FURTHER ENHANCMENT OF ANALGESIC ACTIVITY: ENKEPHALIN ANALOGS WITH TERMINAL GUANIDINO GROUP

S. BAJUSZ, A. Z. RÓNAI, J. I. SZÉKELY, E. MIGLÉCZ and I. BERZÉTEI
Institute for Drug Research, Budapest, PO Box 82, 1325, Hungary

Received 12 September 1979
Revised version received 28 November 1979

1. Introduction

There is a great body of evidence that a basic amino-terminal is essential for the opioid activity of enkephalins, Tyr–Gly–Gly–Phe–Met and –Leu [1], and their analogs. Removal of the amino-group of Tyr results in a practically inactive peptide [2], as does its acetylation [3]. Introduction of two methyl-groups into the terminal amino-moiety brings about a substantial loss in the biological activity [4], while mono-methylation decreases the potency in the mouse vas deferens preparation but enhances activity in the guinea-pig ileum [5]. It was tempting to speculate that interaction between the peptide N-terminus and its counterpart on the receptor (presumably a carboxyl-group) might be favourably influenced by replacing the terminal amino-group with a guanidino function. The resonance stabilized guanidinium cation has the capacity to interact with some bidentate structure in the receptor site establishing hydrogen bridges in addition to the electrostatic interaction. One could anticipate that the improved binding properties of the peptide N-terminus would result in enhanced biological activity. It has also to be taken into account, however, that this replacement leads to a distal shift of the positive charge (~1.2 Å). Such modification at the ϵ-amino group of the lysine side chain in certain peptides, i.e., conversion of lysine into homoarginine residue, makes the lysine bond resistant to trypsin [6]. Thus trypsin seems to be unable to combine simultaneously with both the carbonyl-group and the positive charge of the basic residue when the distance between these is greater than occurs in a lysine or arginine residue. In this context one may recall that papain, which is less specific than trypsin, can hydrolyse homoarginine bonds, though at a lower rate than, for instance, arginine bonds [7]. Thus the guanidino-group introduced into the N-terminus of an enkephalin analog may have dual influence on the peptide. For studying this problem two tetrapeptideamides and two pentapeptideamides related to [D-Met^2, Pro^5]-enkephalinamide [8] have been synthesized.

2. Materials and methods

[D-Met^2, Pro^5]-Enkephalinamide (Ia) was prepared by the stepwise synthesis in solution as in [8]. A similar procedure was used for the synthesis of des-Met^5-[D-Met^2]-enkephalinamide (IIa), des-Met^5-[D-Nle^2]-enkephalinamide (IIIa) and [D-Nle^2, Pro^5]-enkephalinamide (IVa). To obtain the corresponding guanidino-derivatives I–IV, peptideamide acetates Ia–IVa were reacted with I-amidino-3, 5-dimethyl-pyrazole acetate in the presence of triethylamine in ethanol at 60–80°C for 3–4 h. I–IV were purified by precipitation from ethanol/ethyl acetate mixture or by column chromatography on silica gel using a chloroform–25% ammonia–methanol (12:3:8) system for elution. R_F values found in thin-layer chromatography on Kieselgel G in the latter system were 0.92–0.97 for compounds Ia–IVa and 0.65–0.70 for I–IV. Amino acid analysis after acidic and alkaline hydrolyses showed the expected composition. Details of the synthesis were described in [9]. The agonist activities of the compounds were determined in electrically stimulated mouse vas deferens [10,11] and guinea-pig ileum preparations using both the 'single dose' [12] and 'multiple dose' [13] methods in the latter system. Antinociceptive properties were assessed by the tail-flick test [14] as in [15].

