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SOME OF THE α-NH_2-ACETYLATED β-ENDOPRHIN-LIKE MATERIAL IN RAT AND MONKEY PITUITARY AND BRAIN IS ACETYLATED α- AND β-ENDORPHIN

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Summary

There are several studies demonstrating the existence of α-NH_2-acetylated (N-Ac) forms of β-endorphin in the intermediate lobe of several species (1,4,6). These include N-Ac-β-endorphin(1-31), N-Ac-β-endorphin(1-27) and N-Ac-β-endorphin(1-26). The existence of N-acetylation of brain β-endorphin is more controversial (5,6). Using molecular sieving, HPLC, and several radioimmunoassays, either directed at the midportion of β-endorphin or at only N-acetylated opioids, we have studied brain multiple forms (cf. Akil, 1982). We have noted that little or no acetylation of β-endorphin-sized material occurs in hypothalamus, and a small amount of N-acetylation appears to take place in the midbrain and the medulla. These results will be described in detail elsewhere (Akil et al., in preparation), but point the fact that processing of β-endorphin(1-31) in brain is different than either lobe, with the production of β-endorphin(1-27) and β-endorphin(1-26) being more predominant in brain terminal areas than in the neuro-intermediate lobe. In the course of these experiments we noted the existence of a smaller-sized material which reacted with our N-acetyl-β-endorphin antibody. The following study describes the partial characterization of this material as N-Ac-α- and N-Ac-β-endorphin (i.e., the N-acetylated forms of β-endorphin (1-17) and β-endorphin(1-16).

Procedures

Extractions. Brains and pituitaries from white Sprague Dawley rats and from rhesus monkeys were rapidly dissected to separate neurointermediate from anterior lobe and various brain areas. They were rapidly frozen on dry ice until extraction, which took place within 24 hours. The peptides were extracted with acetone:0.2 N HCl (3:1). the pellet washed once and the two supernatants combined and evaporated.

Molecular sieving. A Sephadex G-50 (1.5 X 90 cm) column was developed with 1% formic acid. This column separates 31K, β-LPH, β-endorphin(1-31), β-endorphin(1-27) + β-endorphin(1-26), and γ-endorphin. Fractions (1.2

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ml) were collected and a small aliquot evaporated and subjected to multiple RIA's.

Radioimmunoassays. Three RIA's were employed in these studies. The midportion antiserum (Brenda) has only partial crossreactivity with α- and γ-endorphin, since its major antigenic determinant is β-endorphin(17-27). Thus this antiserum consistently underestimates the amount of α - or γ-endorphin-like material. The RIA is run at a final dilution of 1:40,000. Antiserum Melinda (courtesy of Drs. Richard Mains and Betty Eipper) exhibits full crossreactivity with all α -, β-, and γ-endorphin forms. It was initially raised against β-endorphin, and by using [^{125}I]N-Ac-β-endorphin(1-27), as the tracer, we specify the midportion as the only determinant (i.e., we eliminate NH_2 or COOH-terminus specific antigenic determinants). The N-Ac-specific antiserum, Nancy Beth, is the most relevant to this work. Its antigenic determinant is N-Ac Tyr-Gly-Gly-Phe. Non-acetylated opioids neither bind to this antibody nor displace its binding in micromolar concentrations. This antiserum is used at a final diultion of 1:6000 to 1:8000. Bound and free are separated by double antibody technique.

High Performance Liquid Chromatography (HPLC). The peptides were separated on reverse phase HPLC using a C_8 column with a linear gradient of 40 mM potasium phosphate buffer with 0.1% TEA and acetonitrile. Percent acetonitrile begins at 20% and rises linearly to 45% over a 25 minute period. The flow rate is 1 ml/min. This program separates the oxidized forms of α- and γ-endorphin from their N-acetylated forms and from the des-tyrosine forms. These peptides are also separated from a number of other opioids including β-endorphin(1-31), (1-27), (1-26), dynorphin A and their N-acetylated forms. Fractions from the Sephadex in the appropriate molecular weight range are pooled, dried down, oxidized with H_2O_2 and applied to HPLC. 0.5 ml fractions are collected from the outflow of the HPLC column, dried down and subjected to the radioimmunoassays.

