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Research report

Opioid growth factor and organ development in rat and human embryos

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Abstract

In addition to neurotransmission, the native opioid peptide, [Met^5]enkephalin, is a tonically active inhibitory growth molecule that is termed opioid growth factor (OGF). OGF interacts with the zeta (ζ) opioid receptor to influence cell proliferation and tissue organization. We now identify OGF and the ζ receptor in embryonic derivatives including ectoderm, mesoderm, and endoderm of the rat on gestation day 20. Messenger RNA for preproenkephalin (PPE), the precursor of OGF, was detected in the developing cells, suggesting an autocrine production of this peptide. Acute exposure of the pregnant female to OGF resulted in a decrease in DNA synthesis in cells of organs representing all three germ layers, and did so in a receptor-mediated fashion. The influence of OGF was direct, as evidenced in organ culture studies. Blockade of endogenous opioid interaction using naltrexone (NTX) produced an increase in DNA synthesis, indicating the constitutive and functional nature of opioid activity on growth during prenatal life. Human fetal cells contained OGF and the ζ receptor. These data support the hypothesis that endogenous opioid modulation of organ development is a fundamental principle of mammalian embryogenesis, and that OGF has a profound influence on ontogeny. Irregularities in the role of opioids as growth regulators in relationship to the more than 500,000 newborns suffering from birth defects each year in the US needs to be examined. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Significant birth defects occur in at least 100,000 live-born infants in the US each year, and these major structural anomalies are the leading cause of infant mortality [6,40]. Another 500,000 newborns suffer from minor congenital anomalies [6,40]. In 40–60% of all birth defects, the cause is unknown [40], although many malformations occur during formation of structures [40]. There is increasing evidence that growth factors and receptors play an important role in developmental biology (see reviews in Refs. [8,36,45]), including opioid peptides [2–5,9–20,22–35,38–44,46–53,55,56,58–66,68–75]. With the initial evidence that perturbation of endogenous opioid systems alters body and brain development, as well as modulate neoplasia, the importance of native opioids in neurotransmission [1] was extended and the novel postulate put forth

that opioids serve as growth factors [62–64]. Opioids may influence growth in a variety of ways including: (a) transduction of effects by way of classical opioid receptors such as μ , δ , and κ (e.g., Refs. [2,7,16,24,25,43,44]), (b) modulation in a non-opioid receptor mediated fashion [3,4,7,13,31,32], and (c) mediation of activity by way of novel opioid receptors [9,10,26,27,62–66,68–75].

One growth factor, the native opioid peptide [Met^5]enkephalin, is not only a neuromodulator [1] but also has received considerable attention as a potent regulator of development, cellular renewal, cancer, angiogenesis, and wound healing [5,10,18,20,23,24,26,33,34,47,48,51,65,66,68–70,73–75]. In view of its growth properties, [Met^5]enkephalin has been termed the opioid growth factor (OGF) [66]. OGF is an autocrine-produced and -secreted inhibitory peptide that is not cell, tissue, or organ specific, exhibits activity at physiologically relevant concentrations, does not elicit physical dependence, tolerance, and/or withdrawal, displays a temporal and spatial distribution consistent with specific growth-related effects, is sensitive to opioid antagonists, and has a direct, rapid, prolonged,

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stereospecific, non-cytotoxic, and reversible effect on growth which has been evidenced in tissue culture and in both prokaryotic and eukaryotic organisms. The receptor mediating OGF action is a novel opioid receptor because of its binding characteristics, tissue distribution, cellular location, temporal expression, and function; this nuclear-associated receptor has been termed zeta (ζ). Blockade of the interaction of endogenous opioids with opioid receptors by antagonists such as naltrexone (NTX) enhances developmental events [10,26,41,62–64,66,69–72,74,75], suggesting that growth-related opioid peptides such as OGF are tonically active.

Whereas OGF's influence in postnatal life as to somatic and neurobiological ontogeny is well-known (see review in Ref. [66]), information about the action of OGF during embryogenesis is limited. A number of reports have shown that OGF and gene expression for its prohormone, pre-proenkephalin A (PPE), as well as the ζ opioid receptor, are present during the prenatal period [9,11,14,17,22, 26,27,30,35,37–39,42,46–48,51,56,61]. Maternal opioid receptor blockade using the potent and long acting antagonist NTX has a profound effect on prenatal events, with neonatal body and organ weights increased substantially from control levels [29]. The effects of prenatal opioid receptor blockade also are manifested in the postnatal period, with body and organ weights being elevated from control values [29,71]. Moreover, the ontogeny of physical characteristics, spontaneous motor activity, and sensorimotor reflexes is altered during the preweaning period, as well as motor activity and emotionality in the postweaning period [28,71]. Perturbation of endogenous opioid systems in prenatal life also determines certain aspects of pain sensitivity in reaction to analgesics [71].

