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A SUPERACTIVE ANTINOCICEPTIVE PENTAPEPTIDE, (D-Met^2, Pro^5)-ENKEPHALINAMIDE

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1. Introduction

Our recent study [1] on synthetic analogs of enkephalins (Tyr–Gly–Gly–Phe–*X*, *X* = Met and Leu [2]) has revealed some structural requirements for a pentapeptide to exert antinociceptive property by intravenous application. First, Gly^2 has been replaced by a D-amino acid, the side-chain of which has been supposed to provide an extra binding-site for the receptor [1]. To support this view the D-Met^2 analog was more active both in vitro and in vivo than the D-Ala^2 and even the D-Nle^2 ones [1]. Based on other considerations the replacement of Gly^2 by different D-amino acids has also been accomplished by other workers [3–5]. To protect the fourth peptide bond of enkephalins, i.e., Phe^4–*X*^{5}, against proteolysis Met/Leu^5 has been replaced by Pro, resulting in a further increase of the in vivo biological activity [1]. Finally, an alkyl amide group has been introduced into the COOH-terminus of the molecule with the purpose that it should furnish a further receptor binding-site. Ethyl amide appeared to be superior to amyl amide indicating the size of the alkyl amide-group to be crucial. As the most significant result of the above attempts, ethyl amide of (D-Met^2, Pro^5)-enkephalin has been reported to have 55% of the analgesic activity of morphine by intravenous injection [1].

In this paper we report the synthesis of an even more potent analgesic: (D-Met^2, Pro^5)-enkephalinamide.

Table 1
Relative antinociceptive potency of β-endorphin, Met^5-enkephalin and Pro^5-enkephalinamide analogs by the tail-flick test in rat

Compound	Potency ratio on a molar basis (morphine = 1)	
	Intravenously	Centrally
β-Endorphin	3–4[b]	19.3[a] 21.8[c]
H–Tyr–Gly–Gly–Phe–Met–OH	0[d]	0.02[d]
H–D-Ala–Pro–NH–Amyl	0.1[d]	0.08[d]
H–D-Ala–Pro–NH–Et	0.19[d]	16.9[d]
H–D-Ala–Pro–NH_2	0.22	3.9
H–D-Met–Pro–NH–Et	0.55[d]	16.9[d]
H–Tyr–D-Met–Gly–Phe–Pro–NH_2	5.5	49.8

[a] Ref. [8,9]
[b,c] Determined by the tail-flick test in mice [11] and in rat [10], respectively
[d] Ref. [1]

2. Experimental

Starting from free proline, Boc–Tyr–D-Met–Gly–Phe–Pro–OH was prepared by the stepwise method in solution. The N-protected peptide amide was obtained by dicyclohexylcarbodiimide-condensation of the peptide acid and ammonia which was added as its 1-hydroxybenzotriazole (HOBt) salt. Deblocking gave Tyr–D-Met–Gly–Phe–Pro–NH_2 (thin-layer chromatography: R_F 0.55 in a mixture of ethyl acetate/pyridine/acetic acid/water, 60:20:6:11). Amino acid analysis after acid hydrolysis showed the expected composition. Details of the synthesis of this compound and its analogs (some of the latter are also shown in table 1) were described elsewhere [6]. The new reagent for amidation, NH_3.HOBt, was obtained by dissolving HOBt in aqueous ammonia followed by dilution with acetone, melting point 184–185°C with darkening at 162°C.

Antinociceptive properties were examined by the tail-flick test in rat [7] as described previously [1].

3. Results

Data of table 1 show that (D-Met^2, Pro^5)-enkephalinamide is 5.5-times more potent than morphine when injected intravenously and 49.8-times more active than morphine when applied centrally. Thus for the first time an analgesic of peptide nature has been prepared which is more active than β-endorphin itself [8–11].

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