ENKEPHALIN ANALOGS CONTAINING AMINO SULFONIC ACID AND AMINO PHOSPHONIC ACID RESIDUES AT POSITION 5

S. BAJUSZ, A. Z. RÓNAI, J. I. SZÉKELY, A. TURÁN, A. JUHÁSZ, A. PATTHY, E. MIGLÉCZ and I. BERZÉTEI

Institute for Drug Research Budapest, POB 82, H-1325 Hungary

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1. Introduction

Enkephalins, the endogenous opioid peptides: Tyr–Gly–Gly–Phe–Met and –Leu [1], have been known to show much higher agonist potency in the mouse vas deferens preparation (MVD) than in the guinea-pig ileum assay system (GPI) [2–6]. The high MVD/GPI potency ratio is characteristic of enkephalins and of their synthetic analogs unless the pentapeptides are terminated by an amide function. For instance, potency ratios calculated for Met-enkephalin, its D-Ala^2 analog and [D-Ala^2, Met^5]-enkephalinamide were 9.0, 8.4 and 2.0, respectively [2]. The MVD/GPI ratio for β-endorphin, the 31-residue opioid peptide of the pituitary, is ~1.2 and for non-peptide narcotics, e.g., normorphine and morphine even smaller [2,4–6]. Accordingly, the acidic COOH group at the C-terminus is essential for the 'enkephalinoid' character of pentapeptides. Starting from this consideration we synthesized some analogs of norleucine (Nle)-enkephalin in which the terminal COOH group is replaced by SO_3H and PO_3H_2 group, respectively, i.e., Nle^5 is substituted by α-amino-pentane-sulfonic acid (NleS) and α-amino-pentane-phosphonic acid (NleP), respectively. The dissociation constant of a sulfonic acid or a phosphonic acid is known to be higher than that of the corresponding carboxylic acid. For instance, pK'_a values of taurine and β-alanine are 1.5 and 3.6, respectively [7]. Similarly, pK'_a of glycine is 2.34 while the two pK'_a values of amino-methane-phosphonic acid are 1.85 and 5.35, respectively [8]. The more acidic terminal groups were expected to improve the enkephalin-like activity of the peptides. To study this question the opiate agonist potencies of the new compounds were compared to those of the parent peptides with terminal COOH function.

2. Materials and methods

Nle^5-enkephalin and its analogs containing D-Ala, D-Nle or D-Met at position 2 and NleS, D-NleS, NleP or D-NleP at position 5 were prepared by the conventional synthesis in solution starting from Nle, DL-NleS and DL-NleP, respectively. For obtaining DL-NleS bisulfite compound of *n*-valeraldehyde was reacted with excess ammonium hydroxide in water followed by acidification. DL-NleP was obtained from its benzyloxycarbonyl(Z) derivative by hydrogenolysis. Z-DL-NleP was prepared by analogy with a known procedure [9]; i.e., by reacting benzyl carbamate, *n*-valeraldehyde and PCl_3 in acetic acid. DL-NleS and DL-NleP were acylated with N-protected L-phenylalanine by the mixed anhydride method. The diastereomeric mixture of dipeptides, i.e., Phe–NleS/Phe–D-NleS and Phe–NleP/Phe–D-NleP, respectively, obtained after deblocking, were separated by crystallization. Configuration of NleS and NleP in the dipeptides was determined by the rule of shift, i.e., the optical rotation of the NleS- and NleP-dipeptides were compared with those of the corresponding Nle-dipeptides of known configuration. This assignment was in agreement with the results of digestion of NleS^5-, D-NleS^5-, NleP^5- and D-NleP^5-enkephalins with aminopeptidase M (Röhm GmbH). Enzyme stability of these peptides was also examined against carboxypeptidase A (Miles-Yeda). Details of the synthetic work were described in [10]. The opiate agonist activities of compounds were determined in electrically

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stimulated mouse vas deferens [6,11] and guinea-pig ileum [12] preparations. Antinociceptive properties were assessed by the rat tail-flick test [13] as in [14].

3. Results and discussion

The opiate agonist activities determined in MVD and GPI are summarized in table 1. The peptides involved in this study can be described by the general formula Tyr–Xxx–Gly–Phe–Yyy wherein Xxx stands for Gly, D-Ala, D-Nle or D-Met and Yyy for Nle, NleS, D-NleS, NleP and D-NleP.

