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Marked recovery of functional metabolic activity and laminar volumes in the rat hippocampus and dentate gyrus following postnatal hypothyroid growth retardation: A quantitative cytochrome oxidase study

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Abstract

Similar to cretinism in human children, absence or deficiency of thyroid hormones in rats and mice during early postnatal development results in marked retardation of brain development along with behavioral and cognitive deficits. Less is known about brain recovery from postnatal hypothyroidism. [Farahvar, A., Meisami, E., 2007. Novel two-dimensional morphometric maps and quantitative analysis reveal marked growth and structural recovery of the rat hippocampal regions from early hypothyroid retardation. *Experimental Neurology.*] found, by means of morphometric maps, that surface areas of hippocampal cortex and its CA1–CA4 regions which were significantly reduced in developing hypothyroid rats, show nearly complete growth recovery upon restoration of thyroid function. Here we explore the ability of hippocampal synapse-rich neuronal fiber layers to show recovery from early hypothyroid growth retardation. Rat pups were made hypothyroid from birth to day 25 (weaning) or up to young adulthood (day 90) by a treatment with the reversible goitrogen, PTU (*n*-propylthiouracil), in the drinking water. Recovery was induced by withdrawal of PTU at weaning and analysis of cytochrome oxidase (CytOx)-stained serial sections of the hippocampus and dentate gyrus at the ages of 25 and 90 days. CytOx stains the synapse-rich fiber layers of the hippocampal formation (HCF). Volumetric growth of molecular layer, stratum oriens and radiatum and dentate hilar region showed complete or nearly complete recovery from marked and significant growth retardation induced by early postnatal hypothyroidism. Also the reduced CytOx staining intensity in the hypothyroid rat HCF layers showed marked recovery following hormonal restoration. Results indicate remarkable growth plasticity of the HCF and ability of the synapse-rich fiber layers to show complete recovery of metabolic and functional neural activity from deleterious effects of early hypothyroidism. Mitochondrial CytOx is highly localized to the synapse-rich fiber layers of the HCF and its activity and histochemical staining intensity correlates positively with functional metabolic activity of neural tissue. Thus hippocampus and dentate gyrus neuronal fiber layers and their oxidative activity show remarkable ability to recover from the postnatal hypothyroid growth retardation. The results indicate that some brain regions are less vulnerable to early developmental insults and can recover.

Keywords: Hippocampus; Dentate gyrus; Cytochrome oxidase; Neural activity; Hippocampal growth and development; Laminar volumetric analysis; Postnatal hypothyroidism; Neurologic recovery; Oxidative recovery; Brain retardation; Brain rehabilitation; Brain plasticity; Hippocampal plasticity; Propylthiouracil (PTU); Experimental cretinism

Introduction

Thyroid hormones (THs) are critically needed for normal growth and maturation of the developing central nervous system as evident from the massive neurological, behavioral and cognitive deficiencies experienced by human cretin children, born either with congenital athyroidism or as a result of dietary iodine deficiency (Delange, 1994; Smith et al., 2002). In experimental animals as well, such as rats and mice, a deficiency of THs during the early neonatal period (first 3 weeks after birth) results in profound neurological sequelae, including retarded or abnormal neuronal fiber growth, synaptogenesis and energy metabolism (Atterwill et al., 1985; Bernal, 2002; Dembri et al., 1984; Koibuchi et al., 1996; Satav and

Abbreviations: CytOx, cytochrome oxidase; HCF, hippocampal formation; HC, hippocampus; DG, dentate gyrus; P, postnatal; PTU, *n*-propylthiouracil; THs, thyroid hormones.

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Katyare, 1982; Schwartz and Oppenheimer, 1978; Verity et al., 1976); these neuronal deficiencies are manifested in abnormal sensory and motor development, as well as cognitive and behavioral deficits (Delange, 1994; Dussault and Ruel, 1987; Sokoloff, 1967; Timiras, 1988).

In terms of regional brain development, cerebellum is possibly the most intensively studied model brain structure for showing neurobiological state of developing hypothyroid brain, followed by cerebral cortex. Fewer studies have also investigated the deleterious effects of THs deficiency on the developing hippocampus (HC) and dentate gyrus (DG) (Gould et al., 1991; Lauder and Mugnaini, 1977, 1980; Rabie et al., 1980; Rami and Rabie, 1988; Rami et al., 1987), although more recently several molecular and functional studies have also appeared (Alvarez-Dolado et al., 2001; Gerges and Alkadhi, 2004, Gilbert, 2004; Gilbert and Paczkowski, 2003; Martinez et al., 2001; Matos et al., 2002; Meaney et al., 2000; Roskoden et al., 1999; Sui and Gilbert, 2003; Vaidya et al., 2001, Vara et al., 2002).

