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β-ENDORPHIN IS PRESENT IN ACTIVE AND INACTIVE FORMS IN RAT GASTRIC ANTRUM

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Lipotropin, β-endorphin and a series of peptides related to β-endorphin were extracted from rat antrum and resolved by gel filtration, ion exchange chromatography and high pressure liquid chromatography; the concentrations of the peptides were determined by radioimmunoassay. The major peptide with β-endorphin immunoreactivity present in the antrum was lipotropin but it was accompanied by substantial quantities of β-endorphin in its biologically active form; in addition there were minor quantities of a number of inactive β-endorphin related peptides. The experiments demonstrate that in rat antrum gastrin can be accompanied by both active and inactive forms of β-endorphin. The implications of post-translational processing mechanisms common to gastrin and β-endorphin are discussed.

Opiate alkaloids have long been known to exert potent effects on the contraction of gut (1) and it is likely that endogenous opioid peptides perform similar functions in the intact animal. To examine this possibility several investigations have attempted to establish whether β-endorphin, a potent opioid peptide, occurs in gastric antrum (1,2,3). In experiments with human tissue it was reported that although lipotropin, a precursor of β-endorphin, seemed to be present there was no evidence for the occurrence of β-endorphin (1). In other studies it was concluded that β-endorphin is a major peptide in human antrum while lipotropin was identified as a minor constituent (2,3). More recently evidence has been obtained that peptide with β-endorphin immunoreactivity and

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with the approximate molecular size of β-endorphin does occur in human antrum (4) but it was not established whether the peptide was present in the biologically potent form. In this communication we describe the results of a study on peptides related to β-endorphin in rat antrum, demonstrating the presence of both lipotropin and β-endorphin as well as a number of minor inactive forms of β-endorphin. The data show that inactive as well as active forms of β-endorphin are produced in the antrum, apparently in the same cells as the 17 residue form of gastrin (5).

METHODS

Antra from 14 Sprague Dawley rats, killed by stunning and decapitation, were rinsed in phosphate buffered saline and cut into sections. The tissue was extracted in acid acetone (acetone, H_2O, hydrochloric acid, 40:6:1) containing ^{125}I-labelled bovine marker peptides (6), lipotropin, α,N-acetyl β-endorphin 1-27 (Ac 1-27) and β-endorphin 1-27 (1-27), α,N-acetyl β-endorphin (Ac 1-31) and β-endorphin (1-31). The peptides obtained, after centrifugation at 15,000 rpm and removal of solvent *in vacuo*, were fractionated on a column (120 x 1.5cm) of Sephadex G-75 with 50% acetic acid as eluent. The radioactive fractions were combined into a single fraction, concentrated *in vacuo* and rechromatographed on a 120 x 1cm column of Sephadex G-75 in 50% acetic acid. Lipotropin and β-endorphin related peptides were located by radioimmunoassay (RIA) of portions (200µl) of the eluted fractions (1.5ml) using an antiserum raised against porcine β-endorphin (7).

The fraction containing β-endorphin related peptides obtained by gel filtration was resolved on a column (70 x 0.7cm) of SP-Sephadex C25 (pyridinium form). Chromatography was performed in 50% acetic acid using a linear gradient from 0-1M pyridine, mixer volume 100ml (8). Fractions (1.8ml) were collected and aliquots (300µl) were dried *in vacuo* and analysed by β-endorphin RIA.

Further evidence for the identity of the β-endorphin related peptides isolated by gel filtration was obtained by high pressure liquid chromatography (HPLC). The peptides were resolved on a C18 Microbondapak column (30 x 0.4cm) eluted in 0.01M HCl (95%)-acetonitrile (5%) at 1.5ml per min with a linear gradient (1% per min) to acetonitrile (95%)-0.01M HCl (5%). Fractions (450µl) were collected, aliquots (150µl) were dried *in vacuo* and the peptides located by RIA. The elution positions of standard peptides related to β-endorphin were determined on the same column immediately after the resolution of peptides from the antrum.