Replacement of Nle^5 by NleS or NleP resulted in enhanced biological potency in general. In case of substitution by NleS these increments of activity were nearly equal both in the MVD preparation and in the GPI assay, thus the MVD/GPI ratios changed by ⩽14–42%. Replacement by NleP increased potencies if the substituents at position 2 were Gly, D-Ala and D-Nle, respectively, while diminished activities were found for the $D\text{-}Met^2$ containing molecule. Higher potencies were obtained in MVD than in GPI. Similarly, in the case of $D\text{-}Met^2$ analog the activity measured in MVD was lowered to a lesser extent than in GPI. Thus the bulky, dibasic PO_3H_2 group at the 'C'-terminus seems to be preferred or tolerated by the δ receptors of MVD rather then by the μ receptors of GPI. As a consequence higher MVD/GPI potency ratios could be calculated for the $NleP^5$ analogs than for the corresponding Nle^5- or $NleS^5$-enkephalins.

Replacement by D-NleS or D-NleP resulted in a substantial loss of agonist activities in particular of these measured in MVD. With an amino carboxylic acid at the C-terminus of enkephalins the influence of the configuration seems to be just the opposite. Namely [$D\text{-}Ala^2$, $D\text{-}Leu^5$]-enkephalin was found to be 3-times more active in MVD than its $L\text{-}Leu^5$ analog [2]. In the case of [$D\text{-}Ala^2$, $D\text{-}NleS^5$]-enkephalin and its $L\text{-}NleS^5$ isomer this potency ratio is 0.06.

$NleS^5$- and $NleP^5$-enkephalins containing D-amino acid residue at position 2 possess analgesic activity when giving them intracerebroventricularly. Of these [$D\text{-}Met^2$, $D\text{-}NleP^5$]-enkephalin was the only compound

Table 1
Opioid agonist activities of enkephalin analogs Tyr–Xxx–Gly–Phe–Yyy in MVD and in GPI given in $10^{-6}/IC_{50}$ and their analgesic potencies relative to morphine (= 100)

Tyr–Xxx–Gly–Phe–Yyy	MVD $10^{-6}/IC_{50}^{a}$	GPI $10^{-6}/IC_{50}^{a}$	MVD / GPI	analgesic potency[b]	
				i.c.v.	i.v.
——Gly————Nle	48.49 (1)[c]	2.74 (1)[c]	17.7	–	–
——Gly————NleS	235.89 (4.8)	16.99 (6.2)	13.9	2	2
——Gly————NleP	430.96 (8.9)	7.81 (2.8)	55.2	1	1
——Gly————D-NleS	5.48 (0.1)	0.27 (0.1)	20.3	2	2
——D-Ala————Nle	1000.00 (1)	15.29 (1)	65.4	–	–
——D-Ala————NleS	1786.03 (1.8)	42.81 (2.8)	41.7	2.0	2
——D-Ala————NleP[d]	5100.84 (5.1)	61.16 (4.0)	83.9	15.0	2
——D-Ala————D-NleS[d]	58.10 (0.06)	15.29 (1.0)	3.8	9.8	2
——D-Nle————Nle	1077.24 (1)	16.78 (1)	64.2	–	–
——D-Nle————NleS[d]	4761.90 (4.4)	117.45 (7.0)	40.5	2	2
——D-Nle————NleP	4221.19 (3.9)	26.84 (1.6)	157.3	4.5	2
——D-Nle————D-NleS	10.07 (0.01)	8.39 (0.5)	1.2	2	2
——D-Met————Nle[d]	1667.78 (1)	25.97 (1)	64.2	–	–
——D-Met————NleS	2041.65 (1.2)	54.54 (2.1)	40.5	9.5	2
——D-Met————NleP	618.20 (0.4)	9.35 (0.4)	66.1	25.0	2
——D-Met————D-NleS	5.19 (0.003)	10.39 (0.4)	0.5	6.4	2
——D-Met————D-NleP	7.53 (0.004)	10.65 (0.4)	0.7	52.0	16.8

[a] Dimension is M^{-1}

[b] ED_{50} values of morphine in the tail-flick test in rats were 3.14 nmol/animal after intracerebroventricular (i.c.v.) administration and 1.9 μmol/kg after intravenous injection (i.v.)

