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Pro-Opiomelanocortin mRNA and Peptide Co-Expression in the Developing Rat Pituitary

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KHACHATURIAN, H., S. P. KWAK, M. K.-H. SCHAFER AND S. J. WATSON. *Pro-opiomelanocortin mRNA and peptide co-expression in the developing rat pituitary*. BRAIN RES BULL **26**(2) 195–201, 1991.—Pro-opiomelanocortin (POMC) is synthesized in both the pituitary gland and the brain. Various peptide products of this precursor, namely beta-endorphin, ACTH and alpha-MSH are co-localized in the anterior lobe corticotrophs, all intermediate lobe cells and in hypothalamic neurons. Messenger RNA (mRNA) for POMC has further been shown to exist in these tissues. In this study, we have shown that POMC mRNA, and peptide accumulation as detected by in situ hybridization and immunocytochemistry, respectively, occur simultaneously within the rat pituitary gland during ontogeny and that their maturation occurs in parallel during prenatal and early postnatal development.

Pituitary Development POMC ACTH Gene expression mRNA

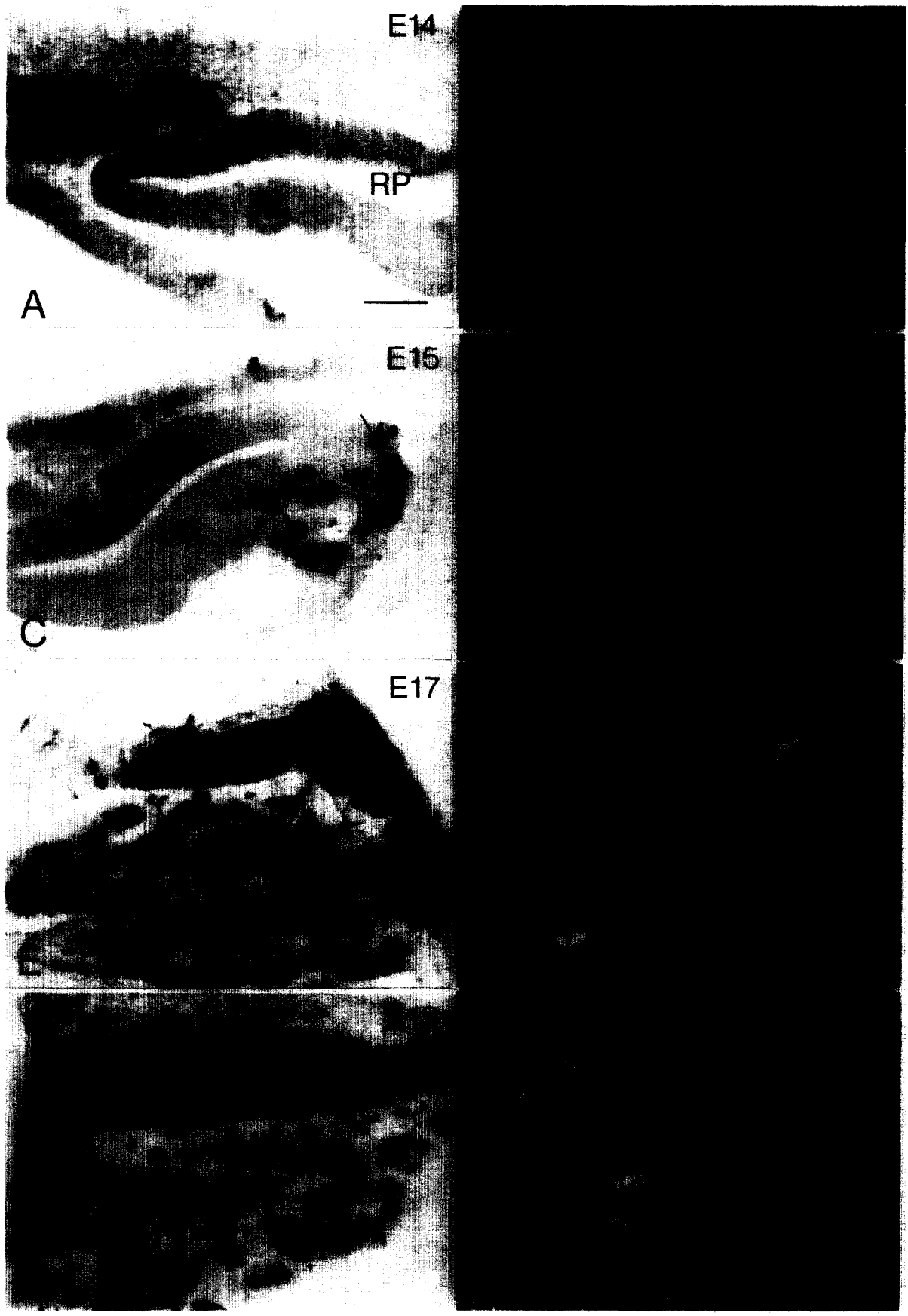
PRO-OPIOMELANOCORTIN (POMC) codes for several bioactive peptides, ACTH, alpha-MSH, beta-endorphin, that are important not only in the adult neuroendocrine function, but also during development (10, 15, 19, 20, 31, 34, 36, 48). POMC is synthesized in both the pituitary gland and the brain (1, 8, 13, 18, 24–26, 34). Furthermore, POMC gene expression has been shown to occur in the brain as well as the pituitary gland (5, 9, 17, 27–30, 43). Developmentally, POMC peptide products are seen very early during gestation in the pituitary and brain (2, 4, 7, 11, 21, 23, 31, 32, 41, 47). Given the known effects of POMC products on nerve cell maturation (45), as well as differential posttranslational processing of POMC during different developmental stages (21), it is of interest to investigate the expression of this important precursor and its mRNA during ontogenetic development. Thus, in the present study, we present evidence for the co-appearance of POMC mRNA and therefore gene expression and POMC peptide products in the rat pituitary gland as early as the immunocytochemical detection of POMC in this gland during ontogenetic development. We further study the development of POMC mRNA and peptide, throughout prenatal and early postnatal period in this gland.

