Effects of Perinatal Exposure to Opioid Agonists and Antagonists on Central Nervous System Development

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Publication info: Pre- and Peri-natal Psychology Journal 5. 4 (Summer 1991): 359-365.

Abstract: None available.

Full Text: Headnote ABSTRACT: The perinatal opioid syndrome has been recognized for over a century. Examination of this phenomena has revealed no pathognomonic symptoms, but rather a constellation of somatic and neurobiological deficits that may continue into adulthood. Research in this area has found that exogenous opioids such as heroin and methadone interact with opioid receptors and influence development. Moreover, a fundamental and important observation shows that endogenous opioid peptides, the counterpart to exogenous opioids, normally modulate developmental events. Opioids are inhibitory growth factors that tonically mediate their action by way of the opioid receptor. Studies now reveal that [Met5]-enkephalin, a naturally occurring neuropeptide derived from proenkephalin A, is the most potent opioid peptide in regulating growth and acts on a newly discovered opioid receptor-the zeta (f) receptor. Cell proliferation appears to be the major target of growth-related opioids. Exogenous opioids such as methadone and heroin also have an affinity for the zeta receptor, and involvement of these compounds with growth can be envisioned. Continued study of endogenous opioid systems and developmental processes, particularly at the cell and molecular level, may permit elucidation of the etiology and pathogenesis of various abnormalities (e.g., mental retardation, tumorigenesis) associated with neural development. Opiates and opiate-like compounds (hereafter collectively referred to as 'opioids') in medicine, as well as within the social setting, date back thousands of years.1 The consumption of opioids, maternally and paternally, has even further ramifications, with citations in the literature dating to 1870's discussing the harmful effect of opioids on the fetus and neonate.2 A great deal of research into the influence of opioid exposure on offspring has been conducted both in basic and clinical science.2-4 In general, opioid exposure has often been linked with growth retardation and neurobiological disorders. However, the number of confounding variables (e.g., problems in prenatal/postnatal care, nutritional status) is of such magnitude and complexity that determination of the etiology and pathogenesis of disabilities related to perinatal opioid exposure are difficult to assess. Certainly, no pathognomonic characteristics involved with the perinatal opioid syndrome have emerged. Rather a constellation of somatic and neurobiological problems are often apparent. During the 1970's, new and important information about opioids was obtained. In 1973, three different laboratories led by Snyder,5 Terenius,6 and Simon,7 discovered receptors for opioids. In 1975, Hughes and Kosterlitz8 provided evidence that the body makes its own morphine-enkephalins and endorphins. Thus the interaction of opioids with opioid receptors mediates a number of biological processes (e.g., analgesia). In relation to the perinatal opioid syndrome, a striking observation was that exogenous opioids such as methadone interface with opioid receptors to influence growth, since concomitant injections of opioid agonists such as methadone, along with opioid antagonists such as naloxone, blocked methadone's retarding effects.9'10 These data indicated that exogenous opioids such as methadone, heroin, and morphine may exert their effect on growth at the level of the opioid receptor. In 1983, a series of experiments reported in Science11 indicated that endogenous opioid peptides may regulate mammalian growth. These findings were extended in other investigations that followed.12-21 Many of these studies were designed to take advantage of the action of opioid antagonists. These compounds selectively block opioid agonists from interacting with the opioid receptor and, depending on either the dosage or schedule of drug administration, induces what is referred to as an "opioid receptor blockade." Observation of the repercussions during opioid receptor blockade reveal the function and tonic importance of these peptides. A daily pattern of short blockade followed by a longer period with the

