

METABOLISM OF THE ANALGESIC PEPTIDE LIPOTROPIN C-FRAGMENT IN RAT STRIATAL SLICES

D. G. SMYTH and C. R. SNELL

National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, England

Received 23 April 1977

1. Introduction

A series of morphinomimetic peptides has been isolated from brain or pituitary [1–6]. Comparison of their abilities to displace specifically bound [^3H]-naloxone from brain opiate receptors showed that C-Fragment (lipotropin 61–91) is much more potent than C'-Fragment (61–87), γ -endorphin (61–77), α -endorphin (61–76) or methionine enkephalin [7]. Investigation of the analgesic properties revealed that the shorter peptides are at most weakly active whereas C-Fragment produces profound and long lasting analgesia [8–11]. In addition, the C-Fragment alone exhibits other central activities, including the stimulation of grooming [12], inhibition of neurotransmitter release [13] and the production of hyperglycaemia [14]. The high potency of C-Fragment in its central actions would seem to imply that this is the physiologically significant agent in brain.

In the present investigation we have shown that the proteolytic degradation of C-Fragment in striatal slices takes place in a specific manner with the formation of products corresponding to the range of opiate active peptides that have been isolated from brain.

2. Materials and methods

2.1. Iodination of peptides

C-Fragment and C'-Fragment were isolated from porcine pituitary [1]. Methionine enkephalin and the heptapeptide 61–67 were synthesised by Dr A. F. Bradbury. γ -Endorphin was obtained by rennin digestion of C-Fragment [15] and 61–73 by carboxypeptidase A digestion of γ -endorphin [17]. The

octadecapeptide 61–78 was prepared by chymotryptic digestion of C-Fragment [15]. The peptides were iodinated by the method of Hunter and Greenwood [16] to give a specific activity of approx. 2 Ci/mmol. Each peptide was purified by gel filtration on Sephadex G-10 (column dimension 2 × 0.2 cm) in 50% (v/v) acetic acid.

2.2. Incubation with striatal slices

Coronal slices (approx. 200 mg wet wt) through the corpus striatum were prepared by section of fresh rat brains 3.5 mm and 2.5 mm rostral to the anterior part of the optic chiasma. The slices were preincubated (45 min, 37°C) in 3 ml saline (NaCl 134 mM, KCl 5 mM, KH_2PO_4 1.25 mM, MgSO_4 2.0 mM, CaCl_2 1.0 mM, NaHCO_3 16 mM, glucose 10 mM) containing the appropriate bacitracin concentration. The slices were transferred and gently agitated at 37°C in 3 ml of saline, or 3 ml of saline 10^{-4} M in bacitracin, containing 6×10^6 cpm of iodinated peptide. Aliquots (0.3 ml) were taken at intervals and an equal volume of glacial acetic acid added. Each sample was submitted to gel filtration on Sephadex G-50 (150 × 0.9 cm) in 50% (v/v) acetic acid and the eluate fractions were monitored for radioactivity. It may be noted that C-Fragment behaves anomalously during gel filtration at neutral pH; in 50% acetic acid C-Fragment and its related peptides behave reproducibly and are recovered in high yield.

3. Results and discussion

3.1. Degradation by endogenous aminopeptidase

The use of iodine-labelled peptides ensured that the incubations could be performed at approaching

physiological concentration (approx. 10^{-12} M); products with the amino terminal region intact could be readily detected since the only iodinated residue in the peptides is the terminal tyrosine.

[125 I]C-Fragment on incubation with striatal slices was degraded slowly, with a half-life of 3.2 h (Fig.1). In the presence of bacitracin, an aminopeptidase inhibitor, the half-life was unchanged, which indicates that the degradation of C-Fragment is not initiated by the attack of an aminopeptidase. γ -Endorphin had a half-life of 1.1 h but in the presence of bacitracin the half-life was increased to 3.6 h, in agreement with the known susceptibility of this peptide to the action of aminopeptidase enzymes [17].