METHOD

Adult male and female Sprague-Dawley rats were used to obtain timed-pregnant females. One male and three females were placed in a cage before 6 p.m., and vaginal smears were examined at 8 a.m. the following day for the presence of sperm. The first day of detection of sperm in vaginal smear was designated day zero of gestation. Pregnant (sperm-positive) rats were placed in individual marked cages, and at various time points they were sacrificed by cervical dislocation to obtain embryonic rat pups. These included embryonic days E14, E15, E17, and E19. Some pregnant rats were kept to term, and the male progeny were sacrificed at the following postnatal days: P1, P7, P14, and P21. The rat pups were flushed through the heart with 4°C normal saline, decapitated, and the head was fixed in 4°C neutral buffered paraformaldehyde. The postnatal rats, as well as an adult male rat, were sacrificed by intracardiac perfusion of the same fixative, and the pituitaries were extracted for further processing. After fixation, all tissues were immersed in 20% sucrose (4°C) for 24 hours, and frozen in liquid nitrogen. Ten micron cryostat sections of the pituitary gland were mounted onto polylysine-coated slides and stored at −80°C. Some sections were processed for peroxidase anti-peroxidase immunocytochemistry using an antiserum to ACTH (23). Close-by sections (nonadjacent) were processed for in situ hybridization histochemistry using a mouse POMC riboprobe (22).

Immunocytochemistry

Sections were incubated (37°C) with an ACTH antiserum (1/2000), the cross-reactivities of which have been fully characterized (23,51). After incubation for 1 hour, the slides were placed in a moist-box and refrigerated for 24 hours. The sections were washed in 0.02 M phosphate-buffered saline (PBS) and incubated with goat anti-rabbit serum (1/1000, Arnel) for 30 minutes followed by refrigeration for 24 hours. After several PBS washes,


E14
RP
A
E15
C
E17

the sections were incubated successively with anti-horseradish peroxidase (anti-HRP), and 4 μg/ml HRP enzyme (Sigma, type VI), each for 40 minutes. After PBS rinse, the sections were reacted in a solution of 0.125 mg/ml diaminobenzidine (Sigma) and 0.03% H_2O_2 for 15 minutes at room temperature. The slides were then washed in distilled water, dehydrated through ethanols and xylenes, and coverslipped in Permount for microscopic observations.

In Situ Hybridization

Mouse POMC cDNA (gift of Dr. J. L. Roberts, Columbia University) was subcloned into SP6. A cRNA probe was prepared using the SP 6 transcription system (Promega). The plasmid was linearized using restriction enzyme NcoI, and was transcribed in the presence of SP6 polymerase and [^{35}S]UTP yielding a probe of 436 bases (complementary to nucleotides 479–915 of the mouse POMC cDNA). Labeled cRNA (specific activity: 10.94×10^6 Ci/mmol) was separated from free label on a Sephadex G50 column. Prior to hybridization, sections were deproteinated in 0.2 N HCl for 15 minutes and washed in 2× SSC (300 mM NaCl, 30 mM Na citrate). Hybridization buffer contained 50% formamide, 10% dextran sulfate, 3× SSC, 1× Denhardts (0.02% ficoll, 0.02% polyvinyl pyrollidone, 10 μg/ml BSA), 100 μg/ml yeast RNA, and 100 μg/ml Salmon perm DNA. The [^{35}S]RNA probe (2 million counts: 8.23×10^{-14} mol./slide) was applied to each section, coverslipped, edges sealed with rubber cement, and incubated at 45°C for 24 hours. Control sections were incubated with an equal concentration of radiolabeled sense strand cRNA which has a sequence identical to that of the mRNA. Slides were washed in 2× SSC, then incubated in RNase A (1 mg/5 ml in 11 mM Tris buffer, 500 mM NaCl) for 30 minutes at 37°C to reduce nonspecific hybridization. Tissues were washed in 0.5× SSC at 45°C for 1 hour, dried, and processed for autoradiography. Kodak NTB-2 nuclear-track emulsion was melted at 42°C and diluted in distilled water (v/v). Slides were individually dipped, allowed to air-dry in dark, and stored at 4°C from 1–5 days. These were then developed in Kodak D19 developer, dehydrated through ethanols and xylenes, and coverslipped for microscopy. Observations and photography were performed on a Leitz Orthoplan microscope.

