Structure-activity Study of Endomorphins Analogs with C-terminal Substitution

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Abstract

Aims: To further wonder the influence of C-terminal residues on the pharmacological activities. Methods: The in vitro and in vivo opioid activities of C-terminal substitution analogs [L-Tic \textsubscript{4}] EM1 and [L-Tic \textsubscript{4}] EM2 were investigated using radioligand binding assay, guinea pig ileum (GPI) assay, mouse vas deferens (MVD) assay, systemic arterial pressure (SAP) assay and tail-flick test. Results: Our data showed that the analogs produced a higher \textdelta-opioid affinity but low \textmu-opioid affinity, dose-dependent but reduced analgesic activities and cardiovascular effect comparing with those of EMs. Moreover, these effects induced by the analogs can be inhibited by naloxone, indicating an opioid mechanism. Conclusion: These results provided suggestive evidences that the substitution of C-terminal residue may play an important role in the regulation of opioid affinities and pharmacological activities.

1. Introduction

Endomorphin 1 (EM1) and endomorphin 2(EM2), the endogenous \textmu-opioid ligands discovered from bovine and human brain in 1997, display various of biological functions [1], albeit with some undesired side-effects, which limited their clinical use and encouraged the studies regarding the chemical modifications on the structures of EMs in order to improve the pharmacological profile of EMs [2-3].
Among them, one hotspot attracting many attentions was the structure-activity relationship of C-terminal phenylalanine (Phe). Numerous studies demonstrated that Phe acts as an important element in determining opioid receptor binding affinity, however, the features that Phe is free to adopt a “bioactive” conformation at the receptor site and that activation can occur independently of the correct orientation and stereochemistry of this residue promote the studies of structure-activity of Phe [4]. In the past decades, a number of synthetic analogs have been developed with the aim to overcome these problems and confer to synthetic peptides advantages properties. Currently, our group has also synthesized a series of EMs analogs by substitution or modification of C-terminal residue to investigate the pharmacological effect of this residue [5-12]. In the present study, to further our knowledge of the influence of C-terminal substitution on the pharmacological activities, we have designed and synthesized the analogs of EMs with substitution of Phe4 by 1, 2, 3, 4-tetrahydroisoquinoline-3-carboxylic acid (Tic), meanwhile, radioligand binding assay, guinea pig ileum (GPI) assay, mouse vas deferens (MVD) assay, systemic arterial pressure (SAP) assay and tail-flick test have been performed to investigate the in vitro and in vivo opioid activities of these analogs. Our results gave the evidences that the substitution of C-terminal residue may play an important role in the regulation of opioid affinities and pharmacological activities.

2. Results and Discussion

Our previous study regarding the investigation of the conformational properties by 1D and 2D 1H NMR spectroscopy [8-9] and molecular modeling [11-12] provided suggestive evidences that the C-terminal residue of EMs affected these analogs conformations markedly, therefore changed the opioid receptor affinity. In the study, to get insight into the important role of C-terminal residue of EMs, we investigated the analogs by substitution of the C-terminal residue by Tic.

With the purpose of revealing the relationship of structure and bioactivities, most efforts have been centered on the substitution side chain modification, backbone modification or deletion. Tic has been utilized as a conformationally restricted analogue of Phe and was established to be a synthon to enhance δ receptor binding affinity and selectivity in many cases [13-14], which posses two potential advantages for replacing Phe in bioactive peptide ligands: constrained peptide backbone conformation and restricted orientation of the aromatic side chain [14]. Our radio-ligand binding assay, GPI assay and MVD assay data showed the EMs analogues exhibited lower μ affinities but higher δ affinity, revealing the transformation of selectivity of [L-Tic4]EMs from μ to δ receptors (shown in Table 1 and 2). Intravenous injection (i.v.) injections of the two analogs in doses of 0.3-100nmol/kg can cause significant dose-dependent decrease in SAP (Fig.1). The fact that the cardiovascular effects of analogs were inhibited by naloxone suggested opioid system is involved in the regulation of cardiovascular effect (Fig.2). Furthermore, the reduced affinity of [L-Tic4]EMs for μ-receptor made less influence on their cardiovascular effects thus the effect probably depend on their high affinity for δ-opioid receptor. We also tested the well documented analgesic effects of the analogs via tail-flick test. These data shown in Fig. 3 revealed that the analogs can produce a dose-dependent but short-lasting antinociceptive effect, probably owing to the much less receptors involved in the antinociception of [L-Tic4] EMs than that of EMs. Moreover, the antinociceptive effects can be inhibited by naloxone, indicating an opioid mechanism (data not shown).