[c] Changes in activity relative to the parent Nle^5 analogs

[d] The most active analogs of Nle^5- $NleS^5$-, D-$NleS^5$- and $NleP^5$-enkephalins

which caused antinociception after systemic administration as well.

Comparison of data given in table 1 also reveals that a significant interdependence exists between residues 2 and 5 of enkephalins, which is manifested in the peptide–receptor interactions either directly or through its contribution to the formation of preferential conformation of the given molecule. The structure of analogs possessing the highest activities in vitro (marked by asterisks) show the most favourable combination of the studied residues at position 2 and 5, respectively, i.e., D-Ala–NleP, D-Ala–D-NleS, D-Nle–NleS and D-Met–Nle, respectively. However, as for the analgesic potency (i.c.v.) of $NleS^5$- and $NleP^5$-enkephalins, $D\text{-}Met^2$ is the most prefered substituent, which, regarding the in vitro potencies is a rather unfavourable one. Similar interrelations could also be observed in other groups of enkephalin analogs [15].

A further fact which was proved by this study is that peptides containing D- or L-NleS or NleP as C-terminal residue were resistant towards carboxypeptidase A. Namely, only unchanged pentapeptides could be detected in the 2 h digest of $NleS^5$-, $D\text{-}NleS^5$-, $NleP^5$- and $D\text{-}NleP^5$-enkephalins while 60–70% of Nle and Phe were released from Nle^5-enkephalin under identical conditions [16]. Thus, replacement of COOH by SO_3H or PO_3H_2 seems to be a tool for preparing carboxypeptidase-resistant peptides with acidic 'C'-terminal of L-configuration.

References

[1] Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A. and Morris, H. R. (1975) Nature 258, 577–579.

[2] Kosterlitz, H. W. (1978) in: Endorphins '78 (Gráf, L. et al. eds) p. 267, Akadémiai Kiadó, Budapest.

[3] Lord, J. A. H., Waterfield, A. A., Hughes, J. and Kosterlitz, H. W. (1976) in: Opiates and endogenous opioid peptides (Kosterlitz, H. W. ed) pp. 275–280 Elsevier/North-Holland, Amsterdam, New York.

[4] Lord, J. A. H., Waterfield, A. A., Hughes, H. and Kosterlitz, H. W. (1977) Nature 267, 495–499.

[5] Rónai, A. Z., Gráf, L., Székely, J. I., Dunai-Kovács, Z. and Bajusz, S. (1977) FEBS Lett. 74, 182–184.

[6] Rónai, A. Z., Berzétei, I., Székely, J. I., Gráf, L. and Bajusz, S. (1979) Pharmacology 18, 18–24.

[7] Greenstein, J. P. and Winitz, M. (1961) in: Chemistry of the amino acids, vol. 1, pp. 486–487, Wiley London, New York.

[8] Fasman, G. D. ed (1976) Handbook of Biochemistry and Molecularbiology pp. 308, 318, CRC Press, (3rd edn) Cleveland, OH.

[9] Oleksyszyn, J., Tyka, R. and Mastalerz, P. (1978) Synthesis 479–480.

[10] Synthesis of these peptides is covered by pending patent application in the name of Institute for Drug Research.

[11] Hughes, J., Kosterlitz, H. W. and Leslie, F. M. (1975) Brit. J. Pharmacol. 53, 371–381.

[12] Kosterlitz, H. W., Lydon, R. J. and Watt, A. J. (1970) Brit. J. Pharmacol. 39, 398–413.

[13] D'Amour, F. E. and Smith, D. L. (1941) J. Pharm. 72, 74–79.

[14] Székely, J. I., Rónai, A. Z., Dunai-Kovács, Z., Miglécz, E., Berzétei, I., Bajusz, S. and Gráf, L. (1977) Eur. J. Pharmacol. 43, 293–294.

[15] Bajusz, S. and Rónai, A. Z. (1980) in preparation.

[16] Edman, P. and Henschen, A. (1975) in: Protein sequence determination (Needleman, S. B. ed) pp. 277–279, Springer, Berlin, Heidelberg, New York.