RESULTS

The ACTH antiserum used in the present study has been extensively characterized under immunocytochemical conditions (23,51). The antiserum recognizes the ACTH-(20–24) region and was not blocked by alpha-MSH, beta-lipotropin, beta-endorphin, or Met-enkephalin. For in situ hybridization control, the specificity of labeling seen with the POMC riboprobe was confirmed by the application of equal concentrations of radiolabeled sense strand cRNA to the sections. No binding was observed with the sense strand probe; this result was expected because the sense strand POMC cRNA has the same sequence as that of the POMC mRNA. The mouse POMC riboprobe showed high specificity for POMC mRNA. It had distinct advantages over oligonucleotides (cDNA) under in situ hybridization conditions. Labeling one out of four nucleotides ([^{35}S]UTP) over the entire 436 bases of cRNA yielded a probe with higher specific activity when compared to the labeling of much shorter sequences of nick-translated cDNA (3). Since RNA-RNA bonds are more stable than DNA-RNA bonds, more stringent washing conditions were applied to reduce nonspecific background. Lastly, posthybridization digestion with RNase A were used to further reduce nonspecific signals.

Analysis of sections processed for ACTH immunocytochemistry revealed a pattern of staining in the anterior lobe corticotrophs and intermediate lobe cells as previously described by a number of investigators (21,41). At the earliest embryonic day analyzed, i.e., E14, no ACTH staining could be detected in either anterior or intermediate lobes of the pituitary gland (Fig. 1A). At embryonic day E15, a few ACTH immunoreactive cells were seen in the anterior lobe, with no immunoreactivity discernable in the intermediate lobe (Fig. 1C). By day E17, the pattern of ACTH staining changed drastically such that a large number of anterior lobe cells and numerous (but not all) intermediate lobe cells exhibited immunoreactivity (Fig. 1E). At E19, the pattern of ACTH immunoreactivity resembled that of the adult pituitary with the notable exception that the cells in both lobes were more densely packed such that the staining pattern was much denser than that normally seen in the adult pituitary (Fig. 1G).

From postnatal days P1 through P21 (data not shown for P7), ACTH immunoreactivity in the anterior and intermediate lobes matured to more adult-like patterns (Fig. 2A, C, E). The major change observed during this period was one of rapid tissue expansion resulting in an apparent "dilution" of the staining intensity in the later stages studied. Finally, the pattern of staining at P21 was identical to that seen in the adult, save for tissue expansion (Fig. 2E, G).

In situ hybridization histochemical labeling showed a very similar pattern of autoradiographic grains to that seen with ACTH immunocytochemistry. No autoradiographic label (i.e., no [^{35}S]cRNA-mRNA binding) was seen in the earliest embryonic days analyzed; at E14 the grain density was not higher than background (Fig. 1B). However, by E15, several labeled cells could be seen in the anterior lobe, corresponding to the same region where immunoreactive ACTH cells reside (Fig. 1D). The pattern of autoradiographic grain densities over the anterior and intermediate lobes at embryonic days E17 and E19, also mirrored the pattern of ACTH staining in these lobes. Labeling became increasingly intense over both lobes from E17 to E19 (Fig. 1F, H). Postnatally, in situ labeling patterns became progressively less intense in the anterior lobe but not the intermediate lobe. At P1, grain density over the anterior lobe was lower than that seen at E19 (Fig. 2B). Likewise, at P7 (data not shown), P14, and P21, and in the adult, autoradiographic grain density over the anterior lobe was less intense when compared to embryonic patterns of labeling (Fig. 2D, F, H). This diminution of label with increasing age corresponded well with the progressively less intense ACTH staining observed under immunocytochemical conditions, a phenomenon attributable perhaps to general tissue expansion with increasing age.