In this study, we addressed the question of whether native opioid peptides play a role in organ and tissue development during prenatal life. Using rats at gestation day 20, initial experiments employed a paradigm in which NTX was administered to pregnant females in order to block opioid receptors. The repercussions on DNA synthesis in organs and tissues from all three embryonic derivatives were monitored. To inquire if the well-known opioid peptide, OGF, subserves a growth function in embryogenesis, the effects of maternal exposure to this peptide on DNA synthesis in the ectoderm, mesoderm, and endoderm were ascertained. OGF's influence on development with respect to a mechanism of receptor-mediation was examined, and the query as to whether this peptide has a direct or indirect action on growth was evaluated by measuring DNA synthesis in organs cultured during exposure to agonists or antagonists. The presence and distribution of OGF and the ζ receptor were studied by immunocytochemistry, whereas the gene expression of this peptide was investigated by *in situ* hybridization. Finally, to begin to obtain information about the human condition, immunocytochemistry was performed on human fetal tissues and the location of OGF and the ζ receptor were determined.

2. Materials and methods

2.1. Animals

Male and female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were mated and the presence of sperm indicated day 1 of pregnancy. Animals were housed in wire-bottom cages and maintained under standard laboratory conditions. All investigations with animals conformed to NIH regulations and the guidelines of the Department of Comparative Medicine of The Pennsylvania State University College of Medicine; methods and protocols were approved by the Institutional Animal Care and Use Committee.

All embryos were collected on day 20 of gestation (E20). Pregnant animals were anesthetized with Nembutal (30 mg/kg; i.p.) and rapidly decapitated. Rat fetuses were immediately removed from the midline portions of each uterine horn and prepared for autoradiography, immunocytochemistry, or *in situ* hybridization.

2.2. DNA synthesis

Four hours prior to collection of fetuses, some pregnant rats were injected with either 10 mg/kg OGF, 30 mg/kg NTX, or 0.5 ml sterile water (vehicle). Radiolabeled thymidine ([³H]thymidine; 20 Ci/mmol specific activity, New England Nuclear) was injected into the pregnant females at 3, 2, and 1 h (0.33 ml per i.p. injection) prior to sacrifice. Fetuses were harvested, fixed in phosphate buffered formalin for 7 days, and processed for paraffin embedding. Six-micron sections were cut and coated with Kodak NTB-2 emulsion; slides were stored at 4°C for 14 days before developing and staining with hematoxylin and eosin (H&E). Two to six pregnant rats were treated with each compound.

At least six fetuses from each mother were collected. Two sections per embryo were examined, and two grids per section were assessed. Twelve different organs were evaluated including: cerebrum (telencephalon), skin, spinal cord, cerebellum, vertebrae, ribs, adrenal cortex, heart, lung, liver, dorsal surface of the tongue, and intestinal crypts. The number of cells with and without silver grains were recorded for each grid. All cells within a field were counted and no effort was made to distinguish cell type. If an organ of interest did not have any grains, the entire fetus was not utilized. Likewise, if organs appeared to have high background, the entire fetus was not analyzed. Labeling indexes (LI) were calculated as the number of cells with grains divided by total number of cells \times 100.

2.3. Organ culture

Immediately following the sacrifice of pregnant females, organs were dissected and placed in serum-free Dulbecco's media containing 10⁻⁶ M concentrations of

either OGF, OGF and the short-acting antagonist naloxone (NAL), NAL alone, NTX, or 0.2 ml of sterile water (vehicle, control) along with radiolabeled thymidine (2 μ Ci/ml) for 4 h. Organ cultures were maintained at 37°C in 5% CO₂/95% air throughout the study, and processed for autoradiography.

2.4. Immunocytochemistry

E20 fetuses were harvested and quickly frozen in isopentane chilled on dry ice. Ten micron sections were collected onto autoclaved, gelatin-coated slides and stored at -20°C for no longer than 2 weeks. Immunocytochemical procedures followed those published earlier [9,56,67]. Antibodies for [Met⁵]enkephalin (OGF) and the ζ receptor are described in earlier reports [9,10,67]. In brief, the polyclonal antibody to [Met⁵]enkephalin (CO-172) recognized 25 ng of [Met⁵]enkephalin, but did not detect as much as 500 ng of β -endorphin or 1 μ g of [Leu⁵]enkephalin, [Met⁵, Arg⁶, Phe⁷]enkephalin, [Met⁵, Arg⁶, Phe⁷, Leu⁸]enkephalin, or dynorphin A1–8. The antibody to the ζ receptor was generated against the 17-kDa polypeptide and revealed four bands of immunoreactivity (32, 30, 17, and 16 kDa) in Western blot analysis. Antibodies were diluted 1:100 in Sorenson's phosphate buffer with 1% normal goat serum and 0.1% Triton X-100, and the secondary antibody (rhodamine-conjugated goat anti-rabbit IgG) was utilized at 1:100. Immunoreactivity was visualized using an Olympus microscope with a rhodamine interference filter. Controls for specificity included adjacent sections processed with antibodies preabsorbed with excess antigen, and specimens treated only with secondary antibody.

