Maturation of endomorphin-2 in the dorsal horn of the medulla and spinal cord of the rat

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Introduction

Endomorphins are endogenous ligands for the mu opioid receptor that were recently isolated from the human [1] and bovine [2] central nervous system. Endomorphin 1 (EM-1; Tyr-Pro-Trp-Phe-NH2) is localized throughout the brain and spinal cord [3]. Endomorphin-2 (EM-2; Tyr-Pro-Phe-Phe-NH2) is densely localized in primary afferents, superficial lamina of the dorsal horn of the spinal cord, and in the trigeminal tract and trigeminal nucleus of the brain stem [3–7], with fibers found in many other sites throughout the brain. EM-2 is probably synthesized in the dorsal root ganglia neurons and transported to superficial lamina of the spinal cord [5,6]. Endomorphins are analgesic, acting through mu opioid receptors [2,8–12]. Taken together, these data suggest that endomorphins are the major endogenous opioid ligands for pre- and postsynaptic spinal mu opioid receptors, and could be critical regulators of a variety of physiological functions.

Opioid-mediated behaviors develop at different ages (see [13] for review). Mu opioid receptors develop early in the rat and mu opiates [14–16] are analgesic in neonates (see [17,18] for reviews). In contrast, stress-induced analgesias, dependent on release of opioid peptides, develop later [19–23]. This latter maturation may be due to differential development of these peptides. Because of the localization of endomorphin-2 in the dorsal horn of the spinal cord and trigeminal nucleus in the adult [3,4], we used the previously characterized antibodies to this peptide and examined its distribution in the spinal cord and hindbrain of infant rats using standard immunocytochemical methods.

Materials and Methods

Research protocols were approved by the Institutional Animal Care and Use Committee and were conducted under the ethical guidelines of the International Association for the Study of Pain, the Society for Neuroscience and the Society for Developmental Psychobiology.

Long Evans hooded rats aged 0, 3, 7, 10–11, 14 and 35–44 days of age bred at New York State Psychiatric Institute were subjects in this study. Housing conditions followed standard methods. Cages were checked daily at ~10.00 h and 17.00 h. Pups found on that day at either time were termed 0 days of age. Pups were deeply anesthetized, and perfused with acrolein/paraformaldehyde [3–5]. Immunocytochemical methods, using a previously characterized antibody to EM-2, were performed on floating 30 μm sections as described previously [3–5]. Most dilutions of the primary antibody were at 1:5000. Homologous preabsorption with 0.01–100 μM of the parent peptide blocked staining. There was some heterologous blocking of staining by the endomorphin-1 peptide, but it occurred at

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THE endomorphins are potent and selective endogenous agonists at the mu opioid receptor. We describe here the postnatal ontogeny of endomorphin-2 like immunoreactivity (EM2-LI) in the dorsal horn of the rat medulla and spinal cord. EM2-LI is dense in the superficial lamina of the dorsal horn of the adolescent and adult rat, with fibers also present in skin and dorsal root ganglia. No staining was noted at 3 days of age or younger. Faint and limited staining was noted by 7 days of age. The density and distribution of the immunoreactivity increased with age, reaching an adult-like distribution by weaning. Stress-induced responses mediated by endogenous opioids occur late in development and may be related to the late appearance of endomorphin-2.
concentrations 1–2 orders of magnitude greater than that for homologous blockade. The standard ABC method was used with diaminobenzidine as the chromogen [24]. Tissue from young animals was assayed with tissue from older animals to ensure that negative results were not likely to be due to assay error. Furthermore, tissues from a single age were assayed at different times, often separated by several weeks.

Results

Spinal cord: We examined the cervical and lumbar dorsal horn: results were the same at both levels. In the adolescent rat (35–44 days of age) the previously described adult pattern of staining was observed [3–7]. Staining was limited largely to a dense plexus in the superficial laminae of the dorsal horn (lamina I and IIo) with a limited number of fibers penetrating ventrally (Fig. 1). In contrast, no staining was seen at 0 and 3 days of age. Staining was noted by 7 days of age and increased in density with increasing age (Fig. 2). Although staining was present at 7 days, the pattern of staining was not mature. For example, the immunoreactivity was limited to the medial aspects of the dorsal horn, and the density of the stain was generally lighter (Fig. 2). The density and distribution of EM2-LI increased gradually with age, and although still immature at 14 days of age they became similar to the adult pattern by 21 days of age.

To compare the maturation of EM2-LI in the spinal cord with that of other opioid peptides, we performed a limited number of experiments in 3-
day-old pups with antibodies to enkephalin and dynorphin [1–8]. Both enkephalin- and dynorphin-
like immunoreactivity were present at 3 days of age in the superficial dorsal horn of the lumbar cord (data not shown).

Medulla: A similar pattern was noted in the dorsal horn of the medulla (adult distribution shown in
Fig. 3). Staining was totally absent at 0 and 3 days of age, first appearing in an immature form at 7 days (Fig. 4), and reaching the adult-like distribution and density by 21 days of age.

Skin and dorsal roots: We noted a limited number of fine fibers in the skin and dorsal root ganglia of the adolescent rat (35–44 days; Fig. 5). We were unable to identify fibers in these tissues in preweaning pups.

Discussion
Endomorphin-2 is distributed in the superficial aspects of the spinal and medullary dorsal horn. It matures relatively late compared to other opioid peptides and receptors. Endomorphin-2 is strategically localized to be involved in the modulation of noxious input. It is analgesic itself when injected intrathecally [1,8,11], and may mediate stress-induced analgesias. Of note, we have found that endomorphin-1, the sibling mu opioid specific en-

FIG. 3. Photomontage of endomorphin-2 like immunoreactivity in trigeminal nucleus of the medulla in the 34-day-old animal. Bar = 100 μm.

FIG. 4. Photomontage of endomorphin-2 like immunoreactivity in trigeminal nucleus of the medulla in the 7-day-old animal. Note the light staining and limited distribution compared with the 34-day-old seen in Fig. 3. Bar = 100 μm.

FIG. 5. Isolated endomorphin-2 like immunoreactive fibers in the glabrous skin of the hindpaw, and in the lumbar dorsal root ganglion (arrowheads). Bar = 25 μm.
Endomorphin-2 is present in regions of the medulla and spinal cord that are involved in the processing of pain. The adult-like distribution of endomorphin-2 is present by 21 days of age, but is not detectable at 3 days of age and younger. The development to the adult pattern is slow over the following 2 weeks. Thus endomorphin-2, like endomorphin-1, but unlike other opioid peptides develops rather late and may explain the delayed appearance of behaviors mediated by endogenous opioids.

References


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