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FIG. 1. Parasagittal sections through the rat pituitary gland on embryonic days E14 (A and B), E15 (C and D), E17 (E and F), and E19 (G and H). These sections were processed for ACTH immunocytochemistry (A, C, E, G), and in situ hybridization histochemistry using a POMC cRNA (B, D, F, H). Note that no ACTH immunoreactivity or specific autoradiographic grains can be seen in either anterior lobe (AL) or intermediate lobe (IL) at E14, but at E15 numerous ACTH cells as well as autoradiographic grains over cells (arrows) can be seen in the same region of the AL. At E17 and E19, ACTH immunoreactivity and autoradiographic grains in both the AL and IL are very intense. RP = Rathke's pouch. Magnification for all sections: Bar (in A) = 50 μm.

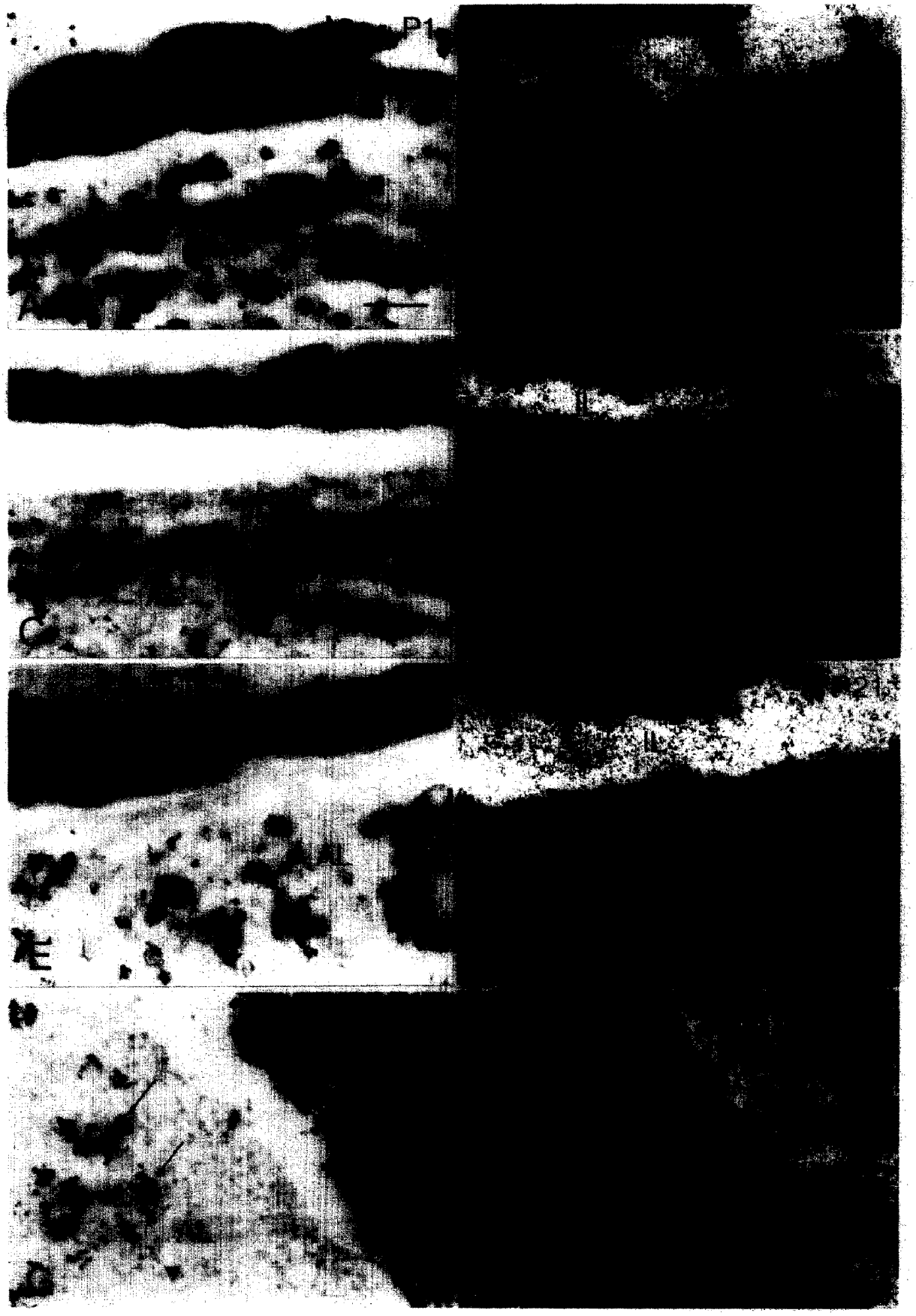
P1
F1
F2

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FIG. 2. Horizontal sections through the rat pituitary gland on postnatal days P1 (A and B), P14 (C and D), P21 (E and F), and in the adult (G and H). Sections A, C, E, and G, were processed for ACTH immunocytochemistry. Sections B, D, F, and H, were processed for in situ hybridization histochemistry using a POMC cRNA. Note that the intensity of ACTH immunoreactivity per unit area of the anterior lobe (AL) is progressively diminished from P1 to P21 and in the adult. In parallel to this, the autoradiographic grain density over the AL cells becomes progressively less intense during the same time period. IL = intermediate lobe. Magnification for all sections: Bar (in A) = 50 μm.