Results

Figure 1 shows the profile of N-acetylated opioid material from intermediate lobe as chromatographed on Sephadex. The first peak represents N-Ac- β-endorphin(1-31), and the second N-Ac- β-endorphin(1-27) and N-Ac- β-endorphin(1-26) as confirmed on HPLC. The third peak represents the unknown material. This material has a molecular weight of approxmiately 1700 to 1800 and is therefore larger than dynorphin(1-8), the enkephalins or the enkephalin heptapeptide or octapeptide. The apparent molecular weight is consistent with either N-Ac-Dynorphin A or N-Ac- α- or γ-endorphin. However, the amount of 45 pmoles/neuro-intermediate lobe is 50 fold higher than the total content of dynorphin(1-17). This led us to consider the hypothesis (proposed by Dr. J. van Ree) that the material may be N-Ac- α- or γ-endorphin, which we then tested with HPLC and multiple RIA's. Similar chromatographic studies have been carried out in brain regions of rat and monkey. In both species, when crude extracts are tested with N-Ac-specific antiserum, evidence of N-acetylation of opioids can be seen in medulla and in midbrain, with little or no evidence of N-acetylation in hypothalamus. However, only 50% or less of the N-acetylated materials was in the molecular weight range of β-endorphin. The remainder is consistent with the weight of N-Ac- α- or γ-endorphin.

Sephadex fractions from the rat neuro-intermediate lobe and from rhesus medulla of the correct molecular weight range were pooled and evaporated. They were resuspended in 1N acetic acid and oxidized with hydrogen peroxide. They were chromatographed on HPLC, and the fractions tested with the N-acetyl-specific serum (Nancy Beth).

FIG. 1

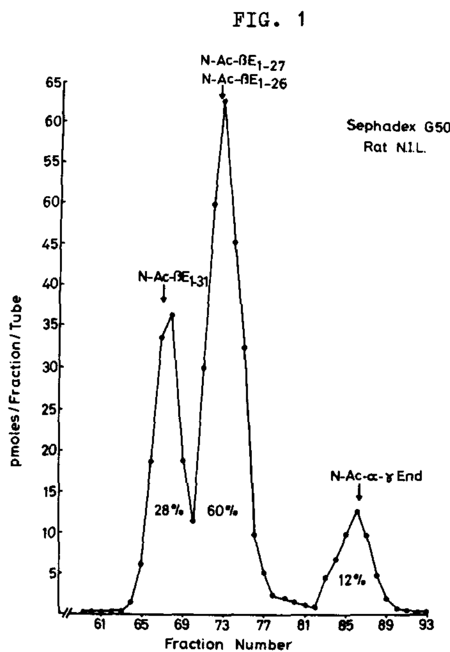

Fig. 1. Profile of N-Acetyl-opioid materials on Sephadex G_{50} assayed with N-Acetyl-specific RIA.

FIG. 2

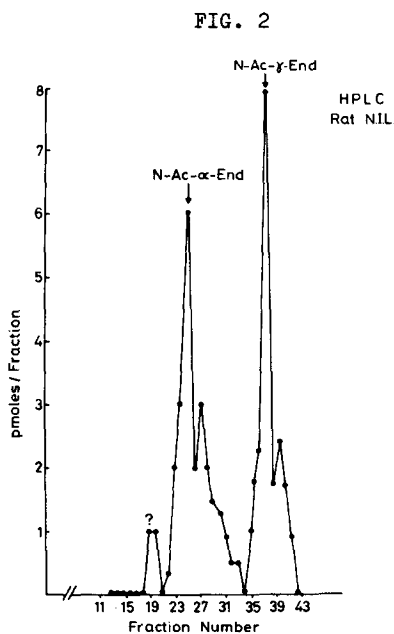

Fig. 2. Reverse phase HPLC profile of fractions #82-90 off Sephadex (MW 1700-1800) RIA with N-Acetyl-specific antiserum.

Figure 2 shows that the neuro-intermediate lobe, when tested with the N-acetyl antibody, exhibits two main peaks which co-chromatograph with N-Ac-α- and N-Ac-γ-endorphin respectively. Other minor peaks are detected. The peaks do not coincide with N-acetylated dynorphin(1-17), the only other opioid of the correct molecular weight. Similar evidence of N-acetyl-α and γ-endorphin was obtained in medulla.

Conclusions

A number of tissues which either produce or store pro-opiomelanocortin appear to process β-endorphin by several steps. The production of N-acetyl-α- or γ-endorphin, as judged by molecular weight, HPLC and multple RIA's would represent a processing through numerous post-translational steps. Whether the production of N-acetyl-α- and γ-endorphin in the intermediate lobe proceeds via the cleavage of N-Ac-β-endorphin(1-27) and N-Ac-β-endorphin(1-26) or whether it can be the result of direct cleavage from β-endorphin(1-31) or its N-acetylated counterpart is undetermined and requires pulse-labelling studies. Even less is known in various brain regions about the orderly steps in the post-translational events leading to γ-endorphin production. However, it does not seem likely that these smaller molecular weight peptides are only extraction artifacts, since they are only seen (and reliably so) in some brain regions which contain β-endorphin and not in others. These N-acetylated peptides are no longer active at the opioid receptors. Nevertheless, they may be active in other systems, since the des-tyrosine counterparts of α- and γ-endorphin have been shown to exhibit pharmacological effects *in vivo* (3).

Acknowledgements

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