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POSTNATAL DEVELOPMENT OF BETA-ENDORPHIN IMMUNOREACTIVITY IN THE MEDULLA OBLONGATA OF RAT

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ABSTRACT

Beta-endorphin (B-END) like immunoreactivity (i.r.) levels were measured by radioimmunoassay in the medulla oblongata of developing rats on postnatal ages P1-P42 at 7 day intervals, and in adult rats. From P1 to P42, B-END i.r. increased from 77.0 ± 1.3 fm to 900.0 ± 21.6 fm per medulla region (Mean ± S.E.M.). Adult levels of B-END i.r. were 852.0 ± 17.0 fm per medulla region. When B-END i.r. was determined per unit protein during this developmental period, a statistically significant change in levels was noted. B-END i.r. dropped from P1 to P7, and then increased from P7 to P14 ($P<0.01$). From P14 through adult, levels did not change significantly. Despite a "drop-out" in the observed immunostaining of B-END neurons in caudal medulla (perikarya in the nucleus tractus solitarius) at P21, radioimmunoassayable levels of this peptide remained constant from P21 through adult per unit protein.

INTRODUCTION

Neurons containing proopiomelanocortin (POMC) peptides, Beta-Endorphin (B-END) and ACTH have been identified in the nucleus tractus solitarius (NTS) of the adult rat medulla oblongata (1-4). Previous immunohistochemical studies of the development of this system have demonstrated the dropping out of perikaryal B-END immunoreactivity (i.r.) in the third postnatal week (1,4). A previous biochemical study of the postnatal ontogeny of B-END i.r. in the medulla demonstrated a steady increase per unit of protein in B-END i.r. in this area (5). In the present study, B-END i.r. is measured in the caudal medulla oblongata during postnatal development using a radioimmunoassay method and the results are compared to it's immunocytochemical presence in the NTS neurons.

MATERIALS AND METHODS

Tissues Extraction: Postnatal (P) rats, taken at 7 day intervals (P1...P42), as well as adults, were sacrificed by decapitation. The medulla oblongata was dissected as a block, placed on dry ice, and stored at -80° C. At the time of processing, each tissue block was homogenized in 1 ml of 0.1N HCl and acetone (1:3). This suspension was centrifuged at 10,000 rpm for 20 min. The super-

natant was removed, lyophilized, and stored at -80° C until the time of the assay.

B-END Radioimmunoassay and Protein Measurement: B-END-like i.r. was determined utilizing the anti-B-END serum ("Barbarella") obtained from Dr. Huda Akil, at a final dilution of 1:20,000. At this dilution the sensitivity of the assay was 20-30 fm/ml. The procedure used has been previously described (6). This antiserum was 100% cross reactive with B-END(1-31),-(1-27), and 98% cross reactive with the N-acetylated forms of the latter peptides. With regard to other peptides, it demonstrated the follwowing levels of cross reactivity: ACTH(1-24) - 0%; alpha-MSH - 1%; desacetyl-alpha-MSH - <2%; Met-enkephalin - <1%; BAM-12P - 0%; BAM22P - 0%; and peptide E - 2%. Levels of soluble protein were determined using the Lowry Technique (7). Statistical analysis of data involved the use of both one-way analysis of variance (ANOVA) and Kruskal-Wallis.

Immunocytochemistry: The neonatal rats were anesthetized and perfused through the heart with 50-100 ml 4% formaldehyde. Some rats were pretreated with colchicine (1 ugm/gm body weight) to enhance perikaryal immunoreactivity. The brains were removed, postfixed for an additional 2 hours, and were stored in 15% sucrose overnight. Each brain was then frozen in liquid nitrogen and sectioned on a cryostat at -20° C. The sections obtained were processed for peroxidase-antiperoxidase immunocytochemistry as described peviously (8). Anti-B-END serum ("Brenda") was obtained from Dr. Stanley Watson.

RESULTS

Radioimmunoassay: Levels of B-END i.r. began at 77.0 ± 1.3 fm per caudal medulla oblongata at P1 and increased steadily to 900.0 ± 21.6 fm per region at P42. This level dropped to 852 ± 17.0 fm per region in adult animals (Figure 1a). When B-END i.r. was expressed per ug protein, there was a significant alteration in the levels of this peptide from P1 through adulthood (Kruskal-Wallis, $P<0.01$) (Figure 1b). From P7 to P14, B-END i.r. dropped from 0.06 ± femtomoles (fm) per ug of protein to 0.04 ± fm/ug protein (ANOVA, $P<0.02$); and from P7 to P14 B-END levels increased from 0.04 ± fm/ug protein to 0.07 ± fm/ug protein (ANOVA, $P<.008$). Throughout the remaining postnatal period (P14-Adult) there were no further significant alterations in the levels of B-END per unit protein.