DISCUSSION

In the present study, we have shown that POMC mRNA expression as determined by in situ hybridization histochemistry, and POMC peptide expression as observed by ACTH immunocytochemistry, are parallel events in both the pituitary anterior and intermediate lobes during prenatal and early postnatal development in the rat. ACTH immunoreactivity was used as a marker for POMC synthesis, since this peptide, along with alpha-MSH, beta-endorphin and others are all derived from POMC and are co-stored in the same cells of the pituitary (6, 38, 52). Furthermore, the ACTH antiserum has been characterized under immunocytochemical conditions (23,51). The mouse POMC riboprobe used in these studies binds with high specificity to POMC mRNA. It has distinct advantages over oligonucleotides (cDNA) under in situ hybridization conditions. Labeling one out of four nucleotides ([^{35}S]UTP) over the entire 436 bases of cRNA yields a probe with higher specific activity when compared to the labeling of much shorter sequences of nick-translated cDNA (3). Furthermore, RNA-RNA bonds are more stable than DNA-RNA bonds, and therefore more stringent washing conditions can be applied to reduce nonspecific background. Lastly, posthybridization digestion with RNase A can further reduce nonspecific signals due to single-stranded probe "stickiness" to tissue. The control conditions used in this study consisted of the application of equal concentrations of radiolabeled sense strand cRNA to the sections. Since the sense strand POMC cRNA has the same sequence as that of the POMC mRNA, no binding was observed, confirming the specificity of the in situ labeling seen with the POMC riboprobe.

Numerous studies have addressed the development of the POMC precursor and its peptides during prenatal and postnatal development (2, 4, 7, 11, 14, 21, 23, 31, 32, 41, 47). All of these studies are in agreement that POMC peptides occur very early in both pituitary and brain during development. For example, beta-endorphin, and ACTH immunoreactivity are seen in the rat hypothalamus as early as embryonic day E12 (21,41). The appearance of POMC peptides in the pituitary, however, is comparatively delayed until embryonic day E15. Nevertheless, POMC peptides are among the earliest peptidergic systems to develop during ontogeny. Even among the opioid peptides, POMC products are seen earlier in both brain and pituitary when compared to either the pro-enkephalin or pro-dynorphin peptides (2, 4, 16, 21, 35, 37, 39, 42, 50). The significance of early POMC synthesis during ontogenetic development has been explored by several investigators. For example, alpha-MSH and other ACTH fragments have been shown to stimulate nerve cell maturation, and thus might fit into the category of neurotrophic substances (45). Furthermore, in the adult rat brain, there is evidence for differences in posttranslational processing of POMC in different regions, an observation that is consistent with the known posttranslational processing differences in POMC between the two lobes of the pituitary (12,13). Acetylation of ACTH fragments in the adult intermediate lobe to yield alpha-MSH is one such example. This process is apparently indigenous to the intermediate lobe cells and occurs readily in the anterior lobe as well as during prenatal and early postnatal development (21). Yet the significance of this early ontogenetic processing of ACTH in the intermediate lobe can only be appreciated when taken in light of the evidence that developing adrenal cortical cells respond to alpha-MSH but not ACTH during early ontogeny (44).

POMC mRNA has been shown to occur in the pituitary of several mammalian species including rat, cat, pig, bull, and man (5, 9, 17, 27–29, 43). The pattern of labeling is identical to that which is seen using POMC peptide immunocytochemistry, i.e., all the intermediate lobe cells and scattered anterior lobe corticotrophs exhibit autoradiographic labeling. During development, there is further evidence that POMC mRNA is present in the Rathke's pouch (embryonic anlage of the pituitary gland) as early as embryonic day E13–E15 localized primarily to the rat anterior lobe (22, 30, 40). Lugo et al. (30) have further noted that POMC mRNA detection preceded the detection of POMC peptide by 0.5 day. The discrepancy in our previous observations (22) and those of Lugo et al. (30) can perhaps be attributed to the differences in birth-dating techniques used to obtain timed-pregnant rats. A recent study of the prenatal ontogeny of POMC in the mouse pituitary gland also showed peptide and mRNA expression very early in this gland (14). Conversely, studies of prolactin and growth hormone mRNA expression have shown a much later prenatal (E19) maturation pattern for both of these hormones in the rat pituitary gland (33,46). The present study establishes the parallel development of POMC peptide immunoreactivity and POMC gene expression in the pituitary anterior and intermediate lobes beginning on embryonic day E15, and reaching adult-like patterns by postnatal day P21.

