DIFFERENTIAL BEHAVIOUR OF LPH-(61–91)-PEPTIDE IN DIFFERENT MODEL SYSTEMS: COMPARISON OF THE OPIOID ACTIVITIES OF LPH-(61–91)-PEPTIDE AND ITS FRAGMENTS

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1. Introduction

An endogeneous pentapeptide with opiate agonist activity, identified as residues 61–65 of porcine pituitary β-lipotropin, has been isolated and characterized by Hughes et al. [1]. Since then, several fragments of the C-terminal portion of β-LPH molecule have been shown to possess morphine-like activity in different assay systems [2–10].

The two in vitro preparations used most frequently for the demonstration of opioid activity of alkaloids, semisynthetic and synthetic narcotic derivatives as well as that of natural and synthetic peptides, are the longitudinal muscle of guinea-pig ileum and the mouse vas deferens [1,4,8,11–17]. The relative potencies of non-peptide narcotics in guinea-pig ileum and mouse vas deferens are rather similar [13,14] with the exception of some cyclazocine-derivatives [17]. Furthermore, there is also a fair agreement so far as the relative potencies of shorter synthetic peptides similar to LPH-(61–65)-peptide, are concerned [16].

The aim of this study was to determine the relative potencies of larger active fragments of β-LPH in these two biological models. The potency ratios were found to be similar in both preparations for LPH-(61–69) and LPH-(61–79)-peptides as compared to LPH-(61–65)-peptide, whilst in the case of LPH-(61–91)-peptide there was an apparent divergence between the two systems.

2. Experimental

LPH-(61–65)-Peptide was synthesized as described previously [4]. LPH-(61–79)-Peptide was obtained by plasmin cleavage of β-LPH [18,19]. LPH-(61–91)-Peptide was prepared from porcine pituitary gland [20]. The longitudinal muscle strip of guinea-pig ileum was prepared as described by Paton and Vizi [21]. The morphine-like activity was measured according to Kosterlitz and Watt [11]. For the comparison of the agonist potencies of the peptides, the single-dose method [11] was not used, but complete dose–response curves were constructed. The mouse vas deferens was prepared according to Hughes et al. [12] and bathed at 31°C in modified Krebs' solution [12]. We have slightly modified the parameters of the stimulation applied. Field electrical stimulation was used, delivered through platinum wire electrodes positioned at the top and the bottom of the organ bath. The upper electrode was a ring of 4 mm diameter. Paired shocks with 100 ms delay between the rectangular pulses of 1 ms were used, delivered at a rate of 0.1 Hz.

3. Results

Table 1 contains the relative agonist potencies of four active β-LPH fragments in guinea-pig ileum and mouse vas deferens. It can be seen, that the relative potencies of LPH-(61–69)- and LPH-(61–79)-peptides are similar in the two in vitro models. The increment in the chain length led to the decrease of the agonist potency in both assay systems.

Introduction of residues 80–91 to the molecule, however, resulted in an apparent divergence between

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Table 1
The relative agonist potencies of four β-LPH fragments in longitudinal muscle strip of guinea-pig ileum and mouse vas deferens

Peptide	Relative agonist potency (Met-enkephalin = 100)		GPI/MDV
	Guinea-pig ileum (GPI)	Mouse vas deferens (MDV)	
LPH-(61–65) (Met-enkephalin)	100[a] (n = 4)	100[b] (n = 14)	–
LPH-(61–69)	44[c] (n = 4)	35[d] (n = 4)	1.3
LPH-(61–79)	39 (n = 7)	35 (n = 4)	1.1
LPH-(61–91)	130 (n = 4)	5 (n = 8)	26.0

[a] The ID_{50} of LPH-(61–65)-peptide was as high as 113.2 ± 22.5 nM (n = 4)
[b] The ID_{50} of LPH-(61–65)-peptide was 4.2 ± 0.4 nM (n = 14)
[c] LPH-(61–69)-peptide was a purified material [6]
[d] A tryptic digest of β-LPH was used [22] without further purification

Purified LPH-(61–69)-peptide and the complete tryptic digest of β-LPH exerted qualitatively and quantitatively identical effects in the biological systems investigated

the two preparations. While in the longitudinal muscle strip of guinea-pig ileum the agonist activity of LPH-(61–91)-peptide was higher than that of LPH-(61–79)-peptide, there was a further loss in the potency in mouse vas deferens. Tryptic cleavage of LPH-(61–91)-peptide led to the appearance of the agonist potency corresponding to LPH-(61–69)-peptide in both preparations. None of the compounds studied possessed measurable antagonist activity in any of the systems used.

4. Discussion

According to our previous study on the analgesic activities of these peptides [8], increase of the chain length from residues 61–65 to residues 61–69 and 61–79 enhanced analgesic potency and the introduction of residues 80–91, led to a further dramatic increase in the analgesic activity.

Previous speculations on the conformation of peptides possessing opiate activity are in agreement concerning the significance of the tyramine moiety of the compounds at sequence position 61 [23–26]. Further receptor binding sites of hydrophobic character are provided by the Phe_{64} and Met_{65} side-chains. Furthermore, a well-defined secondary structure was proposed for LPH-(61–65)-peptide, characterized by a β-turn, which is stabilized by a hydrogen-bond between the carbonyl-group of Gly_{62} and the imino-group Met_{65} [25,26] or between the CO of Tyr_{61} and the NH of Phe_{64} [23].