2.5. In situ hybridization

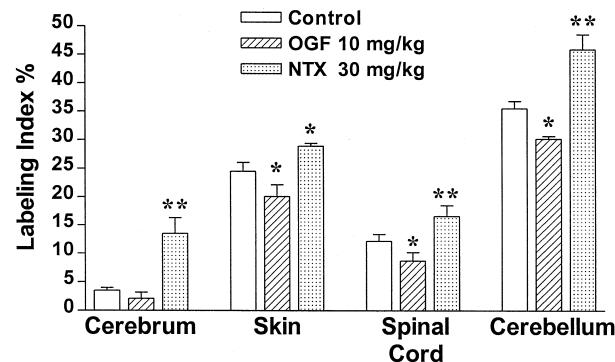
In situ hybridization methods followed those outlined earlier [30,61]. Frozen sections were collected on un-subbed, autoclaved slides and used either immediately or within 1 week of collection (storage at -20°C). Animals were sectioned in the median or paramedian plane in order to sample organs. Sections were treated as described previously [30,61] and incubated with γ -[³⁵S]dATP-labeled PPE antisense or sense (control) oligonucleotides.

The plasmid containing the PPE sequence was provided by Dr. Steven Sabol [57], and was derived from the pRPE2 clone. Oligonucleotide probes were synthesized by the Core Macromolecular Facility at The Milton S. Hershey Medical Center. The antisense PPE probe corresponded to nucleotides 388–435 of the open reading frame for rat PPE [57]; the sense oligoprobe corresponded to the sense sequence. All sequences were checked for redundancy using BLAST.

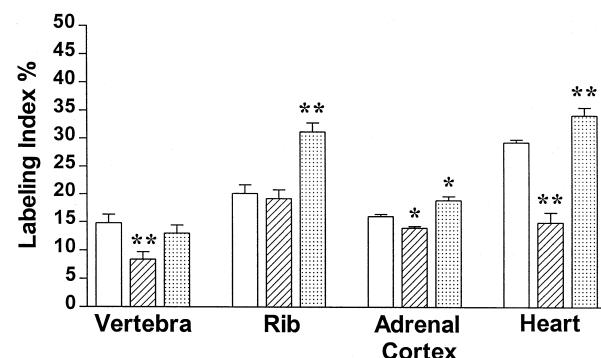
Tissue sections were dipped in distilled water and air-dried for 24 h prior to being exposed to Kodak XAR-5 film for 7–14 days. Some slides were immediately coated

with Kodak NTB-2 emulsion and stored at 4°C for up to 8 weeks. Emulsion-coated slides were developed with Kodak D-19, counterstained with H&E, and viewed with an Olympus BH-2 microscope equipped for dark-field and phase-contrast microscopy.

Ectodermal Derivatives



Mesodermal Derivatives



Endodermal Derivatives

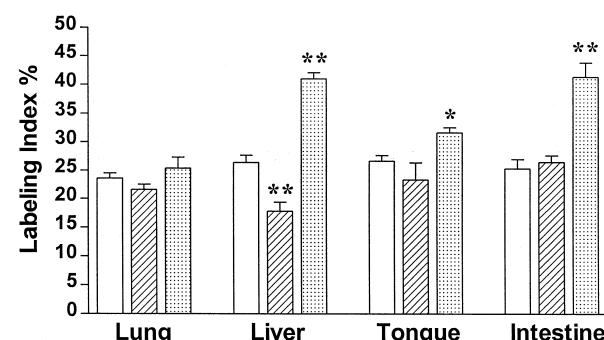


Fig. 1. The LI for organs collected from E20 rat embryos that were maternally exposed for 4 h to either 10 mg/kg OGF, 30 mg/kg NTX, or sterile water. Pregnant female rats were injected i.p. with tritiated thymidine at three 1-h intervals prior to being sacrificed. Bars represent means \pm S.E. of percent labeled cells in organs collected from at least six embryos taken from three different rats; four organs representative of each germ layer were examined. Significantly different from controls at * $p < 0.05$ and ** $p < 0.01$.

Heart In Vitro

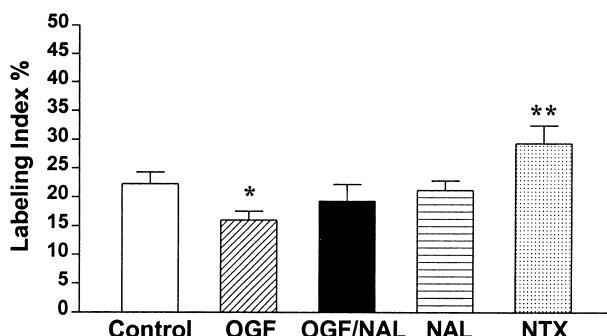


Fig. 2. The LI of myocardium from E20 rat hearts placed in a tissue culture media containing 10^{-6} M concentrations of OGF, OGF and naloxone (NAL), NAL alone, NTX, or 0.2 ml of sterile water (CO). Bars represent mean LI \pm S.E. Significantly different from controls at * p < 0.05 and ** p < 0.01.

2.6. Human tissues

A 31-week-old human fetus that had congenital hypoplasia of the lung was obtained at autopsy from the Department of Pathology, The Penn State Geisinger Health System. All procedures were approved by the Clinical Investigation Committee prior to obtaining several organs including skin from the forearm, muscle, and intestine.

Tissues were immediately frozen and processed for immunocytochemistry.

