Lipotropin: precursor to two biologically active peptides.

A.F. Bradbury, D.G. Smyth and C.R. Snell.

National Institute for Medical Research,

Mill Hill, London, NW7 1AA.

Received February 23,1976

SUMMARY

Lipotropin appears to be the common precursor to β-MSH, a peptide with lipolytic activity, and C-Fragment, a peptide with potent opiate activity. The product formed is determined by the specificity of the activating enzymes.

The amino acid sequence of β-MSH, the 18 residue melanocyte stimulating hormone, is contained within the central region of lipotropin (LPH), a 91 residue polypeptide. On this basis Li and his colleagues[1] suggested that LPH might be the prohormone of β-MSH. Bertagna, Lis and Gilardeau[2], on the other hand, were unable to demonstrate conversion of LPH to β-MSH *in vitro* using pulse labelling techniques. If LPH is the precursor of β-MSH, formation of the hormone should be accompanied by release of the contiguous fragments of the prohormone and the fragments remain in the secretory particle of the gland. To obtain evidence on the biosynthetic origin of β-MSH, we have isolated peptides from pituitary in a search for the N- and C-fragments of the prohormone.

Table 1. Polypeptides isolated from the pituitary gland of the pig.

LPH Residue Number		Yield (Umole)
1-91	LPH	1.1
1-58	γ-LPH	1.7
1-38	N-fragment	0.7
41-58	β-MSH	1.6
61-87	C -fragment	0.7
61-91	C-fragment	0.9

Pituitary glands were obtained from 1200 pigs and after homogenization and precipitation according to Li[1] ion exchange chromatography and gel filtration yielded a series of homogeneous peptides:[3] among those present were the constituent fragments of lipotropin, comprising the biologically active peptide β-MSH and the N- and C-fragments (Table 1). Each of the peptides appears to have been formed by cleavage of the lipotropin chain at the carboxyl side of paired basic residues (Fig.1) and the basic amino acids removed by the action of a carboxypeptidase B enzyme. It is notable that none of the fragments was formed by cleavage at single basic residues. The specificity of the pituitary enzymes involved is thus identical with the specificity of the pancreatic enzymes that generate insulin from proinsulin.[4] This supports the view that the LPH fragments are released in a

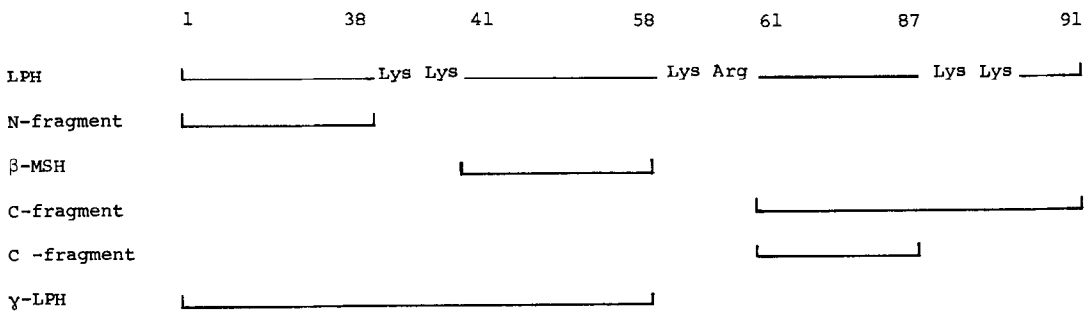

Fig. 1.

Peptide fragments of lipotropin isolated from porcine pituitary glands.

highly organised process and were not formed by exposure to diverse degrading enzymes during isolation.

The specificity of the pituitary enzyme that activates lipotropin was compared with the specificity of trypsin by studying their actions on the synthetic hexapeptide (I),

I. Lys Asp Lys Arg Tyr Gly

which corresponds to the sequence from positions 57-62 in LPH. It was observed that the peptide bond between arginine and tyrosine was cleaved rapidly and specifically, both by trypsin and by the trypsin-like enzyme isolated from secretory granules of the pituitary. Specific cleavage of the same peptide bond took place when lipotropin was digested under mild conditions either with trypsin (enzyme substrate ratio 1:4000, 30 min., pH 7.4, 37°C) or with the pituitary enzyme; and the same specificity was implicit in the finding that the C-fragment of LPH (residues 61-91) and the complementary polypeptide γ-LPH (residues 1-58) were present in substantial quantity in

the gland. On this evidence it seems likely that in the pituitary the first cleavage of LPH is at the arginyl tyrosine bond at positions 60-61.