absence of blockade can provide vital information as to endogenous opioid action. Opioid antagonists elicit above normal concentrations of opioids, as well as an increase in opioid receptor number and supersensitivity to opioid agonists.22-24 During the interval when an opioid antagonist is no longer present, the higher levels of opioids can interact with the greater number of receptors and produce a heightened response. Using the paradigm of daily intermittent and complete blockade, it was found that animals receiving a complete opioid receptor blockade demonstrate accelerated somatic and neurobiological development. This portion of the experiment revealed that endogenous opioids must be extremely important in regulating growth through inhibitory mechanisms and that this action must be tonic, otherwise removal of the influence of endogenous opioids should have little effect. Daily intermittent blockade retarded growth. A key experiment was to see if drug dosage was of importance, or if the duration of opioid receptor blockade was the vital element in regulating growth. By taking a dosage of opioid antagonist that, when given once daily, inhibited growth and dividing the total dosage into smaller aliquots to be given periodically each day (i.e., invoke a complete opioid receptor blockade), the question of dosage/duration could be addressed. The results showed that a daily series of low dosages of opioid antagonist stimulated development. Thus, the duration of opioid receptor blockade determined the course of developmental events. Additional proofs of the involvement of opioid-opioid receptor interaction were conducted, including the demonstration of stereospecificity. In these experiments, the (-) isomer of naloxone proved to be effective in altering growth, but not the (+) isomer.21 Although utilization of opioid antagonist paradigms has proven extremely important in revealing the role of endogenous opioids in growth, and circumvents the problem of the short-half life of opioid peptides in in vivo experiments, the underlying mechanism should involve both opioid agonist and antagonist paradigms. The work with opioid antagonists had shown that opioid agonists are inhibitory growth factors, and the changes in cell number and maturation of dendrites and synaptic spines suggested that cell proliferation and cell differentiation are regulated by opioids. Of course it must be kept in mind that changes in cell differentiation may be a consequence of opioid action on cell proliferation. To investigate the role of opioids in regard to cell replication and exploring the fundamental action(s) of opioids, studies were conducted in which DNA synthesis was examined.18 Using the cerebellar cortex of 6-day old rats and [3H]-thymidine to monitor DNA synthesis, animals were treated with naltrexone, a potent opioid antagonist. When naltrexone is present, a significant increase in [3H]-thymidine was noted in contrast to control specimens. Animals subjected to [Met5]-enkephalin, an opioid peptide, exhibited a marked reduction in cells incorporating [^sup 3^H]-thymidine; this effect was blocked by concommitant exposure to naloxone (an opioid antagonist with considerably less potency and duration than naltrexone). These data indicate that the endogenous opioids regulate cell proliferation, acting as inhibitory trophic factors. The results also show that opioidreceptor interaction and the influence on cell replication must be an active and ongoing process, since interruption of opioid-receptor interfacing by the use of potent opioid antagonists such as naltrexone produces an increase in DNA synthesis. To inquire further as to the special relationship of endogenous opioids to developmental events, immunocytochemical procedures were used to determine the presence of opioid peptides in the developing brain.25 Using the cerebellum, an area reported to have few enkephalinergic cells (i.e., Golgi II neurons) or fibers in adult rats, sections of preweaning and adult animals were stained with antibodies to [Met^sup 5^]-enkephalin. The results show that a germinative matrix, the external germinal (granule) layer, was highly immunoreactive; stain was associated with the cytoplasm of these cells but not the cell nucleus. A mosaic of staining occurred in the internal granule layer where the majority of external germinal cells translocate to differentiate as internal granule neurons. These results show that germinative cell populations exhibit opioid peptide immunoreactivity, but as these cells differentiate, they lose this immunoreactivity. This would suggest that some opioids bear a unique relationship to neuro-ontogeny, and may subserve a regulatory function. To address the question of which opioid peptide(s) is(are) involved with growth, tissue culture studies using neuroblastoma were employed.26 These cells actively proliferate in tissue culture, and opioid peptides can be added to the medium. Utilizing this strategy, we