3. Results and discussion

The structure of the newly synthesized compounds and that of [D-Met^2, Pro^5]-enkephalinamide [8] can be described by the general formula X–Tyr–Y–Gly–Phe–Q, wherein Q represents the L-prolineamide moiety or an amino group, Y stands for D-Met or D-Nle and X for hydrogen or an amidino-group. Thus the N-terminal residue of the guanidino-terminated and the regular amino-terminated peptides can be abbreviated as H_2N–C (NH)–Tyr and H–Tyr, respectively. The opiate activities of the peptides assessed in two in vitro assays and in an in vivo test for analgesia are summarized in table 1. Regarding the agonist activities in vitro it is apparent that guanidination improved the biological potencies of pentapeptides as well as the tetrapeptides. The higher potencies were obtained in the guinea-pig ileum preparation. Assessment of ID_{50} values by the 'single dose' and 'multiple dose' methods gave different results. The highest deviation (and the highest values for agonist activity) was obtained for the guanidino derivative of [D-Met^2, Pro^5]-enkephalinamide. These results may raise the intriguing question [12] whether or not the guanidino-terminated peptides are of mixed, agonist–antagonist character. It also appears that D-Met^2-containing peptides possess somewhat higher activity than the D-Nle^2 analogs in vitro.

However, with respect to analgesic activity, the influence of guanidination markedly differs from that found in the in vitro assay systems. A guanidino-terminal seems to be advantageous only in the tetrapeptides. Given intracerebroventricularly (i.c.v.) the guanidino derivative of des-Met^5-[D-Met^2]-enkephalinamide (II) was the most active compound showing nearly 20 times higher potency than the amino terminated analog, IIa, and 200-times higher potency than morphine. When the peptides were applied intravenously (i.v.) the guanidino derivative of des-Met^5-[D-Nle^2]-enkephalinamide (III) proved to be the most potent analgesic, being ~2-times more

Table 1
Opiate activities of Met-enkephalin and its analogs relative to normorphine[a] (= 1, in vitro) and morphine[b] (= 1, in vivo) on a molar basis

Number of compound	Peptide X–Tyr–Y–Gly–Phe–Q	GPI[c]	GPI[d]	MVD[e]	TFR[f]	TFR[g]
Ia	H–D-Met–Pro-NH_2	7.9	7.9	7.9	64.6	4.2
I	H_2N-C/NH/–D-Met–Pro-NH_2	823.0	173.0	25.0	10.5	0.65
IIa	H–D-Met–NH_2	17.0	17.0	4.1	12.1	2.2
II	H_2N-C/NH/–D-Met–NH_2	211.0	79.0	21.0	210.0	4.4
IIIa	H–D-Nle–NH_2	21.7	21.7	8.4	16.4	1.2
III	H_2N-C/NH/–D-Nle–NH_2	126.0	79.0	18.0	20.0	7.95
IVa	H–D-Nle–Pro-NH_2	17.7	17.7	11.6	28.8	1.6
IV	H_2N-C/NH/–D-Nle–Pro-NH_2	79.0	21.0	17.0	1.1	0.6
	H–Tyr–Gly–Gly–Phe–Met-OH Met-enkephalin	0.95	0.95	26.1	<0.01	0

[a] ID_{50} value was 172.8 nM in the guinea-pig ileum
[b] ID_{50} values in the rat tail-flick test (depending on the season) were 1.82–2.41/μM/kg on (i.v.) application and 1.15–6.8 nM/animal on i.c.v. administration, respectively [14,15]
[c] 'Single dose' method in the guinea-pig ileum [12]
[d] 'Multiple dose' method in the guinea-pig ileum [13]
[e] Mouse vas deferens preparation [10,11] where the ID_{50} of normorphine was as high as 183.0 nM
[f] Tail-flick test in the rat after i.c.v. application
[g] Tail-flick test in the rat after i.v. application

active than [D-Met^2, Pro^5]-enkephalinamide (Ia) and ~8-times more potent than morphine. In the case of pentapeptides (Ia–I, IVa–IV) guanidination resulted in a substantial loss of analgesic activity.