Tryptic hydrolysis of the synthetic heptapeptide (II),

II. Ala Glu Lys Lys Asp Glu Gly

which corresponds to the sequence from positions 37-43 in porcine LPH, took place 300 times more slowly than hydrolysis of the hexapeptide. The cleavage occurred specifically between the two lysine residues and not between lysine and aspartic acid. Since β-MSH has aspartic acid at the NH_2-terminus, it seems that the pituitary enzyme which releases β-MSH from lipotropin *in vivo* has a different specificity from trypsin. Indeed the pituitary enzyme was found to have a molecular weight in the region of 100,000, compared with the value of 23,000 for trypsin; the optimum pH of the isolated enzyme was higher than trypsin; and the action of the pituitary enzyme on the synthetic peptides was unaffected by soya bean trypsin inhibitor. In view of these differences it was not surprising that no β-MSH could be detected when lipotropin was digested heavily by trypsin: cleavage at trypsin sensitive sites within the β-MSH sequence takes place before cleavage at the paired lysine residues on the N-terminal side of the hormone. The presence of the N-fragment of LPH (residues 1-38) in the pituitary, however, does confirm that β-MSH is released *in vivo* by enzymic hydrolysis of lipotropin.

Whether the pituitary enzyme has an absolute requirement for consecutive basic residues under *in vivo*

conditions is not known. With trypsin the rate of cleavage between Arg and Tyr in the hexapeptide (I) decreased only slightly when the substrate was acetylated at the ϵ-NH_2 group of the adjacent lysine residue. With the pituitary enzyme, cleavage of the acetylated peptide did occur but the rate was substantially less than that for the peptide with paired basic residues. These results indicate that the specificity exhibited *in vivo* by the pituitary enzyme for the paired basic residues of LPH is only partially explained by the intrinsic specificity: the conformation of the prohormone may be an additional factor. According to empirical rules for predicting secondary structure from a knowledge of sequence,[5,6,7] a β-bend comprising the residues Tyr.Gly.Gly.Phe (residues 61-64) lies adjacent to the enzymically susceptible site in LPH. With this structure located on the outside of the molecule, the peptide bond between arginine and tyrosine at positions 60 and 61 would understandably be accessible to enzymic attack.

The C-fragment of LPH (residues 61-91), which is endogenous to pituitary,[3] has the same sequence at the NH_2-terminus as that reported for a peptide with opiate activity, termed methionine enkephalin (III)

III. Tyr Gly Gly Phe Met

which was isolated from upper regions of pig brain.[8] From the correspondence of primary structure it seems likely that methionine enkephalin is formed *in vivo* by proteolytic cleavage of the lipotropin C-fragment. It has now been

found that C-Fragment itself has an affinity for brain opiate receptor, several times greater than that reported for the pentapeptide.[9] It is thus clear that two potential biological activities reside within the structure of the lipotropin molecule. The enzyme that releases C-fragment from lipotropin could also be involved in the formation of β-MSH but further study will be necessary to establish whether the two activities are developed in the same cell or whether specific activation of lipotropin takes place in cells that have different enzymic complements.

References

1. Li, C.H., Barnafi, L., Chretien, M. and Chung, D. (1965) Nature, 208, 1093-1094.

2. Bertagna, X., Lis, M. and Gilardeau, C., (1974) Can. J. Biochem., 52, 349-358.

3. Bradbury, A.F., Smyth, D.G. and Snell, C.R. (1975) Prohormones of β-MSH and ACTH: Structure and Activation, in "The Peptide Hormones: Molecular and Cellular Aspects", CIBA Foundation Symposium No. 41, in press.

4. Kemmler, W., Peterson, J.D., Rubenstein, A.H. and Steiner, D.F. (1972) Diabetes, 21, 572-583.

5. Chou, P.Y. and Fasman, G.D. (1974) Biochemistry, 13, 211-222.

6. Chou, P.Y. and Fasman, G.D. (1974) Biochemistry, 13 222-245.

7. Lewis, P.N., Momany, F.A. and Scheraga, H.A. (1971) Proc. Natl. Acad. Sci. U.S.A., 68, 2293-2297.

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

9. Bradbury, A.F., Smyth, D.G. and Snell, C.R. Birdsall, N.J.M. and Hulme, E.C. (in press) Nature.