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Research report

Increased expression of insulin-like growth factor-I (IGF-I) during embryonic development produces neocortical overgrowth with differentially greater effects on specific cytoarchitectonic areas and cortical layers

Rebecca D. Hodge[a], A. Joseph D'Ercole[b], John R. O'Kusky[a,*]

[a]*Department of Pathology and Laboratory Medicine, University of British Columbia, B.C. Research Institute for Children's and Women's Health, 950 West 28th Avenue, Vancouver, British Columbia, Canada, V5Z 4H4*

[b]*Department of Pediatrics, Division of Endocrinology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7220, USA*

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Abstract

The in vivo actions of insulin-like growth factor-I (IGF-I) on the growth and development of the cerebral cortex were investigated in transgenic (Tg) mice that overexpress IGF-I in the brain, beginning as early as embryonic day (E) 13. Compared to non-Tg littermate controls, Tg mice at postnatal day (P) 12 exhibited significant increases in total cortical volume (31%) and in total neuron number (27%). The numerical density of neurons did not differ significantly between Tg and control mice, except in layer I. Comparing cytoarchitectonic areas in Tg mice, significantly greater increases in cortical volume were found for the motor cortex (42%), compared to somatosensory cortex (35%). Similarly, greater increases in total neuron number were found for motor cortex (44%) compared to somatosensory cortex (28%). Comparing individual cortical layers in Tg mice, the greatest increase in neuron number was found in layer I for both motor (93%) and somatosensory (76%) regions, followed by layer V (36–53%) > II/III (26–47%) > VI (26–37%) > IV (22–34%). Our results demonstrate that increased expression of IGF-I in vivo during embryonic and early postnatal development produces substantial overgrowth of the neocortex. IGF-I-mediated growth and development exhibits differential effects in some cytoarchitectonic areas and in lamina-specific neuron populations, most notably the neurons of layer I.

Theme: Development and regeneration
Topic: Neurotrophic factors: biological effects

Keywords: Neurogenesis; Motor cortex; Somatosensory cortex; Cajal-Retzius cells; Stereology; Transgenic mice

1. Introduction

Insulin-like growth factor I (IGF-I), a 70-amino acid peptide, is a member of the insulin superfamily. IGF-I triggers its actions by binding to the type 1 IGF receptor (IGF1R), initiating a cascade of intracellular events ultimately leading to the transcription of genes whose products are responsible for regulating processes such as cell cycle progression and cellular differentiation (for reviews, see Refs. [26,36]). Evidence from recent in vitro studies indicates that IGF-I has a role in embryonic central nervous system (CNS) development. IGF-I augments neural stem cell proliferation [4,17,24] and promotes neuron survival [7,23,58,60]. IGF-I also influences oligodendrocyte development in vitro by promoting oligodendrocyte precursor proliferation and differentiation [35,42,47] and oligodendrocyte survival [8,62].

Mutant mouse models of IGF-I function strongly support a role for IGF-I in brain growth and development (for review, see Ref. [20]). The brain weights of mice carrying a

* Corresponding author. Fax: +1 604 875 3597.
E-mail address: jrokusky@interchange.ubc.ca (J.R. O'Kusky).

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null mutation of the IGF-I gene (IGF-I knockout mice) are significantly reduced, as are the volume and number of neurons in many brain regions [11,38]. Conversely, Tg mice that overexpress IGF-I in the brain exhibit increased brain weight and volume, as well as regional increases in neuron number [12,19,31,41,49,52,61]. These studies clearly indicate that IGF-I contributes to the regulation of brain development.

Relatively few studies, either in vitro or in vivo, have specifically addressed the effects of IGF-I on cerebral cortex development. Both IGF-I and IGF1R are expressed in the primitive cerebral cortex of rodents during embryonic development [6,15]. IGF1R is expressed by postmitotic neurons and neural stem cells in the ventricular zone of the developing cerebral cortex [15], while in situ hybridization studies indicate that IGF-I mRNA is expressed throughout the embryonic cortex [6]. High levels of IGF-I and IGF1R expression also are apparent in the rodent cerebral cortex during the first 3 weeks of postnatal life [9,14,15].