To interpret the above phenomenon, it is possible that the shift of the positive charge at the N-terminus of peptides altered the position (or spatial arrangement) of the fifth residue, i.e., Pro–NH_2, to such an extent that for analgesia its absence was more favourable than its presence.

The effect of guanidination on the analgesic potencies is also influenced by the residue at position 2. In addition, this influence seems to depend strongly on the route of application. For instance, guanidination of the D-Met^2 pentapeptide caused similar decrease of potencies assessed either i.c.v. or i.v. injections, respectively. In the case of the D-Nle^2 peptide the potency on i.c.v. injection was diminished to a much greater extent than on i.v. application. Regarding the tetrapeptides, the potency determined after i.c.v. administration was increased by >1 order of magnitude in the case of the D-Met containing compound but remained practically unchanged in the D-Nle^2 analog. On i.v. injections guanidination of the D-Met^2 and D-Nle^2 tetrapeptides resulted in an increase of the analgesic potency by a factor of 6.6 and 2, respectively. Because of the close similarity of the D-Met and D-Nle residues their complex effects on the analgesic potencies of the peptides can hardly be explained by the alteration of the stability and transport properties alone, some further highly specific interactions might be also involved in this phenomenon.

It may be noticed that the guanidino terminated tetrapeptideamide II and [D-Met^2, Pro^5]-enkephalinamide (Ia) show an analgesic effect in the CFLP mouse also after oral administration. Their potency ratios in the acetic acid writhing test are as follows: 8 and 14 for Ia and II, respectively, relative to morphine (= 100) on a molar basis. In this context [D-Met^2, Pro^5]-enkephalinamide (Ia) has been reported inactive in the mouse when given orally [16]. Presumably strain differences and diversity of analgesic testing may explain this discrepancy.

Finally, from the structure–activity relationships presented by this study it can be concluded that the complexity of events in the CNS resulting in analgesia cannot be modelled satisfactorily by the in vitro methods.

Acknowledgements

We are grateful to Z. Mohai for her skillful assistance in the synthetic work and to A. Patthy for amino acid analyses.

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] Morgan, B. A., Smith, C. F. C., Waterfield, A. A., Hughes, J. and Kosterlitz, H. W. (1976) J. Pharm. Pharmacol. 28, 660–661.
[3] Ling, N. and Guillemin, R. (1976) Proc. Natl. Acad. Sci. USA 73, 3308–3310.
[4] Frederickson, R. C. (1977) Life Sci. 21, 23–42.
[5] Lord, J. A. H., Waterfield, A. A., Hughes, J. and Kosterlitz, H. W. (1977) Nature 267, 495–499.
[6] Weil, L. and Telka, M. (1957) Arch. Biochem. Biophys. 71, 473–474.
[7] Shields, G. S., Hill, R. L. and Smith, E. L. (1959) J. Biol. Chem. 234, 1747–1753.
[8] Bajusz, S., Rónai, A. Z., Székely, J. I., Gráf, L., Dunai-Kovács, Z. and Berzétei, I. (1977) FEBS Lett. 76, 91–92; Belgian patent no. 858, 453.
[9] Synthesis of these peptides is covered by pending patent application in the name of Institute for Drug Research.
[10] Hughes, J., Kosterlitz, H. W. and Leslie, F. M. (1975) Br. J. Pharmacol. 53, 371–381.
[11] Rónai, A. Z., Berzétei, I., Székely, J. I., Gráf, L. and Bajusz, S. (1979) Pharmacology 18, 18–24.
[12] Kosterlitz, H. W. and Watt, A. J. (1968) Brit. J. Pharmacol. 33, 266–276.
[13] Kosterlitz, H. W., Lydon, R. J. and Watt, A. J. (1970) Brit. J. Pharmacol. 39, 398–413.
[14] D'Amour, F. E. and Smith, D. L. (1941) J. Pharm. 72, 74–79.
[15] 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.
[16] Roemer, D. and Pless, J. (1979) Life Sci. 24, 621–624.