RESULTS AND DISCUSSION

β-Endorphin has been shown to exist in a number of forms that react with β-endorphin antisera. These related peptides,

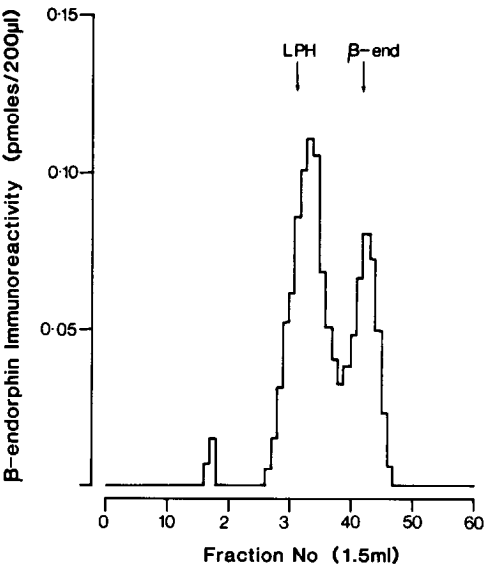

Figure 1. Gel filtration of β-endorphin related peptides extracted from rat antrum. The peptides were fractionated on a column of Sephadex G75 in 50% acetic acid. The elution position of porcine lipotropin is indicated by the first arrow (L) and that of bovine β-endorphin related peptides by the second arrow (R).

which have been isolated from pituitary and chemically identified, are β-endorphin 1-31, β-endorphin 1-27, β-endorphin 1-26 and the corresponding α,N-acetyl derivatives (9). Of the six peptides, however, only β-endorphin 1-31 possesses potent opiate properties and it is therefore important that peptide identified as 'immunoreactive β-endorphin' should be resolved chromatographically and the different forms present determined individually.

As a first step towards identifying β-endorphin related peptides in rat antrum, the tissue was extracted under acidic conditions and gel filtration carried out to separate peptides that had the approximate molecular size of β-endorphin from lipotropin and material of higher molecular weight (Figure 1). This chromatographic step, performed under conditions that reduced the possibility of aggregation, demonstrated that peptide with β-endorphin immunoreactivity emerged from the column

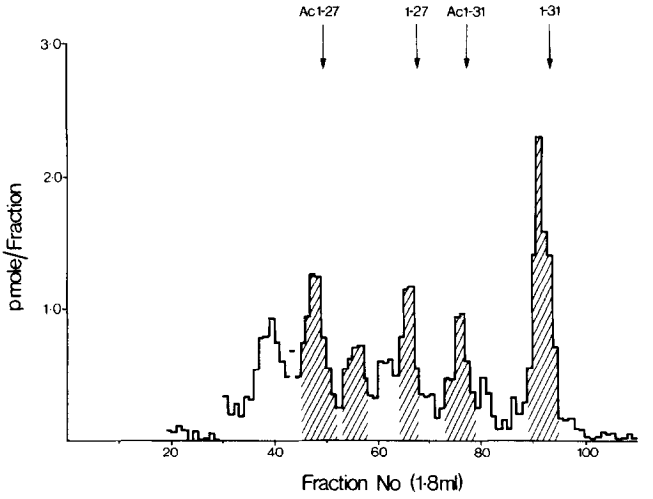

Figure 2. Ion exchange chromatography of β-endorphin related peptides extracted from rat antrum. After gel filtration as in Figure 1, the fraction containing immunoreactive peptides with the approximate size of β-endorphin was resolved on a column of SP Sephadex C25. The conditions are described in the text. The elution Positions of the bovine marker peptides are indicated by arrows.

slightly later than $|^{125}I|$ porcine lipotropin, consistent with the known molecular size of rat lipotropin (10). In addition a second fraction of peptides with β-endorphin immunoreactivity eluted from the column in the same position as $|^{125}I|$-β-endorphin. This fraction would include the 26, 27 and 31 residue forms of β-endorphin which are known to chromatograph as a single component during gel filtration (11).