3. Results

3.1. DNA synthesis and NTX

To ascertain whether endogenous opioids function in cell generation during embryogenesis, the effects of interrupting opioid receptor interfacing by NTX on DNA synthesis in a variety of organs of ectodermal, mesodermal, and endodermal derivation were studied (Fig. 1). Using a paradigm of acute NTX administration that blocked receptors in the pregnant rat [28,29,71] and was known to cross the placental barrier [60], LIs were assessed in tissues 4 h after drug exposure. NTX elevated the LI of the cerebrum, skin, spinal cord, cerebellum, rib, adrenal cortex, heart, liver, tongue, and intestine by 16 to 63% above control values; LIs in the vertebra and lung of NTX-treated fetuses were normal.

3.2. DNA synthesis and OGF

In order to test the influence of OGF on embryonic development, pregnant rats were subjected to an acute administration of this peptide and the LIs of organs determined (Fig. 1). Transplacental exposure to OGF for 4 h

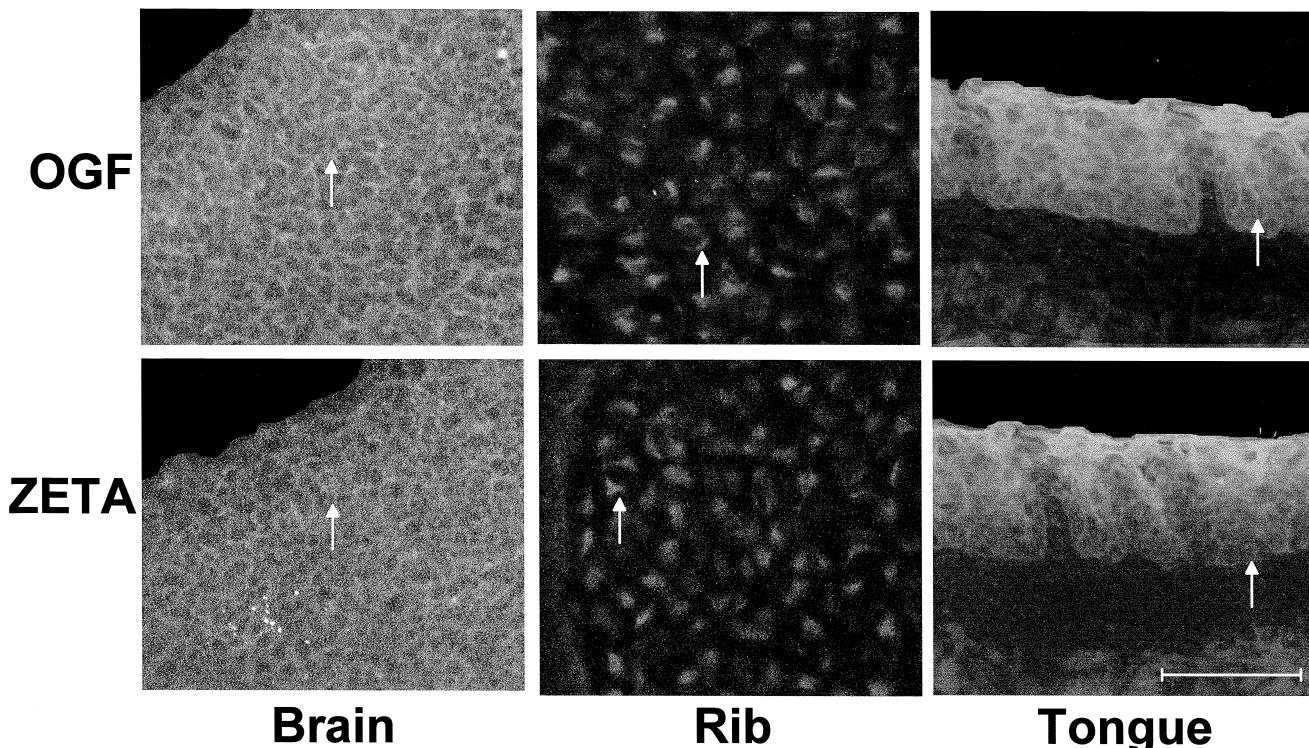


Fig. 3. Immunocytochemical preparations of E20 rat embryonic brain (cerebrum), rib, and tongue. Tissues representative of each dermal derivative were incubated with antibodies to OGF ([Met⁵]enkephalin) or to the ζ opioid receptor. Arrows indicate immunoreactivity. Sections stained with antibodies preabsorbed with their respective antigens did not exhibit immunofluorescence (data not shown). Scale bar = 40 μ m.

depressed DNA synthesis in the skin, spinal cord, cerebellum, vertebra, adrenal cortex, heart, and liver by 13% to 49%. The LIs were lower in the cerebrum, rib, lung, tongue, and intestine of OGF-exposed rats, but these results did not reach statistical significance.

