Life Sciences, Vol. 33, Sup. I, 1983, pp. 57-60 Pergamon Press
Printed in the U.S.A.

POSTNATAL ONTOGENY OF ACETYLATED AND NON-ACETYLATED B-ENDORPHIN IN RAT PITUITARY

Norman E. Alessi, Henry Khachaturian,
Stanley Watson and Huda Akil

Mental Health Research Institute
University of Michigan
Ann Arbor, Michigan 48109

(Received in final form June 26, 1983)

Summary

Extracts of the anterior lobe and intermediate lobe of postnatal (P) (Day P1, P7, P14, P21, P28, P35, P42) and adult male Spraque-Dawley rats were analyzed by both a Beta-endorphin (B-END) radioimmunoassay and a radioimmunoassay for N-acetyl-B-END. In the anterior lobe, on P1, less than 2% of the adult level of B-END was present. By P42 this level had increased to 21% of adult levels. In the intermediate lobe, on P1, the B-END levels were less than 0.1% of the adult level, and by P42 this level approached approximately 45% of the adult levels. N-acetylated B-END was identified in both anterior lobe and intermediate lobe from P1 through adulthood. In the anterior lobe at P1, N-acetyl-B-END immunoreactivity contributes approximately 25% of the total B-END immunoreactivity. This level drops to less than 10% by P21, and to adult-like levels by P42 (less than 5%). On the other hand, in the intermediate lobe, the N-acetyl-B-END levels start at 70% of the total B-END immunoreactivity at P1 and by P14 reaches adult-like proportions of 90% or more of the total B-END immunoreactive material.

The differential post-translational processing of B-Endorphin (B-END) in the anterior and intermediate lobes of the pituitary in adult rat has been well characterized (1,2,3). An important post-translational process in the intermediate pituitary is the N-acetylation of different B-END derivatives. In fact, several different N-acetylated derivatives of B-END have been shown to occur in the intermediate pituiary of adults from a number of different species. The N-acetylation at the amino-terminal tyrosine residue is an important modification which results in almost a complete loss in analgesic potency and over a 1000-fold drop in the ability to displace [^{3}H]-B-END from brain binding sites (4,5).

To date, the ontogenetic studies of B-END have primarily focused on the development of immunohistological staining of B-END-containing cells in the embryo, or the differential development of B-END as compared to other opioid peptides (6,7,8). These studies have clearly demonstrated the early presence of B-END staining in the diencephalic area (arcuate nucleus) at E12, and the subsequent and differential development of B-END in the anterior lobe (E15) and intermediate lobe (E17). Now with the development of antibodies specific for the acetylated forms of B-END, this modification can be investigated to determine not only its physiological significance but

0024-3205/83 $3.00 + .00

its alterations during development.

The current study investigates the ontogeny of both B-END immunoreactivity and N-acetylated B-END immunoreactivity in the anterior and intermediate lobes of the pituitary during postnatal development in the male rat.

Methods

Dissection/Tissue Preparation: Postnatal male Sprague-Dawley rats were sacrificed by decapitation at one week age intervals from day P1 to P42, as were adults. Brains were immediately dissected from the skull, and the pituitaries were removed and micro-dissected. The intermediate lobe was peeled from the anterior lobes, then the medial portion of the anterior lobe was dissected and discarded. Immediately following the dissection, all specimens were frozen and stored at -80°C until further processing. At that time each pituitary specimen (intermediate lobe or anterior lobe) was placed in 2 ml 0.1N HCl and Acetone (1:3) at 4°C, then homogenized for 10 sec using a Polytron. After aliquots of this solution were taken for protein determination, these specimens were centrifuged at 10,000 rpm for 20 min. The supernatant was taken, lyophilized, and stored at -20°C until the time of assay.

B-END RADIOIMMUNOASSAY. The antiserum "Brenda" used in this assay is directed to the midportion of B-END. At a final antibody concentration of 1:30,000, this results in 100% cross-reactivity with all forms of B-END, Proopiomelanocortin and B-Lipotropin and the N-acetylated forms of B-END. All tracers are iodinated by the standard chloramine-T method. The IC_{50} with this antibody is 30 fm/assay tube.

N-acetyl-B-END Radioimmunoassay. The Radioimmunoassay for N-acetylated forms of B-END uses the antibody, from the rabbit "Nancy-Beth" at a final concentration of 1:6,000. This antiserum relies completely on N-acetylation of the tyrosine-glycine-glycine-phenylalanine core as its recognition site and detects equally all N-acetylated opioids. The assay conditions are similar to those of B-END. The IC_{50} is 200 fm/assay tube.

Results

Table 1 shows the B-END and N-acetylated B-END immunoreactivity contained within the extracts of the anterior lobe and intermediate lobe from each stated age. From P1 to P42 the levels of B-END increase 12 fold in the anterior lobe and 214 fold in the intermediate lobe. From P42 to adulthood in the anterior lobe, B-END increases a further 5 fold, resulting in a total increase of greater than 50 fold from P1 to adulthood. In the intermediate lobe the total increase from P1 to adulthood is greater than 470 fold. Further, the patterns of increase in the intermediate lobe is different than that in the anterior lobe. Following comparable rates of increase in B-END levels from P1 to P7, the levels of B-END increases at an accelerated rate in the intermediate lobe whereas in the anterior lobe a marked increase in B-END levels does not occur until the onset of puberty (P35-P42).