The group of peptides with the approximate size of β-endorphin was shown by ion exchange chromatography to include several immunoreactive components but the major peptide was β-endorphin 1-31 (Figure 2). Essentially the same result was obtained when the β-endorphin related peptides were resolved by HPLC; β-endorphin was again shown to be the principal component (Figure 3). This indicates that the β-endorphin in rat antrum occurs principally as the biologically active peptide. Since corticotropin (ACTH), and by inference β-endorphin, is

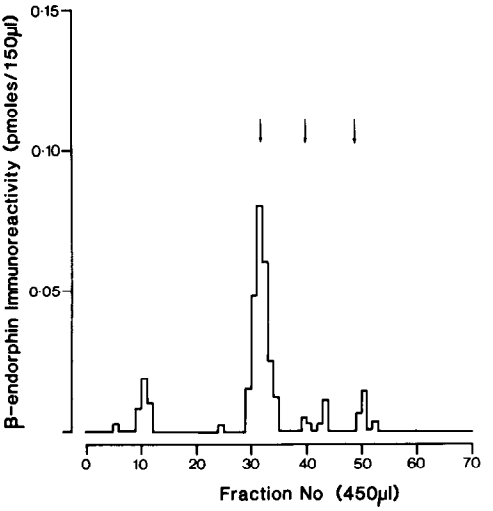

Figure 3. High pressure liquid chromatography of β-endorphin related peptides obtained by gel filtration. The peptides were resolved on a C_{18} Microbondapak column eluted in 10^{-2}M HCl with an acetonitrile gradient, as described in the text. The arrows indicate the elution positions (L-R) of bovine marker peptides: β-endorphin 1-31, β-endorphin 1-27 + acetyl β-endorphin 1-31, and acetyl β-endorphin 1-27.

elaborated in the same cells as gastrin (12), it appears that in the antrum gastrin is accompanied by lipotropin and by β-endorphin in its biologically active form. Thus the 17 residue form of gastrin, which is the major form in the antrum (13), could occur in company with either lipotropin or β-endorphin or with both peptides. Further study will be necessary to determine whether the processing reactions go to completion or whether the end products of processing co-exist in the same secretory granules as the biosynthetic intermediates.

In addition to the β-endorphin in the antrum there were small amounts of a number of β-endorphin related peptides which were attributed to β-endorphin 1-26 and β-endorphin 1-27 (Peaks 3 and 4) and acetylated or sulphated derivatives of these peptides (Peaks 1, 2 and 5). Sulphated derivatives of β-endorphin have not so far been reported but by analogy with sulphated leucine enkephalin (14) it is anticipated that they would be

virtually inactive as opiates; similarly the N-acetylated derivatives of β-endorphin are known to be devoid of opiate activity (15). Thus the minor forms of β-endorphin that occur in the antrum lack the potent opiate activity of the 31 residue peptide.

It is clear that the pattern of processing of β-endorphin in the antrum is similar to the pattern that prevails in the anterior pituitary; in both cases lipotropin, which is inactive as an opiate, can be a major product. In contrast, the larger forms of gastrin are not major peptides in the antrum and they retain the full activity of the 17 residue hormone; conversion of the 34 residue to the 17 residue form leads only to a diminished duration of action (16). From the results of the present experiments it is suggested that variations in the degree of prohormone processing, particularly with respect to the formation of β-endorphin from lipotropin, may influence the relative concentrations of β-endorphin and gastrin present in the same antral cell. Thus a previous suggestion that the biosynthetic precursor of gastrin may undergo parallel processing with the precursor of ACTH and β-endorphin (5) can be interpreted in terms of activation and inactivation of the two hormones.

Recent evidence has indicated that post-translational processing reactions are subject to regulatory control (17). If the processing reactions in the antrum prove sensitive to specific signals, the mechanisms involved may confer a form of 'fine tuning' to the activities of two peptides that are released concomitantly. Such mechanisms could be of functional importance in gastric physiology.

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