In conclusion, the results of the present study indicated that C-terminal substitution produced different pharmacological activities both in vitro and in vivo assays. It was noteworthy that the analogs exerted weaker antinociceptive effects, but decreased the undesirable cardiovascular side effects. These data gave the evidences that C-terminal residue was essential for opioid pharmacological activities of EMs analogs. The substitution of Phe4 by Tic may play an important role in the regulation of opioid affinities and activities. The present study would be helpful in the development of suitable μ-opioid or δ-opioid
therapeutics.

Table 1 Opioid Receptor Binding Affinities of EMs and [L-Tic4] EMs Analogues a

<table>
<thead>
<tr>
<th>Compounds</th>
<th>( K_i \pm SE \text{(nM)}^b )</th>
<th>( K_i \pm SE \text{(nM)}^c )</th>
<th>( \frac{K_i(\delta)}{K_i(\mu)} )</th>
</tr>
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<tbody>
<tr>
<td>EM1</td>
<td>4.55 ± 0.06</td>
<td>5093 ± 621</td>
<td>1119.3</td>
</tr>
<tr>
<td>EM2</td>
<td>8.23 ± 0.15</td>
<td>&gt;10,000</td>
<td>&gt;1215</td>
</tr>
<tr>
<td>[L-Tic(^4)]EM1</td>
<td>1130.2 ± 126.5</td>
<td>634.4 ± 25.5</td>
<td>0.56</td>
</tr>
<tr>
<td>[L-Tic(^5)]EM2</td>
<td>4365.5 ± 325.1</td>
<td>831.8 ± 35.4</td>
<td>0.19</td>
</tr>
</tbody>
</table>

a. values are the mean of 8 experiments ± S.E.M.

b. \( ^3\text{H]DAMGO} \)
c. \( ^3\text{H]DPDPE} \)

Table 2 GPI And MVD Assay Data of Ems and [L-Tic4] EMs

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC(_{50}) ± SE (nM)(^a)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GPI (( \delta ))</td>
<td>MVD/GPI</td>
</tr>
<tr>
<td>EM1</td>
<td>3.41 ± 0.3</td>
<td>47.40 ± 9.75</td>
</tr>
<tr>
<td>EM2</td>
<td>5.68 ± 1.44</td>
<td>23.30 ± 6.57</td>
</tr>
<tr>
<td>[L-Tic(^4)]EM1</td>
<td>268.99 ± 23.68</td>
<td>18.59 ± 11.80</td>
</tr>
<tr>
<td>[L-Tic(^5)]EM2</td>
<td>934.85 ± 61.75</td>
<td>3.44 ± 2.38</td>
</tr>
</tbody>
</table>

a. values are the mean of 8 experiments ± S.E.M.

Figure 1. Bar graphs comparison decrease of SAP and HR in response to i.v. injections of [L-Tic4]EMs in anethetized rat (n = 4–6). The decrease of SAP and HR induced by [L-Tic4] EMs in different doses (a, b), values are shown as the Means ± S.E.M. Double asterisk (**) represent very significant results (p<0.01) in comparison with the control.
Figure 2. Antagonism by Naloxone of the Vasorelaxant Effect (%Relaxation)

Figure 3. The Antinociceptive Effect of [L-Tic4] EMs on the Nociceptive threshold measured by Tail-Flick Test in mice. The results are presented as % of the Maximal Positive Effect (MPE). 8–10 animals were used at each dose level, asterisk (*) represent significant results (p<0.05) in comparison with the control.

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References