3.3. Receptor mediation and direct influence of OGF

To address questions about receptor mediation and the direct actions of OGF, tissues from E20 rat embryos were

excised and placed in serum-free Dulbecco's media containing either OGF, OGF and NAL, NAL alone, NTX, or sterile water (control) along with radiolabeled thymidine ($2 \mu\text{Ci}/\text{ml}$) for 4 h. Representative organs from each dermal derivative were assessed. In the case of the heart (Fig. 2), OGF depressed DNA synthesis by 30% from control levels. This suppression of DNA synthesis by OGF was eliminated by concurrent incubation with the short-acting antagonist, NAL. Addition of NAL at this concentration did not alter cell replicative events. Continuous disruption

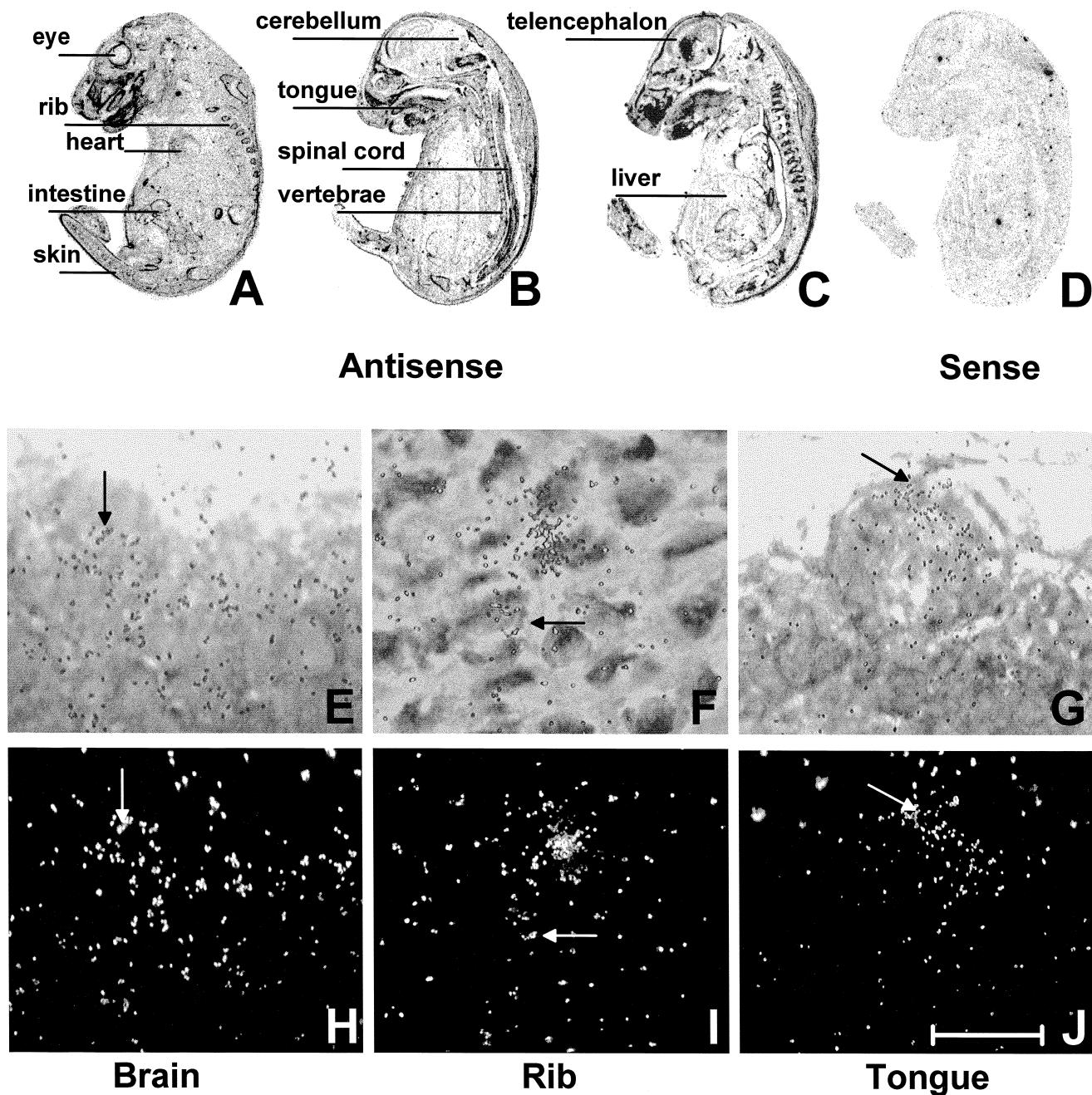


Fig. 4. Distribution of PPE mRNA in sagittal sections of E20 rat fetuses. Photomicrographs were taken from film autoradiographs of sections hybridized with [^{35}S]antisense PPE oligoprobe (A–C) or sense probe (D). Images of embryos are magnified 4 times. Paired phase contrast (E,F,G) and darkfield (H,I,J) photomicrographs of one organ representative of ectodermal (cerebrum), mesodermal (rib), and endodermal (tongue) derivatives are presented. Arrows indicate the pattern of silver grains associated with the same cells. Scale bar for E–J = 40 μm .

of endogenous opioids with opioid receptors by exposure to NTX significantly increased the LI in the heart by 42% from control levels.