The degradations are mediated by extracellular enzymes in the slices and do not involve an active uptake system as even after prolonged incubation there was no enrichment of labelled peptide in the tissue. The results indicate that the extracellular degradations of C-Fragment in striatum is not initiated by attack of an aminopeptidase; as with the proteolysis catalysed by brain membranes [15] the first step involves attack by an endopeptidase.

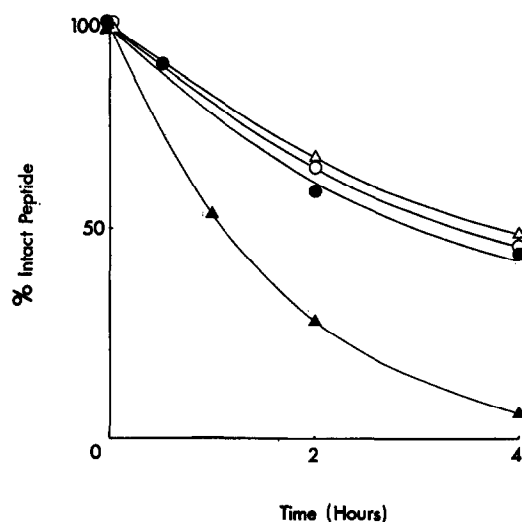


Fig.1. Effect of bacitracin on the metabolism of C-Fragment and γ -endorphin. [125 I]C-Fragment (\circ and \bullet) or γ -endorphin (Δ and \blacktriangle) were incubated in the absence and presence of 10^{-4} M bacitracin respectively. The incubation mixture was examined as described in Materials and methods. Intact peptide was calculated from the ratio of counts corresponding to degraded peptide to total counts eluted.

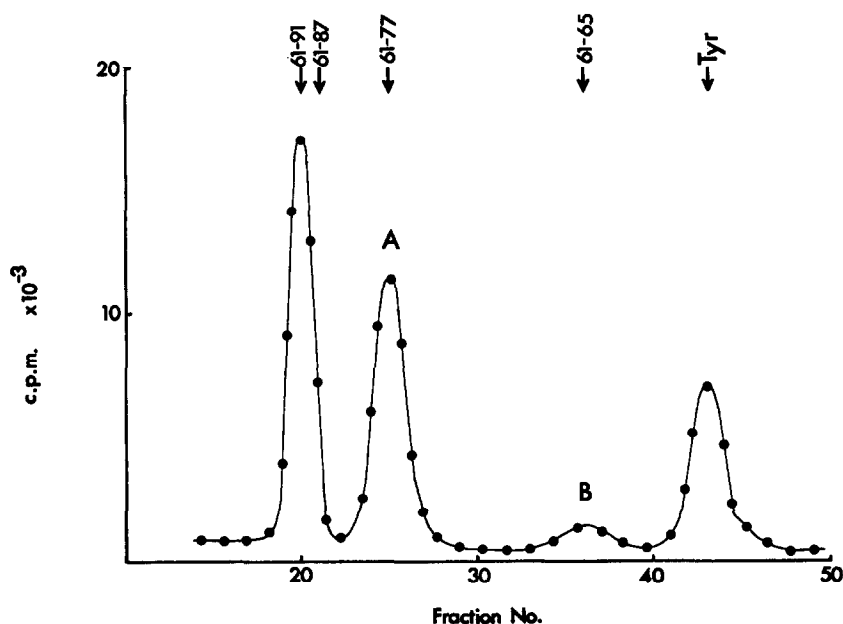


Fig.2. Gel filtration of [125 I]C-Fragment after incubation with striatal slices in the presence of 10^{-4} M bacitracin. An 0.3 ml aliquot taken during incubation at 4 h was diluted with an equal volume of glacial acetic acid and filtration was performed on Sephadex G-50 (150 \times 0.9 cm) in 50% (v/v) acetic acid, fraction size 2.2 ml. The radioactivity in each fraction was measured in a well-type γ -counter. The arrows indicate the elution positions of the authentic iodine labelled peptides.