N-acetylation of B-END is present at birth in the anterior lobe and intermediate lobe. At P1, the N-acetylation of B-END in the anterior pituitary is substantial, making up almost 25% of the total B-END material. This level of acetylation drops to approximately 9% of the total B-END level by P28, then to adult-like levels (less than 5%) by P42. In the intermediate lobe, the acetylation of B-END begins at 70% at P1, and continues to make up a high percentage of B-END throughout development.

TABLE I

B-Endorphin/N-Acetyl B-Endorphin IR

Anterior Pituitary

Postnatal Day	P1	P7	P14	P21	P28	P35	P42	Adult
B-Endorphin IR (pm/lobe)	4.24 ± .96 (5)	14.5 ± 2.5 (6)	14.5 ± 3.0 (6)	19.5 ± 1.4 (6)	28.8 ± 7.8 (5)	35.3 ± 50.0 (6)	50.0 ± 3.7 (5)	242.4 ± 20.7 (5)
N-Acetyl B-Endorphin IR (pm/lobe)	1.1 ± .5 (6)	1.85 ± 0.25 (6)	2.15 ± 0.19 (6)	2.98 ± 0.72 (6)	2.31 ± 0.57 (6)	2.36 ± 0.19 (5)	2.4 ± 0.60 (3)	12.0 ± 12.0 (5)

Intermediate Pituitary

Postnatal Day	P1	P7	P14	P21	P28	P35	P42	Adult
B-Endorphin IR (pm/lobe)	2.28 ± 0.39 (5)	20.15 ± 3.5 (6)	18.75 ± 1.8 (4)	32.5 ± 2.2 (5)	128.5 ± 17.4 (6)	262.5 ± 24.4 (6)	487.5 ± 39.4 (6)	1,080 ± 64.0 (5)
N-Acetyl B-Endorphin IR (pm/lobe)	1.6 ± 0.48 (6)	12.7 ± 1.4 (6)	44.7 ± 4.8 (5)	71.63 ± 15.8 (6)	156.0 ± 5.6 (5)	217.0 ± 28.1 (5)	380.0 ± 68.1 (6)	1,008 ± 128.6 (5)

B-Endorphin/N-acetyl B-Endorphin Immunoreactivity (IR) in the anterior and intermediate lobes of postnatal (P) and adult Sprague-Dawley rats. Postnatal ages are shown in days. Values expressed as $\bar{x} \pm$ S.E.M.

Discussion

This study clearly demonstrates the differences in the postnatal development of B-END immunoreactivity and N-acetylated B-END immunoreactivity in the anterior and intermediate lobes of the male rat pituitary. The level of B-END immunoreactivity at P1 is greater in the anterior lobe than intermediate lobe. By P7, the levels of B-END immunoreactivity in the intermediate lobe have begun to surpass the levels in the anterior lobe. This pattern continues, with the levels of B-END in the intermediate lobe rising more rapidly throughout development. On the other hand, the level of B-END in the anterior lobe rises slowly and does not change rapidly until near or after puberty (P35-42).

N-acetylation of B-END is found throughout postnatal development in both the anterior lobe and the intermediate lobe. Also, immunohistological staining for a-MSH (8) and other radioimmunoassay data (unpublished) indicate that the N-acetylation of proopiomelanocortin related peptides begins in utero. Of further interest is the relatively high level of N-acetylated B-END in the anterior pituitary at P1 which subsequently decreases to adult levels by P28. A parallel process which occurs in the anterior pituitary corticotrophs is the gradual postnatal decrease in alpha-MSH immunostaining from P1 to P14, and the subsequent loss of immunorectivity by P28. This suggests that during development there is a period during which the anterior pituitary processes proopiomelanocortin peptides much the same way as the intermediate pituitary. These differences in post-translational alterations in the processing of B-END during development also suggests that there are factors which influence these processes in the anterior lobe in utero and following birth, but soon subside. To what degree these factors can subsequently become physiologically reactivated is unknown, as is the significance of the presence of N-acetylated peptides in the anterior pituitary during development.

Acknowledgements

This work was supported by a Research Fellowship, Department of Psychiatry (NEA), and NIDA grant DA02265 to S.J.W and H.A.

References

1. R.E. MAINS and B.A. EIPPER, J. Biol. Chem., 256:5683-5688 (1981).
2. S. ZAKARIAN and D. SMYTH, Proc. Natl. Acad. Sci. USA, 76:5972-5976 (1979).
3. H. AKIL, Y. UEDA, H.S. LIN and S.J. WATSON, Neuropeptides, 1:429-446, 1981.
4. J.F.W. DEAKIN, J.O. DOSTROVSKY and D.G. SMYTH, Biochem. J., 189:501-506 (1980).
5. H. AKIL, E. YOUNG, S.J. WATSON and D. COY, Peptides, 2:284-292 (1981).
6. D.G. SCHWARTZBERG and D.K. NAKANE, Endocrinology, 100:855-864 (1982).
7. A BAYON, W.J. SHOEMAKER, F.E. BLOOM, A. MAUSS and R. GUILLEMIN, Brain Research, 179:93-101 (1979).
8. H. KHACHATURIAN, N.E. ALESSI, N. MUNFAKH, and S.J. WATSON. Paper presented at this symposium.