discovered that [Met^sup 5^]-enkephalin, a naturally occurring opioid peptide derived from proenkephalin A, was the most potent opioid related to growth. Concentrations of 10-10M were capable of significantly reducing growth. Further investigation showed that both DNA synthesis and mitosis were influenced by this opioid peptide. Other studies indicated that these neuroblastoma cells produce this peptide, and that regulation of in vitro growth is controlled by this secreted peptide. A number of opioid receptors have been described, including mu, delta, kappa, sigma, and epsilon. In order to assess which opioid receptor is involved in growth, or if a new opioid receptor was associated with cell proliferation, we utilized radiolabeled [Met^sup 5^]-enkephalin, the opioid peptide found to regulate growth, in receptor binding assays. [Met^sup 5^]-enkephalin was found to bind to neuroblastoma cell homogenates obtained from tissue culture in a specific and saturable manner, with a binding affinity (Kd) of 1.6 nM and a binding capacity (B^sub max^) of 48 fmol/mg protein; 14,000 receptors per cell were estimated.27 Binding was dependent on protein concentration, time, temperature, and pH, and was sensitive to 100 nM, but not 5 nM, Na+, Ca^sup ++^, and Mg^sup ++^. Optimal binding required protease inhibitors, and pretreatment of the tumor cell homogenates with trypsin markedly reduced [^sup 3^H]-[Met^sup 5^]-enkephalin binding, suggesting that the binding site was proteinaceous in character. Displacement experiments indicated that [Met^sup 5^]-enkephalin was the most potent displacer of [^sup 3^H]-[Met^sup 5^]-enkephalin. Moreover, these displacement experiments showed that ligands selective for other opioid receptors were not very potent at binding sites for [Met^sup 5^]-enkephalin, indicating that this receptor was a new opioid receptor type. In view of the relationship of this receptor to the proliferation of cells, this receptor was named from the Greek word zoe (life): zeta (f), the sixth letter of the Greek alphabet. Thus, a number of interesting points emerge from the finding that an endogenous opioid-[Met^sup 5^]-enkephalin-serves as a naturally occurring growth inhibitory factor and that its action is mediated by the zeta opioid receptor. The binding affinity of the receptor and the concentration of opioid peptide required to affect growth are similar (i.e., in the nanomolar range), revealing the physiological importance of this endogenous opioid system. Finally, displacement studies using receptor binding analysis showed that exogenous opioids interacted with the zeta receptor at the micromolar range; earlier work also showed that compounds such as methadone and heroin at the micromolar range inhibited growth both in vitro and in vivo. Thus, exogenous opioids involved in the perinatal opioid syndrome may well act to inhibit growth through the zeta opioid receptor, and take advantage of this naturally occurring growth-regulatory mechanism. In summary, studies with exogenous opioids and the developing organism showed that opioids retarded growth. Further work revealed that endogenous opioids also regulate growth, and do so as inhibitory agents. With the exciting knowledge that an opioid receptor related to growth may be the mechanism utilized by the exogenous opioids, clinical and basic science phenomena reported earlier can now be explained. Cell and molecular biological studies should reveal the details of this interaction, and may be used to design strategies to rescue-or prevent- the problems associated with the perinatal opioid syndrome. References REFERENCE NOTES 1. Blum RH, A history of opium. In: Blum RH and Associates, eds. Society and Drugs. I. Social and cultural observations. San Francisco: Jossey-Bass, 1969:45-58. 2. Zagon IS, McLaughlin PJ, Weaver DJ, Zagon E. Opiates, endorphins, and the developing organism: a comprehensive bibliography. Neurosci Biobehav Rev 1982; 6:439-479. 3. Zagon IS, McLaughlin PJ. Endogenous opioid systems and neurobehavioral development. In: Rodgers RJ, Cooper SJ, eds. Endorphins, Opiates and Behavioral Processes. Chichester: John Wiley and Sons, 1988:287-309. 4. Zagon IS, McLaughlin PJ. An overview of the neurobehavioral sequelae of perinatal opioid exposure. In: Yanai J, ed. Neurobehavioral Teratology. Amsterdam: Elsevier, 1984:197-234. 5. Pert CB, Snyder SH. Opiate receptor: a demonstration in nervous tissue. Science 1973; 179:1011-1014. 6. Terenius L. Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. Acta Pharmacol Toxicol 1973; 32:317-320. 7. Simon EJ, Miller JM, Edelman I. Stereospecific binding of the potent narcotic analgesic PH]etorphine to rat brain homogenate. Proc Natl Acad Sci 1973; 70:1947-1949. 8. Hughes JA, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR. Identification of the pentapeptides from the brain with

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Publication title: Pre- and Peri-natal Psychology Journal

Volume: 5

Issue: 4

Pages: 359-365

Number of pages: 7

Publication year: 1991

Publication date: Summer 1991

Year: 1991

Publisher: Association for Pre&Perinatal Psychology and Health

Place of publication: New York

Country of publication: United States

Journal subject: Medical Sciences--Obstetrics And Gynecology, Psychology, Birth Control

ISSN: 08833095

Source type: Scholarly Journals

Language of publication: English

Document type: General Information

ProQuest document ID: 198680100

Document URL: http://search.proquest.com/docview/198680100?accountid=36557

Last updated: 2010-06-06

Database: ProQuest Public Health