Immunocytochemistry: B-END i.r. was localized to both perikarya and fibers of caudal (commissural) NTS on days P1 (Figure 2), P7, P14, and P21 with gradually decreasing staining intensity. No immunostaining was observed beyond P21 without colchicine pretreatment. However, increasingly more B-END-stained fibers could be seen in caudal medulla on later postnatal stages. It is currently not known what proportion of these fibers originate from NTS neurons as opposed to the B-END neurons residing in the arcuate hypothalamic nucleus and projecting to the ventral portion of the medulla.

DISCUSSION

Based on the present findings, it is apparent that the levels of B-END i.r. does not decrease with maturation in the medulla oblongata as might be suggested by previous immunohistochemical findings of a diminution of B-END

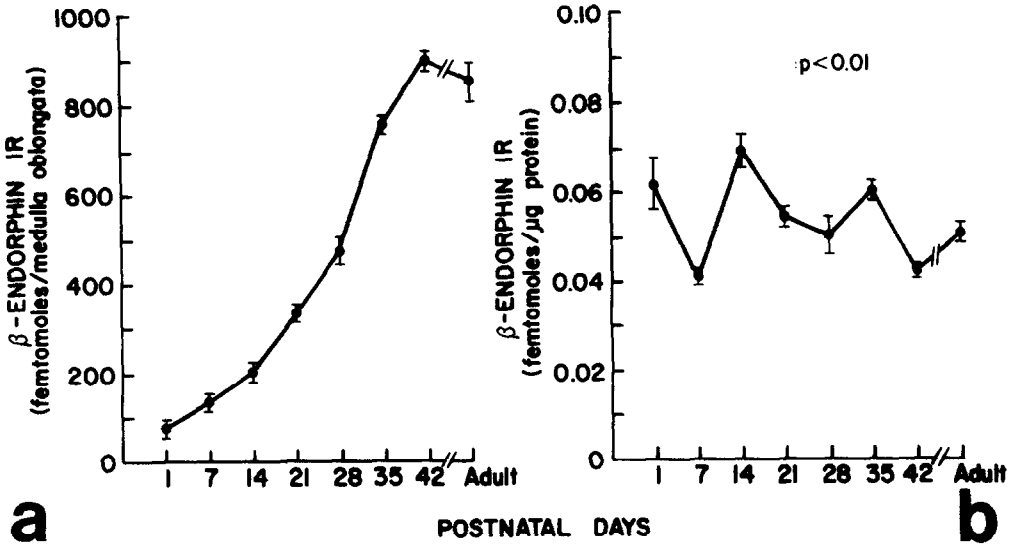

Fig. 1. a) Pattern of B-END i.r. material per medulla oblongata of developing rats. Results are expressed as Mean ± S.E.M. for 6 animals. b) Pattern of B-END i.r. material per ug of protein in each medulla oblongata region analyzed. Mean ± S.E.M. for 6 animals (Kruskal-Wallis).

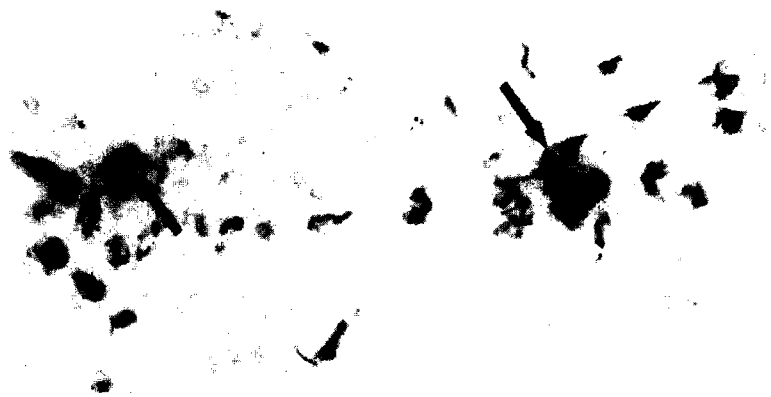

Fig. 2. B-END immunoreactive perikarya (arrows) in the NTS. The tissue was obtained from a neonatal rat (P1) pretreated with colchicine (see Methods) to enhance perikaryal immunoreactivity.

staining intensity in NTS perikarya. The stability in B-END i.r. as measured by radioimmunoassay, can be taken to imply that the "drop-out" noted with immunocytochemistry is due to a simple loss of peptide stores in individual perikarya below the sensitivity levels of the antisera used. On the other hand, the gradually increasing numbers of immunoreactive fibers seen in the caudal medulla could account for the stable radioimmunoassayable levels of this peptide throughout maturation. Exactly what proportion of the medullary B-END fibers originate from the NTS perikarya is at present not known, since some of these fibers could possibly belong to the arcuate hypothalamic POMC neuronal projection system (9).

When the stability of the levels of B-END i.r. in medulla is compared to the marked increases in the pituitary levels during the same period (6), a differential role of B-END across tissues is suggested. Based on these observations, it would appear that B-END in the medulla may act as a neurotransmitter/neuromodulator, whereas the high levels of peptide released from the pituitary is more indicative of its hormone-like action. Further studies of the maturational patterns of other POMC related peptides, i.e., ACTH and alpha-MSH will no doubt shed further light upon the role of these substances during development as well as in the adult.

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