3.4. Immunocytochemistry

The presence of OGF and ζ opioid receptor in a variety of rodent embryonic tissues/organs was determined by immunocytochemistry using polyclonal antibodies (Fig. 3). Cytoplasmic staining for both OGF and the ζ receptor was noted in cells associated with ectodermal (cerebrum, skin, spinal cord, cerebellum), mesodermal (vertebra, rib, adrenal cortex, heart), and endodermal (lung, liver, tongue, intestine) derived organs. Immunoreactivity was not detected in the nucleus. Tissue samples incubated with antibodies

preabsorbed with excess antigen to either OGF or ζ receptor peptides had no staining (data not shown).

3.5. Gene expression of preproenkephalin

To examine the source of OGF, *in situ* hybridization studies were performed to localize PPE mRNA in E20 rat embryos (Fig. 4). PPE mRNA was detected in film autoradiography of the entire animal, with notable activity found in many organ systems. Sections processed for emulsion autoradiography with the antisense probe revealed the presence of mRNA localized to proliferating and differentiating cell populations. Sections incubated with sense probe had no silver grains, indicating the specificity of the *in situ* hybridization preparations.

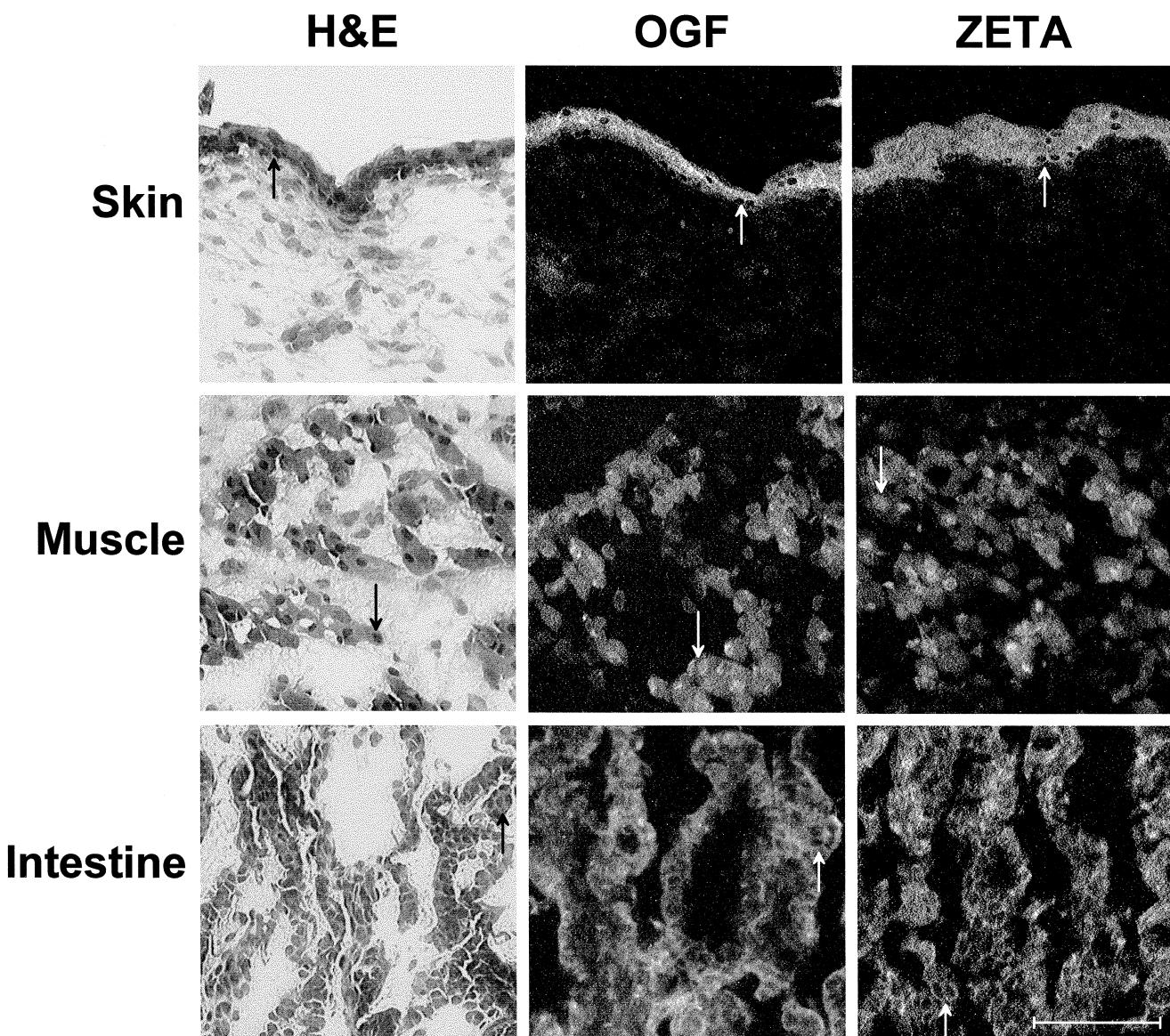


Fig. 5. Immunocytochemical preparations of specimens of human skin, muscle, and intestine from a 31-week-old fetus that had congenital hypoplasia of the lung. Tissues were stained with antibodies to either OGF or the ζ opioid receptor and rhodamine-conjugated goat anti-rabbit IgG. Sections stained with H&E are included for tissue identification. Arrows indicate cellular elements or immunoreactivity. Bar = 35 μ m.