Postnatally, from P1 to P21, both peptide and mRNA labeling was progressively diminished in intensity in the pituitary anterior lobe but not the intermediate lobe. This can best be explained in terms of rapid tissue expansion in the same period of time, thus making the label per unit area appear less intense with advancing age. In the intermediate lobe, mRNA labeling continued to increase slightly in density throughout postnatal ages studied, consistent with the findings of other investigators (49).

Finally, the observation that both in situ hybridization histochemistry and immunocytochemistry detect mRNA and peptide labeling, respectively, on the same embryonic day (i.e., E15), suggests that POMC gene transcription and subsequent translation occur within a very short time in the pituitary gland during embryogenesis. It is important to note that the results of the present study were expected since POMC peptide expression and mRNA expression have already been shown independently to first occur in embryonic pituitary cells at approximately the same gestational age (21, 22, 40). Furthermore, this study does not specifically pinpoint the exact time of either POMC mRNA or peptide expression. Rather, this study confirms the functional status of peptide-synthesizing apparatus within POMC cells at the same time when the POMC gene is transcribed into mRNA.

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REFERENCES

1. Akil, H.; Watson, S. J.; Young, E.; Lewis, M. E.; Khachaturian, H.; Walker, J. M. Endogenous opioids: biology and function. Annu. Rev. Neurosci. 7:223–255; 1984.
2. Alessi, N. E.; Khachaturian, H.; Watson, S. J.; Akil, H. Postnatal ontogeny of acetylated and nonacetylated beta-endorphin in rat pituitary. Life Sci. 33(Suppl. I):57–60; 1983.
3. Angerer, L. M.; Stoler, M. H.; Angerer, R. C. In situ hybridization with RNA probes: an annotated recipe. In: Valentino, K. I.; Eberwine, J. H.; Barchas, J. D., eds. In situ hybridization. New York: Oxford University Press; 1987:42–70.
4. Bayon, A.; Shoemaker, W. J.; Bloom, F. E.; Mauss, A.; Guillemin, R. Perinatal development of the endorphin- and enkephalin-containing systems in the rat brain. Brain Res. 179:93–101; 1979.
5. Bloch, B.; Le Guellec, D.; Keyzer, Y. Detection of the messenger RNAs coding for the opioid peptide precursors in pituitary and adrenal by 'in situ' hybridization: study in several mammal species. Neurosci. Lett. 33:141–148; 1985.
6. Bloom, B.; Battenberg, E.; Rossier, J.; Ling, N.; Leppaluoto, T. M.; Vargo, T. M.; Guillemin, R. Endorphins are located in the intermediate and anterior lobes of the pituitary gland, not in the neurohypophysis. Life Sci. 20:43–48; 1977.
7. Bloom, F. E.; Bayon, A.; Battenberg, E.; French, E.; Koda, L.; Koob, G. Endorphins: developmental, cellular and behavioral aspects. Adv. Biochem. Psychopharmacol. 22:619–632; 1980.
8. Chretien, M.; Seidah, N. Chemistry and biosynthesis of pro-opiomelanocortin: ACTH, MSH's endorphins and their related peptides. Mol. Cell. Endocrinol. 21:101–127; 1981.
9. Civelli, O.; Birnberg, N.; Comb, M.; Douglass, J.; Lissitzky, L. C.; Uhler, M.; Herbert, E. Regulation of opioid gene expression. Peptides 4:651–656; 1983.
10. Clarke, G.; Wood, P.; Merrick, L.; Lincoln, D. W. Opiate inhibition of peptide release from the neurohumoral terminals of hypothalamic neurons. Nature 282:746–748; 1979.
11. Daikoku, S.; Chikamori, M.; Adachi, T.; Okamura, Y.; Nishiyama, T.; Tsutuo, Y. Ontogenesis of hypothalamic immunoreactive ACTH cells in vivo and in vitro: role of Rathke's pouch. Dev. Biol. 97:81–88; 1983.
12. Dores, R. M.; Jain, M.; Akil, H. Characterization of the forms of β-endorphin and α-MSH in the caudal medulla of the rat and guinea pig. Brain Res. 377:251–260; 1986.
13. Eipper, B. A.; Mains, R. E. Structure and function of proadrenocorticotropin/endorphin and related peptides. Endocr. Rev. 1:274–262; 1980.
14. Elkabes, S.; Loh, Y. P.; Nieburgs, A.; Wray, S. Prenatal ontogenesis of pro-opiomelanocortin in the mouse central nervous system and pituitary gland: an in situ hybridization and immunocytochemical study. Dev. Brain Res. 46:85–95; 1989.
15. Ferri, S.; Cocchi, D.; Locatelli, V.; Spampinato, S.; Miller, E. ACTH-(1–24) counteracts the prolactin-releasing effects of an opioid. Eur. J. Pharmacol. 77:143–145; 1982.
16. Gall, C. Ontogeny of dynorphin-like immunoreactivity in the hippocampal formation of the rat. Brain Res. 307:327–331; 1984.
17. Gee, C. E.; Chen, C. L. C.; Robert, J. L.; Thompson, R.; Watson, S. J. Identification of pro-opiomelanocortin neurons in rat hypothalamus by in situ cDNA-mRNA hybridization. Nature 306:374–376; 1983.
18. Herbert, E.; Budarf, M.; Phillips, M.; Rosa, P.; Policastro, P.; Oates, E.; Roberts, J. L.; Seidah, N.; Chretien, M. The presence of a presequence in the common precursor to ACTH and endorphin and the role of glycosylation in the processing of the precursor and secretion of ACTH and endorphin. Ann. NY Acad. Sci. 343:79–93; 1980.