3.2. Degradation by endogenous endopeptidases

The degradation of C-Fragment by endopeptidases was readily followed by gel filtration on Sephadex G-50 (fig.2). The disappearance of C-Fragment was accompanied by the appearance of tyrosine and two peaks (A and B) due to intermediate peptides containing the intact NH₂-terminal region. The tyrosine must arise from the action of an aminopeptidase on the intermediate peptides as C-Fragment is resistant under these conditions. The addition of bacitracin enhanced the formation of the peptides in Fraction A at the expense of tyrosine. In the absence of bacitracin, the maximum level of the intermediate peptides was approx. 10%. Fraction A was rechromatographed on SP-Sephadex C-25 (fig. 3) and found to elute with authentic [¹³¹I]γ-endorphin; a minor component eluted in the position of α-endorphin. It is not known whether α-endorphin is formed directly from C-Fragment by the action of an endopeptidase or whether it arises by loss of the COOH-terminal amino

acid from γ-endorphin by the action of a carboxypeptidase. Peak B (fig.2), which contains peptides shorter than the endorphins, was subjected to further chromatography. The principal product was methionine enkephalin and there was also a small amount of hexa- or heptapeptide (fig.4). It is apparent that the smaller peptides are formed only slowly as the yield of methionine enkephalin was not greatly increased by the presence of 5×10^{-3} M bacitracin.

The degradation of C-Fragment in striatal slices leads to the formation of γ-endorphin, α-endorphin and methionine enkephalin as specific intermediates. The extent to which these peptides are formed in vivo will depend on the relative distribution of the degrading enzymes. It remains to be seen whether the endorphins and enkephalin also occur intracellularly as part of a neurotransmitter system but the evidence of the present experiments is that these peptides can be generated extracellularly. It may be that the endorphins and enkephalin are not elaborated to perform a specific physiological function but represent degradation products of the potent opiate active peptide C-Fragment.

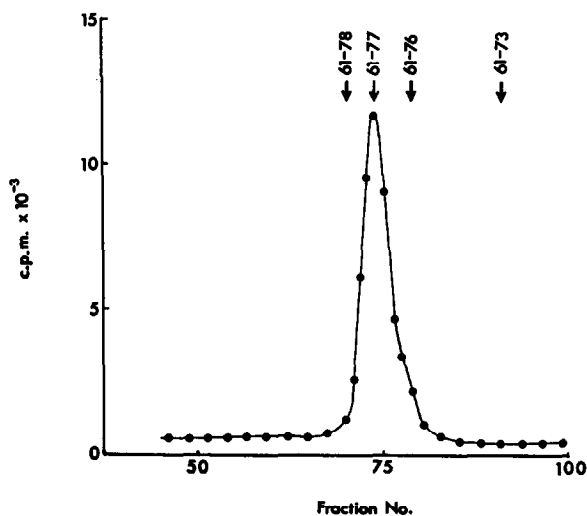


Fig.3. Identification of γ-endorphin as the first degradation product formed from C-Fragment in striatal slices. The peptide fraction Peak I, obtained by gel filtration of [¹²⁵I]C-Fragment incubated with striatal slice in the presence of bacitracin, was evaporated in vacuo and chromatographed with authentic [¹³¹I]γ-endorphin on SP Sephadex C-25 in 0.05 M phosphate, pH 4.5, with a linear gradient from 0–0.5 M NaCl (200 ml mixer). The elution position of the 61–73, 61–76 and 61–78 peptides were established by chromatography of standard mixtures containing these peptides labelled with either ¹²⁵I or ¹³¹I.