Very few studies have focused on the possibility of recovery of the hippocampal formation (HCF), i.e., HC proper and DG, from early postnatal (P) hypothyroid-induced deficiencies. This is surprising in light of the prominent role that HCF plays in normal cognitive functions (Cohen and Eichenbaum, 1993; Olton et al., 1979; Zola-Morgan et al., 1986) and the profound mental retardation associated with severe cases of neurological cretinism (Delange, 1994; Smith et al., 2002). There is therefore a need to know the possibility and extent of recovery from such deficiencies.

In a recent study, we have presented evidence for essentially complete recovery of the surface area of the rat HC cortical sheet from the marked and significant retarding effects of THs deficiency during the early postnatal period (Farahvar and Meisami, 2007). However, the details of laminar growth of HCF fiber- and synapse-rich layers as well as the possibility of metabolic and oxidative recovery of the rat HCF layers from this form of severe developmental brain retardation have not been adequately examined.

In the present study, densitometric quantification of the various CytOx-stained HCF layers allowed for an assessment of the relative changes in functional metabolic activity of this brain region as a result of short-term and long-term developmental hypothyroidism. In addition, we attempted to determine the extent of functional metabolic recovery that accompanies the accelerated structural growth process that has been observed in our previous study (Farahvar and Meisami, 2007).

The specific goals of the present study were therefore the following. First we examined the growth of the neuronal fiber- and synapse-rich laminae of the HCF by measuring the changes in the volumes of these layers in control and hypothyroid animals at postnatal (P) days P25 and P90, to assess the effects of short-term (days P1–P25) and long-term (days P1–P90) hypothyroidism. We also examined the same parameters in animals kept hypothyroid from birth to weaning and then allowed to rehabilitate and recover from this condition starting at day P25 (weaning) and examined at day P90 (young adulthood) for the degree of recovery of HCF laminar growth. These studies were carried out using serial sections of the rat HCF, stained histochemically for CytOx. This method delineates the various synapse-rich and neuronal fiber layers of the hippocampal formation and allows for careful volumetric analysis of these specific layers.

Our second goal was to assess the state of functional metabolic activity of the HCF in the hypothyroid and recovery rat groups. This was done by means of quantitative densitometric determination of CytOx-stained serial sections. Histochemical staining for CytOx has been utilized in neurobiological and neurological studies, as an endogenous metabolic marker to indicate relative levels of neuronal activity in brain tissue regions and layers. The mature brain depends almost exclusively on oxidative metabolism of glucose for energy. Numerous studies have shown a strong correlation between the level of functional activity in the brain and local energy metabolism, as indicated by relative levels of histochemical and cytochemical staining intensity for CytOx (Di Rocco et al., 1989; Hevner et al., 1995; Kageyama and Wong-Riley, 1982; Wong-Riley, 1989).

We hope that these studies will not only expand and confirm our mapping and morphometric studies of the HC cortical regions (CA1–CA4) in the hypothyroid and rehabilitated rats (Farahvar and Meisami, 2007), but also offer insight into the effects of THs depletion and recovery from this condition on the state of oxidative metabolism in the HCF, including HC proper and dentate gyrus (DG), in particular on the neuronal fiber layers containing axons, dendrites and synapses.

Materials and methods

Animals

Pregnant albino rats of the Sprague-Dawley strain (Holtzman, Wisconsin) were kept in separate plastic cages until delivery. At birth, the litter size was reduced to eight pups per mother per cage. The conditions of the colony were kept constant (25 °C, 12-h light–dark cycle). Food (Purina rat chow) and water were provided *ad libitum*. The litters were divided into two groups, one control and one experimental hypothyroid (see below). Pups of both groups were weighed regularly 2–3 times per week and allowed to suckle until day P25 (weaning). After weaning, the animals were separated by sex and the males were kept 2 per cage and weighed twice weekly until P90 (young adulthood). Only male rats were utilized in these studies. Animal care and treatments were according to the approved protocols of the University of Illinois, Urbana-Champaign and standard NIH (National Institutes of Health) guidelines.

Induction of hypothyroidism and recovery

Experimental hypothyroidism was induced in the growing pups by administration of the reversible goitrogen PTU, 6-*n*-propyl-2-thiouracil (Sigma, St. Louis) at 0.1% w/vol