IGF-I has been shown to promote the survival of cultured cerebral cortical neurons [60]. In a line of Tg mice in which IGF-I was overexpressed postnatally in the brain, Gutierrez-Ospina and collaborators [31] documented increases in cortical surface area, as well as total barrel area and neuron number within individual barrels in the posterior medial barrel subfield of the somatosensory cortex. In IGF-I knockout mice, the thickness of the cerebral cortex is reduced and cell density is increased in all regions of the cortex compared to normal mice. The apparent number of projection neurons in layers III and V in the parietal and temporal regions of the cerebral cortex of IGF-I knockout mice, however, does not differ from normal mice [11]. These studies indicate that IGF-I influences the development of the cerebral cortex and suggest a differential effect of IGF-I on specific neuronal populations and/or functional areas of the cerebral cortex.

In the present study, the in vivo effects of IGF-I on the prenatal and early postnatal development of the cerebral cortex were investigated in a new line of Tg mice, termed nestin/IGF-I Tg mice, in which IGF-I is overexpressed in the brain during embryonic development. The transgene was composed of (1) regulatory elements that include the second intron of the human nestin gene and the minimal promoter of herpes simplex virus intermediate early gene ICP4, (2) the human IGF-IA cDNA fused to a signal sequence from rat somatostatin, and (3) a sequence containing polyadenylation signals and sites derived from the human growth hormone gene [52]. In nestin/IGF-I Tg mice, IGF-I overexpression begins as early as embryonic day (E) 13 and continues into postnatal life, with regional expression being the highest in the cerebral cortex [52]. Stereological analyses were conducted to determine the effect of IGF-I on the total volume and mean thickness of the neocortex, and on the numerical density and total number of neurons in the cortex. Separate analyses were conducted in individual cortical laminae for two different cytoarchitectonic regions, the primary motor and primary somatosensory cortices.

2. Materials and methods

2.1. Nestin/IGF-I transgenic mice

The details for the construction of the nestin/IGF-I transgene have been reported previously [52]. Heterozygous nestin/IGF-I Tg male mice were bred with normal C57BL/6 female mice (Charles River Laboratories, Wilmington, MA) to produce litters consisting of approximately half Tg mice and half normal non-Tg controls. Tg mice were routinely identified by polymerase chain reaction (PCR) of tail genomic DNA. Nestin/IGF-I Tg mice were generated at the mouse facility of the University of North Carolina at Chapel Hill. Mice were housed at 22°C with alternating 12-h light and dark cycles. All procedures were conducted in accordance with guidelines set out by institutional review committees of the University of North Carolina at Chapel Hill and the University of British Columbia. In brief summary, expression of the nestin/IGF-I transgene, as judged by in situ hybridization histochemistry, begins by at least E13, the earliest time point evaluated [52]. At this stage, transgene expression was highest in the neuroepithelium, ventricular zone, and cerebellar primordium. The presumptive hippocampus and developing cortical plate also showed increased IGF-I expression. By P0, the highest level of transgene IGF-I expression was seen in the cerebral cortex and hippocampus. At this time, IGF-I protein concentrations are increased by about 60% in the cerebral cortex. Northern blot analysis of whole brain demonstrated increasing transgene expression with a peak occurring at P5 and tapering off thereafter [52]. No changes were observed in the expression of nestin in the Tg mice. Specifically, we did not detect alterations in proportion of nestin-expressing cells or in nestin immunostaining patterns in these Tg mice [52].

2.2. Tissue collection and processing

On P12, six Tg and six control mice were deeply anaesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Individual mice were perfused through the left ventricle with a fixative solution containing 4% paraformaldehyde and 1% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for 45 min at a perfusion pressure of 90–100 mm Hg. Brains were removed, weighed and stored in additional fixative solution at 4°C for 48–72 h. Prior to sectioning, the tissue blocks were cryoprotected by immersion in a solution of 15% sucrose in 0.1M phosphate buffer at 4°C overnight. Serial frozen sections were cut at 30 μm in the coronal plane through the entire rostrocaudal extent of the