3.6. OGF and the ζ receptor in the human fetus

In order to secure information as to whether OGF and the ζ receptor were present in the human fetus, a variety of human tissues were processed for immunocytochemistry (Fig. 5). Staining of OGF and the ζ receptor was observed in skin, muscle, and intestine, organs that are representative of ectodermal, mesodermal, and endodermal derivatives, respectively. Immunoreactivity was localized to the cytoplasm, with no staining found in the nucleus. Sections incubated with preabsorbed antibody had no immunoreactivity (data not shown).

4. Discussion

In this study, we provide evidence for the first time that native opioid peptides serve to regulate DNA synthesis in a wide variety of organ systems during prenatal life. The LIs of fetal organs representative of ectoderm, mesoderm, and endoderm were elevated in response to maternal exposure to an opioid receptor blockade. These results suggest that interference with opioid receptor interaction in the fetus has a profound impact on cell generative processes, and that endogenous opioids must be negative growth regulators. Because an opioid antagonist elicited this increase in DNA synthesis, one may conclude that these naturally occurring opioids restrain growth at the level of the opioid receptor. Moreover, these endogenous opioids are constantly available because interruption of peptide receptor interfacing had repercussions within 4 h of the initiation of blockade. It should be noted that of the 12 organs/tissues studied, vertebra and lung did not have an increase in the LI after exposure to NTX. These results may be interpreted as (a) the schedule of DNA synthesis in these organs and tissues [54] was not appropriate at 20 days of gestation to reveal function, or (b) vertebra and/or lung is(are) not subject to endogenous opioid stimulated transduction with regard to DNA synthesis. In both cases, however, OGF, the ζ receptor, and PPE mRNA were detected, indicating that the machinery for cell regulation by opioids was in place. One may reason that these data suggest that the timetable of development for vertebra and lung was not in line with the singular day of examination — gestation day 20. Further work is required to clarify this issue. In any event, these observations that opioids regulate DNA synthesis in the fetus extend earlier reports that endogenous opioids and opioid receptors, as well as gene expression for opioid peptides, can be observed in the prenatal period [9,11,12,14,17,22,26,27,30,36–39,42,46–48,51,55,56,61,76].

Given that the opioid antagonist, NTX, used in these experiments serves to block all types of opioid receptors from interacting with endogenous opioids [21], the question arises as to which opioid peptide(s) function(s) in DNA synthesis during prenatal life. We chose the opioid

peptide, [Met^5]enkephalin, known as OGF, because of its extensive characterization as a ubiquitous growth factor in neural and non-neural systems (see review in Ref. [66]). This endogenous opioid (and in some cases closely related peptides such as [Leu^5]enkephalin, octapeptide, and heptapeptide) has been found to be selective in influencing DNA synthesis and/or growth of a wide variety of cells and tissues in vitro and/or in vivo in a receptor-mediated fashion [5,9–11,18,20,23–26,33,34,47,50–52,65,66,70,73–75]. Although a strong case can be made that a principal endogenous opioid peptide modulating prenatal organ growth is the pentapeptide, [Met^5]enkephalin, other opioid compounds have been reported to alter cell proliferation [2–4,7,13,16,19,31,32,48,49,53], albeit most frequently of neural cells in a tissue culture environment and after chronic drug exposure. Thus, it may be that opioids in addition to OGF could participate in development and, taken one step further, that different organs and/or biological processes (e.g., proliferation, differentiation, migration) are targeted by different opioids. Nevertheless, this study presents evidence that at least one native opioid peptide, OGF, is present and plays a role in organogenesis.

Subsequent to knowing that OGF functions in prenatal life with respect to DNA synthesis, a series of experiments were employed to reveal more about the mechanism of this peptide. Using a tissue culture system which removed confounding effects from maternal and fetal circulations, we learned that OGF activity was direct and independent of peptide interactions from the mother. Secondly, OGF influenced DNA synthesis in a receptor-mediated fashion because concomitant exposure to NAL, an antagonist that is of considerable less potency than NTX, neutralized peptide activity at a concentration that had no effect alone. These findings are in concert with, and extend, those in earlier reports that have documented the direct and receptor-mediated modulation by OGF on DNA synthesis in homeostatic situations in the postnatal organism [10,26,56,65,66,73–75].