19. Holaday, J. W.; Loh, H. H. Neurobiology of beta-endorphin and related peptides. In: Li, C. H., ed. Hormonal proteins and peptides. vol. 10. New York: Academic Press; 1981:202–291.
20. Iversen, L. L.; Iversen, S. D.; Bloom, F. E. Opiate receptors influence vasopressin release from nerve terminals in rat neurohypophysis. Nature 284:350–351; 1980.
21. Khachaturian, H.; Alessi, N. E.; Munfakh, N.; Watson, S. J. Ontogeny of opioid and related peptides in the rat CNS and related peptides in the rat CNS and pituitary: an immunocytochemical study. Life Sci. 33(Suppl. I):61–64; 1983.
22. Khachaturian, H.; Kwak, S. P.; Schafer, M. K.-H.; Watson, S. J. Pro-opiomelanocortin gene expression in rat pituitary during development: an in situ hybridization study. Soc. Neurosci. Abstr. 13:1249; 1987.
23. Khachaturian, H.; Lewis, M. E.; Alessi, N. E.; Watson, S. J. Time of origin of opioid peptide-containing neurons in the rat hypothalamus. J. Comp. Neurol. 236:538–546; 1985.
24. Khachaturian, H.; Lewis, M. E.; Dores, R. M.; Mansour, A.; Akil, H.; Watson, S. J. Comparative studies of pro-opiomelanocortin peptides in brain and pituitary. In: De Wied, D.; Ferrari, W., eds. Central actions of ACTH and related peptides. Padova: Liviana Press; 1986:15–31.
25. Khachaturian, H.; Lewis, M. E.; Tsou, K.; Watson, S. J. β-endorphin, α-MSH, ACTH, and related peptides. In: Bjorklund, A.; Hokfelt, T., eds. Handbook of chemical neuroanatomy: GABA and neuropeptides in the CNS. vol. 4, part 1. Amsterdam: Elsevier Science Publishers; 1985:216–272.
26. Krieger, D. I.; Licotta, A. S.; Brownstein, M. J.; Zimmerman, E. A. ACTH, beta-lipotropin, and related peptides in the brain, pituitary and blood. Recent Prog. Horm. Res. 36:277–345; 1980.
27. Lewis, M. E.; Khachaturian, H.; Schafer, M. K.-H.; Watson, S. J. Anatomical approaches to the study of neuropeptides and related mRNA in the central nervous system. In: Martin, J. B.; Barchas, J. D., eds. Neuropeptides in neurologic and psychiatric disease. New York: Raven Press; 1986:79–109.
28. Lewis, M. E.; Sherman, T. G.; Burke, S.; Akil, H.; Davis, L. G.; Arentzen, R.; Watson, S. J. Detection of proopiomelanocortin mRNA by in situ hybridization with an oligonucleotide probe. Proc. Natl. Acad. Sci. USA 83:5419–5423; 1986.
29. Lewis, M. E.; Sherman, T. G.; Watson, S. J. In situ hybridization histochemistry and synthetic oligonucleotides: Strategies and methods. Peptides 6(Suppl. 2):75–87; 1985.
30. Lugo, D. I.; Roberts, J. L.; Pintar, J. E. Analysis of POMC gene expression during prenatal development of the rat pituitary gland. Mol. Endocrinol. 3:1313–1324; 1989.
31. McDowell, J.; Kitchen, I. Development of opioid systems: peptides, receptors and pharmacology. Brain Res. Rev. 12:397–421; 1987.
32. Ng, T. B.; Ho, W. K. K.; Tam, P. P. L. Brain and pituitary beta-endorphin levels at different developmental stages of the rat. Int. J. Prot. Res. 24:141–146; 1984.
33. Nogami, H.; Suzuki, K.; Enomoto, H.; Ishikawa, H. Studies on the development of growth hormone and prolactin cells in the rat pituitary gland by in situ hybridization. Cell Tissue Res. 255:23–28; 1989.
34. O'Donohue, T. L.; Dorsa, D. M. The opiomelanotropinergic neuronal and endocrine systems. Peptides 3:353–395; 1982.
35. Palmer, M. R.; Miller, R. J.; Olson, L.; Seiger, A. Prenatal ontogeny of neurons with enkephalin-like immunoreactivity in the rat central nervous system: an immunocytochemical mapping investigation. Med. Biol. 60:61–82; 1982.
36. Parvizi, N.; Ellendorf, F. Beta-endorphin alters luteinizing hormone secretion via the amygdala but not the hypothalamus. Nature 286: 812–813; 1980.
37. Patey, G.; de la Baume, C.; Schwartz, J. C. Ontogenesis of enkephalinergic systems in the rat brain: postnatal changes in enkephalin levels, receptors and degrading enzyme activities. Life Sci. 27:245–252; 1980.
38. Pelletier, G.; Leclerc, R.; LaBrie, F.; Cote, J.; Chretien, M.; Lis, M. Immunohistochemical localization of beta-lipotropin hormone in the pituitary gland. Endocrinology 100:770–776; 1977.
39. Pickel, V. M.; Sumal, K. K.; Miller, R. J. Early prenatal development of substance P and enkephalin-containing neurons in the rat. J. Comp. Neurol. 210:411–422; 1982.
40. Pintar, J. E.; Lugo, D. I. Pro-opiomelanocortin gene expression, prohormone processing, and secretion during rat pituitary development. Ann. NY Acad. Sci. 512:318–327; 1987.
41. Schwatzberg, D. G.; Nakane, P. K. Ontogenesis of adrenocorticotropin-related peptide determinants in the hypothalamus and pituitary gland of the rat. Endocrinology 110:855–864; 1982.
42. Seizinger, B. R.; Liebish, D. C.; Grimm, C.; Herz, A. Ontogenetic development of the pro-enkephalin B opioid peptide system in the rat pituitary. Neuroendocrinology 39:414–422; 1984.

