NEW ENDOMORPHIN ANALOGUES WITH 2`,6`-DIMETHYL-Tyr AND e-β-MePhe SUBTITUTIONS

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Endomorphins (endomorphin-1: H-Tyr-Pro-Trp-Phe-NH_2 and endomorphin-2: H-Tyr-Pro-Phe-Phe-NH_2) are the likely endogenous ligands of μ-opioid receptors [1]. These peptides showed high affinity and selectivity for μ-opioid receptors in rat and mouse brain homogenates and in recombinant μ-opioid receptors in direct and indirect radioreceptor binding assays. In order to improve their biological activity we designed and synthesized new analogues with unnatural amino acids such as 2',6'-dimethyltyrosine (Dmt), in position 1 and eritro-β-methylphenilalanine (e-β-MePhe) in position 4. Competitive radioreceptor binding assays indicated that four endomorphin analogues had high affinity for μ- and δ-opioid receptors in mouse brain membranes. (Dmt-Pro-Trp-(2S,3S)-β-MePhe-NH_2 $K_{i\mu}$ = 0.97 nM $K_{i\delta}$ = 12.53 nM, Dmt-Pro-Trp-(2R,3R)-β-MePhe-NH_2 $K_{i\mu}$ = 7.47 nM $K_{i\delta}$ = 145 nM, Dmt-Pro-Phe-(2S,3S)-β-MePhe-NH_2 $K_{i\mu}$ = 0.75 nM $K_{i\delta}$ = 35.48 nM, Dmt-Pro-Phe-(2R,3R)-β-MePhe-NH_2 $K_{i\mu}$ = 3.08 nM $K_{i\delta}$ = 76.40 nM). Some Dmt-endomorphin analogues stimulated [^{35}S]GTPγS binding in rat brain membranes, but these showed mixed μ agonist /δ antagonist properties using known specific μ and δ antagonists. Preliminary results supported these findings in recombinant cell membranes expressing the human μ- or human δ-opioid receptors. We found that two analougs were better analgetics than the analogous Dmt-endomorphins by the tail-flick tests. (Dmt-Pro-Trp-(2S,3S)-β-MePhe-NH_2 A_{50} = 0.026 nmol, Dmt-Pro-Trp-Phe-NH_2 A_{50} = 0.134 nmol, Dmt-Pro-Phe-(2S,3S)-β-MePhe-NH_2 A_{50} = 0.138 nmol, Dmt-Pro-Phe-Phe-NH_2 A_{50} = 0.64 nmol).

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[1] Zadina, J.E., Hackler, L., Ge, L.J., Kastin, A.J.: Nature 386, 499-502 (1997)