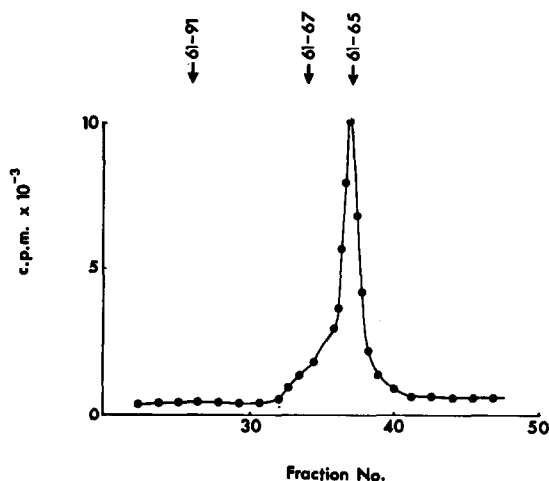


Fig.4. Identification of methionine enkephalin as the principal component of the small peptide fraction formed by degradation of C-Fragment in striatal slices. The peptide fraction Peak II, obtained by gel filtration of [¹²⁵I]C-Fragment on Sephadex G-50, incubated with a striatal slice in the presence of bacitracin, was evaporated in vacuo and chromatographed on Sephadex G-15 (150 × 0.9 cm) in 50% (v/v) acetic acid, fraction size 1.4 ml. The column was calibrated with [¹²⁵I]C-Fragment, the heptapeptide 61–67 and methionine enkephalin (61–65).

References

- [1] Bradbury, A. F., Smyth, D. G. and Snell, C. R. (1975) in: *Peptides: Chemistry, Structure and Biology* (Walter, R. and Meienhofer, J. eds) pp. 609–615, Ann Arbor Sci.
- [2] Hughes, J. et al. (1975) *Nature* 258, 577–579.
- [3] Bradbury, A. F., Smyth, D. G. and Snell, C. R. (1976) in: *Polypeptide Hormones: Molecular and Cellular Aspects*, CIBA Found. Symp. No. 41, pp. 61–75, Elsevier/North-Holland Biomedical Press, Amsterdam.
- [4] Bradbury, A. F., Feldberg, W. S., Smyth, D. G. and Snell, C. R. (1976) in: *Opiates and Endogenous Opioid Peptides* (Kosterlitz, H. W. ed.) pp. 9–17, Elsevier/North-Holland Biomedical Press, Amsterdam.
- [5] Guilleman, R., Ling, N. and Burgus, R. (1976) *C. R. Acad. Sci. Paris, Ser. D.* 282, 783–785.
- [6] Simantov, R. and Snyder, S. H. (1976) *Life Sci.* 18, 781–788.
- [7] Bradbury, A. F., Smyth, D. G. and Snell, C. R., Birdsall, N. J. M. and Hulme, E. C. (1976) *Nature* 260, 793–795.
- [8] Feldberg, W. S. and Smyth, D. G. (1976) *J. Physiol.* 260, 30–31.
- [9] Van Ree, J. M., de Wied, D., Bradbury, A. F., Hulme, E. C., Smyth, D. G. and Snell, C. R. (1976) *Nature* 264, 792–794.
- [10] Loh, H. H., Tseng, L. F., Wei, E. and Li, C. H. (1976) *Proc. Natl. Acad. Sci. USA* 73, 2895–2898.
- [11] Bradbury, A. F., Smyth, D. G., Snell, C. R., Deakin, J. F. W. and Wendlandt, S. (1977) *Biochem. Biophys. Res. Commun.* 74, 748–754.
- [12] Gispen, W. H., Wiegant, V. M., Bradbury, A. F., Hulme, E. C., Smyth, D. G. and Snell, C. R. (1976) *Nature* 264, 794–795.
- [13] Loh, H. H., Brase, D. A., Sampath-Khanna, S., Mar, J. B. and Way, E. L. (1976) *Nature* 264, 564–567.
- [14] Feldberg, W. S. and Smyth, D. G. (1977) *J. Physiol.* in the press.
- [15] Austen, B. M. and Smyth, D. G. (1977) *Biochem. Biophys. Res. Commun.* in the press.
- [16] Hunter, W. M. and Greenwood, F. C. (1962) *Nature* 194, 495–499.
- [17] Austen, B. M. and Smyth, D. G. (1977) *Biochem. Biophys. Res. Commun.* submitted.