43. Sherman, T. G.; Kelsey, J. E.; Khachaturian, H.; Burke, S.; Akil, H.; Watson, S. J. Opioid peptides and vasopressin: the application of in situ hybridization to studies of the hypothalamus and pituitary. In: Uhl, G. R., ed. In situ hybridization in brain. New York: Plenum Press; 1986:49–62.
44. Silman, R. E.; Holland, D.; Chard, T.; Lowry, P. J.; Hope, J.; Robinson, J. S.; Thorburn, G. D. The ACTH 'family tree' of the rhesus monkey changes with development. Nature 276:526–528; 1978.
45. Swaab, D. F.; Ter Borg, J. P. Development of peptidergic systems in the rat brain. In: The fetus and independent life. Ciba Foundation Symposium 86. London: Pitman; 1981:271–294.
46. Tong, Y. A.; Zhao, H. F.; Labrie, F.; Pelletier, G. Ontogeny of prolactin mRNA in the rat pituitary gland as evaluated by in situ hybridization. Mol. Cell. Endocrinol. 67:11–6; 1989.
47. Tsang, D.; Ng, S. C.; Ho, K. P. O. Ontogenesis of opiate binding sites and radioimmunoassayable beta-endorphin and enkephalin in regions of rat brain. Dev. Brain Res. 5:257–261; 1982.
48. Van Wimersma Greidanus, T. B.; Van Ree, J. M.; Goedemans, H. J. H.; Van Dam, A. F.; Andringa-Bakker, E. A. D.; De Wied, D. Effects of beta-endorphin fragments of plasma levels of vasopressin. Life Sci. 29:783–788; 1981.
49. Wang, Y. Q.; Sato, M.; Morita, Y.; Noguchi, K.; Kiyama, H.; Tohyama, M. Postnatal ontogeny of POMC gene expression in the rat pituitary: an analysis by in situ hybridization histochemistry. Dev. Brain. Res. 47:53–58; 1989.
50. Wardlaw, S. L.; Frantz, A. G. Brain beta-endorphin during pregnancy, parturition, and the postpartum period. Endocrinology 113: 1664–1668; 1983.
51. Watson, S. J.; Richard, C. W., III.; Barchas, J. D. Adrenocorticotropin in rat brain: immunocytochemical localization in cells and axons. Science 200:1180–1182; 1978.
52. Weber, E.; Voigt, R.; Martin, R. Concomitant storage of ACTH and endorphin like immunoreactivity in the secretory granule of anterior pituitary corticotrophs. Brain Res. 157:385–390; 1978.