Estimating the total contribution of OGF on the growth of organs needs to take into account a number of factors. First, the activity of endogenous OGF can be roughly calculated (assuming that OGF is the only endogenous opioid accounting for alterations in DNA synthesis) for each organ by data on the effects of opioid receptor blockade using NTX. Thus, with the exception of vertebra and lung which showed no effect of NTX on LI, opioid antagonist exposure elicited changes ranging from 16% (heart) to 300% (cerebrum). Second, the capacity of exogenous OGF, presumably acting by saturating sites where endogenous peptide is absent, needs to be given attention. Therefore, with the exceptions of little alterations in LI of rib, lung, tongue and intestine, application of OGF decreased the LI in the range from 13% (adrenal cortex) to 49% (heart). Hence, summing the effects of endogenous and exogenous OGF activity, when possible, yielded cumulative effects on LI ranging from 31% (adrenal cortex)

to 338% (cerebrum). Furthermore, these changes were observed after a short exposure (4 h) of OGF or NTX, and only at one age (gestation day 20). More research as to the significance of opioid activity on each day of gestation and for each organ needs to be performed. In addition, although we know that NTX passes the placenta [60], and assume that exogenously administered OGF enters the placenta, we have little knowledge about the duration of NTX or OGF action in the fetus. Once again, dose-response experiments are needed to explore the full magnitude of opioid activity on DNA synthesis in the prenatal animal.

The interaction of the opioid receptor with OGF to transduce growth-related activities in the fetus requires clarification. OGF, [*Met*⁵]enkephalin, can interact with a variety of classical opioid receptors such as μ , δ , and κ [21], and these receptors have been identified in organs during prenatal life as well as in preimplantation embryos [12,22,76]. The binding characteristics of OGF to developing/neoplastic cells and tissues, however, indicate a binding site for which peptides selective for classical opioid receptors are not very competitive. Thus, concentrations of DPDPE, DAMGO, and EKC that are approximately 750, $> 10^{-3}$ M, and 160-fold, respectively, greater than OGF are needed to displace radioactive OGF [59]. Additionally, OGF is not very potent in displacing ligands selective for classical opioid receptors in binding studies with developing tissues [58]. Moreover, these ligands have no influence on DNA synthesis and/or growth [26,65]. Therefore, one could argue persuasively that OGF interacts with the ζ receptor to control organogenesis. This viewpoint is supported by immunocytochemical localization of the ζ receptor in cells of the organs examined in the present investigation.

The source of OGF with regard to regulating DNA synthesis in the fetus as demonstrated herein is unclear. In the present study, however, *in situ* hybridization techniques revealed gene expression for PPE mRNA in cells of organs representative of the endoderm, mesoderm, and ectoderm. These results imply that at least these cells in the fetus are capable of transcribing the message for enkephalin. Following translation and posttranslational modification, PPE mRNA would be expected to yield OGF, suggesting a self-regulating system. The autocrine nature of growth control in organogenesis observed in this investigation, supports and extends earlier observations suggesting a parallel situation in adult, healing, and neoplastic tissues [11,14,15,22,25,27,30,35,38,39,42,46,51,52, 56,61,68–70,73–75].

Based on evidence showing that OGF has a direct and tonic influence on embryogenesis, one would predict that both OGF and its receptor, ζ , should reside in developing cells during prenatal life. Indeed, peptide and receptor were found in the cytoplasm but not in the nucleoplasm of cells composing these prenatal organs; these results are consonant with previous reports on cellular distribution of

OGF and the ζ receptor in a wide variety of cells [9,10,26,56,66,68–70,73–75]. While at first glance these findings are at odds with those from binding reactions which indicate a nuclear association for the ζ receptor, the antibodies to OGF and the ζ receptor recognize sites of production in the cytoplasm as well as a putative location on the outer nuclear envelope.

Given the regulation of organogenesis by OGF as suggested by the present studies using acute paradigms, the consequences of continual perturbation during prenatal life on the disposition of organs, and the physical and behavioral abilities of the organism, are of considerable interest. The repercussions of persistent opioid receptor blockade during gestation on fetal, neonatal, and adult outcome [28,29,71] have shown that the body weights of neonatal pups delivered by mothers that were given NTX were significantly greater (8%) than control animals, and that the weights of the brain, heart, kidney, liver, and muscle for neonates exposed *in utero* to NTX were 14 to 65% greater than normal. Thus, alterations in body and organ weights after chronic exposure to NTX may be hypothesized to correlate with alterations in DNA synthesis. Finally, the imprint of alterations from the endogenous opioid system related to growth during gestation had considerable implications on behavioral responses, certain aspects of pain sensitivity, and the density of some opioid receptors in the postnatal period [28,71].

In order to begin translating the information about endogenous opioids and the regulation of rodent development to the human condition, the present study investigated whether OGF and/or ζ receptor were associated with cells in the human fetus. Immunocytochemical studies indicate that both OGF and the ζ receptor were observed in organs representative of ectodermal, mesodermal, and endodermal derivation. Thus, if the situation in humans parallels that in the rat, then OGF may function to tonically inhibit DNA synthesis in the human fetus. If this is the case, then one may consider how irregularities of OGF and/or the ζ receptor are determinants of birth defects. For example, overexpression of either peptide or receptor, either at the transcriptional or translational level, would have the effect of decreasing the number of cells undergoing DNA synthesis and diminish cellular content. Of course the repercussions of such an event would be reliant on the timing of the disturbance in relationship to the schedule of organogenesis, and dysfunction would presumably involve those organ systems positioned within the window of vulnerability. Thus, the hypothesis may be proposed that the etiology and pathogenesis of birth disorders could be associated with endogenous opioid regulation of cell generation.

Acknowledgements

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