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POMC IN RHESUS ANTERIOR PITUITARY AND PLASMA: EVIDENCE OF N-ACETYLATED β-ENDORPHIN AND α-MSH

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Summary

Pro-opiomelanocortin (POMC) related peptides have been studied in rat tissue and plasma, but they have not been well characterized in the rhesus monkey. Since monkey pituitary may be more similar to the human pituitary than the rat, we have characterized POMC related peptides by immunocytochemical, multiple radioimmunoassays (RIA's) and molecular sieving chromatography.

Immunocytochemical staining demonstrated N-acetylated- β-endorphin (N-Ac- β-End) and α-MSH in a few corticotrophs. RIA's of crude anterior pituitary extract and molecular sieving chromatography demonstrates that the major portion is β-End sized with a significant proportion being N-acetylated and an α-MSH peak. Molecular sieving chromatography of extracted plasma demonstrated a similar pattern to that seen in the anterior pituitary. These data suggest that rhesus monkey processes POMC differently than rat or man.

The rhesus monkey (Macaca mulatta) has been studied extensively as an animal model for a number of clinically relevant phenomena such as opiate addiction, withdrawal and cross tolerance between classes of opiates which have led to important observations not initially obtained in rodents and to notions of multiple opiate receptor types. The rhesus has also been a favorite model for the study of certain endocrine systems which can be affected by opiates and endorphins (1,2). Yet, there is little information on the nature of the POMC system of the rhesus pituitary and plasma. Therefore, the distribution and characterization of POMC related peptides β-End, ACTH, N-Ac- β-End and α-MSH in rhesus pituitary and plasma were studied by immunohistochemical techniques, multiple RIA's and molecular-sieving chromatography.

POMC undergoes different post-translational processing in the anterior lobe (AL) versus the IL (IL) of rat and pig pituitary (3-9). The primary products of POMC synthesis in AL is ACTH, β-LPH, the opiate anterior inactive intermediate precursor of β-End and a small portion of β-End. In the IL, ACTH is further processed to α-MSH and β-LPH is almost completely processed to β-End(1-31). β-End may be further modified by cleavage of the last 4-5 amino acid residues at the C-terminus and the addition of an acetyl group to the N-terminus. These modifications affect the opiate-like activity of the peptides.

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Methods

Samples were collected from six monkeys anesthetized with 30 mg/kg body weight of pentobarbital. Blood was collected directly from the heart or the abdominal aorta into a polyproplylene syringe, quickly transferred to EDTA vacutainers and chilled on ice. It was immediately centrifuged at 4°C and plasma was pipetted into plastic vials. The pH was adjusted to pH 2 with 1 N HCl and frozen on dry ice. The animal was then sacrificed with a large dose of pentobarbital, and the pituitary removed. A conservative dissection of the AL from the intermediate and posterior lobe was done on ice. Tissue samples were then immediately frozen on dry ice. Animals for immunocytochemical studies were anesthetized as described above, however, they were intubated and maintained on a respirator while being perfused with formalin for 30 minutes prior to sacrifice.

Standard PAP immunocytochemistry was carried out using antisera against β-End; N-Ac- β-End, ACTH and α-MSH (described elsewhere). N-Ac- β-End is not blocked by β-End nor is α-MSH blocked by ACTH indicating that these antisera are specific for the modified terminal amino acids. Two animals which were studied immunocytochemically received 1500 μg of colchicine intraventricularly 24 hrs prior to sacrifice.

The biochemical methods used in this study are standard in our laboratory. The β-End RIA is a midportion directed antibody with the antigenic determinant between amino acid residues 17 to 27 of β-End when [^{125}I] β-End(1-27) is the tracer. At the final concentration of 1:30,000 the assay is 100% crossreactive with POMC, β-LPH, all forms of β-End.

The N-Ac- β-endorphin RIA relies completely on N-acetylation of the tyrosine-glycine-glycine-phenylalanine core as its recognition site. At a final concentration of 1:6,000 with a tracer of [^{125}I] β-End(1-27) it is 100% crossreactive with all N-acetylated forms of β-End.

The RIA for α-MSH uses an antibody specific for α-MSH (a generous gift of Martin, Ulm, Germany). At a final concentration of 1:30,000 with a [^{125}I] α-MSH tracer, it is 100% crossreactive with α-MSH and 70% crossreactive with ACTH(1-13) NH_2. It is not crossreactive with ACTH(1-13) or ACTH(4-10). (For complete details of the assays see Akil et al., 1981 (3) and Cahill et al., 1983 (10)).

A Sephadex G-50 (superfine) column 1.5 X 90 cm, equilibrated and eluted with 1% formic acid was used for molecular sieving chromatography. The flow rate averaged 4 ml/hr and 1.2 ml fractions were collected. The full report of the calibration of this column is reported elsewhere(10,11). This column separates POMC, β-LPH, β-End(1-31), β-End(1-27), and β-MSH.

Plasma samples were extracted by the Sep-Pak C_{18} method which is fully described elsewhere (10). The recovery rate of all the POMC related peptides is over 80% with this method. Tissue samples were extracted by the standard acid/acetone method; the recovery from tissue is 70% (3).

Results

Immunocytochemical studies of the whole pituitary of rhesus monkey indicate that there are multiple cells in AL which stain for N-acetylated- β-End and α-MSH. This finding is supported by biochemical studies of the AL of the pituitary which indicates that approximately 25% of the β-End-sized material in AL is N-acetylated and a substantial α-MSH

sized and immunoreactive peak is present after molecular sieving. The majority of β-End-like immunoreactivity is β-End sized with a small portion of it being β-LPH sized. Molecular sieving chromatography of plasma indicates that the majority of the β-End-like immunoreactivity is β-End sized with 35% to 50% of it being N-acetylated. A large α -MSH immunoreactive peak is also observed. Intraventricular pretreatment with colchicine reduced the total amount of β -End-like immunoreactivity by about 50%. The amount of N-acetylated- β-End-like material was reduced below detectable limits as was α-MSH-like material.

Discussion

These findings suggest that POMC is processed differently in the rhesus monkey AL than it is in the rat or the pig. In the human, the majority of β-End-like immunoreactivity in human plasma has been reported to be β-LPH sized with only a small portion of β-End sized material (9, 12). It is presumed that the plasma profile observed in the human is reflective of the POMC processing which occurs in the AL since the human does not contain an IL. Therefore, these findings suggest a substantial species difference in POMC processing between the human and the monkey. Since the monkey is often selected for study because of its likeness to man, these differences should be considered when experimental results are to be interpreted across species.

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