma

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References