Practically no information has been available so far as the steric structure of larger lipotropin fragments is concerned. It might be speculated that the relatively weaker potency of LPH-(61–69)- and LPH-(61–79)-peptides, as compared to that of LPH-(61–65)-peptide in isolated systems, may be due to a steric hindrance of the opiate receptor–active site interaction caused by the additional residues of the parent peptides [8]. Some of our unpublished data show, in agreement with the proposal of Hambrook et al. [27], that the common NH_2-terminal Tyr residue of all the peptides studied, is highly accessible to the action of aminopeptidase M. That is, this residue could easily be removed from the peptide chains by the enzyme. In addition, aminopeptidase M completely hydrolyzed LPH-(61–65)- and LPH-(61–79)-peptides. The segment between residues 62–79 in LPH-(61–91)-peptide, however, proved to be partially protected against the action of aminopeptidase M, which may be regarded as an indication of an intramolecular

interaction between this portion and the C-terminal part of the molecule. This change in the steric structure may be in favour of binding to the receptors in longitudinal muscle strip and, at the same time, disadvantageous for the interaction of the molecule with the receptors in mouse vas deferens. In the in vivo efficacy of the peptides, factors other than the affinity to the receptors (i.e., protection of the molecules from rapid inactivation [5,8,27] and the access of the peptides to the receptor sites) have to be taken into consideration.

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] Li, C. H. and Chung, D. (1976) Proc. Natl. Acad. Sci. USA 73, 1145–1148.

[3] Guillemin, R., Ling, N. and Burgus, R. (1976) C.R. hebd. Seanc. Acad. Sci. Paris, D 282, 783–785.

[4] Gráf, L., Rónai, A. Z., Bajusz, S., Cseh, G. and Székely, J. I. (1976) FEBS Lett. 64, 181–184.

[5] Bradbury, A. F., Smyth, D. G., Snell, C. R., Birdsall, N. J. N. and Hulme, E. C. (1976) Nature 260, 793–795.

[6] Cox, B. M., Goldstein, A. and Li, C. H. (1967) Proc. Natl. Acad. Sci. USA 73, 1821–1823.

[7] Li, C. H., Lemaire, S., Yamashiro, D. and Doneen, B. A. (1976) Biochem. Biophys. Res. Commun. 71, 19–27.

[8] Gráf, L., Székely, J. I., Rónai, A. Z., Dunai-Kovács, Zs. and Bajusz, S. (1976) Nature 263, 240–241.

[9] Guillemin, R., Ling, N., Burgus, R. and Lazarus, L. R. (1976) Abstr. VIIth. Int. Congr. of the Int. Soc. Psychoneuroendocrin., Strasbourg, p. 28.

[10] Feldberg, W. and Smyth, D. G. (1977) J. Physiol. London in press.

[11] Kosterlitz, H. W. and Watt, A. J. (1968) Brit. J. Pharmacol. 33, 266–276.

[12] Hughes, J., Kosterlitz, H. W. and Leslie, F. M (1974) Brit. J. Pharmacol. 51, 139P–140P.

[13] Kosterlitz, H. W. and Waterfield, A. A. (1975) Annu. Rev. Pharmacol. 15, 29–47.

[14] Kosterlitz, H. W. (1975) in: Opiate Receptor Mechanisms (Snyder, S. H. and Matthysse, S. eds) pp. 68–73, MIT Press, Massachusetts.

[15] Hughes, J. (1975) Brain Res. 88, 295–308.

[16] Morgan, B. A., Smith, C. F. C., Waterfield, A. A., Hughes, J. and Kosterlitz, H. W. (1976) J. Pharm. Pharmac. 28, 660–661.

[17] Kosterlitz, H. W., Leslie, F. M. and Waterfield, A. A. (1975) in: Opiate Receptor Mechanisms (Snyder, S. H. and Matthysse, S. eds) pp. 68–73, MIT Press, Massachusetts.

[18] Gráf, L. and Li, C. H. (1973) Biochem. Biophys. Res. Commun. 53, 1304–1309.

[19] Gráf, L. (1977) Acta Biochim. Biophys. Acad. Sci. Hung. in press.

[20] Gráf, L., Barát, E. and Patthy, A. (1976) Acta Biochim. Biophys. Acad. Sci. Hung. 11, 121–122.

[21] Paton, W. D. M. and Vizi, E. S. (1969) Brit. J. Pharmacol. 35, 10–28.

[22] Rónai, A. Z., Székely, J. I., Gráf, L., Dunai-Kovács, Zs. and Bajusz, S. (1976) Life Sci. 19, 733–738.

[23] Bradbury, A. F., Smyth, D. G. and Snell, C. R. (1976) Nature 260, 165–166.

[24] Horn, A. S. and Rodgers, J. R. (1976) Nature 260, 795–797.

[25] Roques, B. P., Garbay-Jaureguiberry, C., Oberlin, R., Anteunis, M. and Lala, A. K. (1976) Nature 262, 778–779.

[26] Jones, R. C. and Gibbons, W. A. (1976) Nature 262, 779–782.

[27] Hambrook, J. M., Morgan, B. A., Rance, J. J. and Smith, C. F. (1976) Nature 262, 283.