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Research Report

Inherited tertiary hypothyroidism in Sprague–Dawley rats

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ABSTRACT

Thyroid hormones (THs) are important in the development and maturation of the central nervous system (CNS). The significant actions of THs during CNS development occur at the time when TH levels are lower than those in the mother and the hypothalamic-thyroid (HPT) axis is not fully functional. In the developing rat nervous system, primarily the cerebellum, the first three postnatal weeks represent a period of significant sensitivity to thyroid hormones. This study presents a spontaneous, inherited recessive hypothyroidism in Sprague–Dawley rats with devastating functional consequences to the development of the CNS. The clinical signs develop around 14 day's postnatal (dpn) and are characterized by ataxia, spasticity, weight loss and hypercholesterolemia. The afflicted rats died at 30 days due to severe neurological deficits. The deterioration affects the entire CNS and is characterized by progressive neuronal morphological and biochemical changes, demyelination and astrogliosis. The cerebellum, brain stem, neocortex, hippocampus and adrenal gland medulla appear to be most affected. Thyroid Stimulating Hormone (TSH), T3 and T4 levels were significantly lower in hypothyroid rats than control. Immunohistochemistry and RT-PCR demonstrated a reduction of Thyrotropin Releasing Hormone (TRH) in the hypothalamus of hypothyroid rats. The weight of both thyroid and pituitary glands were significantly less in hypothyroid rats than the corresponding normal littermate controls. Transmission electron microscopy demonstrates consistent postsynaptic dendritic, synaptic and spine alterative changes in the brain of hypothyroid rats. These data suggest that we discovered a tertiary form of inherited hypothyroidism involving the hypothalamus.

1. Introduction

The two principle thyroid hormones are T3 or L-3,5,3′-triiodothyronine and T4 or L -3,5,3′,5′-tetraiodothyronine. T3 is the biologically active hormone and T4, the major thyroid hormone that is secreted from the thyroid gland, is considered a precursor or prohormone (Choksi et al., 2003).The thyroid gland and thyroid hormones are central to human and animal development. The essential function of T3 is to regulate carbohydrate and protein metabolism in all cells. Changes in T3 can affect all organ systems of the body with profound effects on the cardiovascular, nervous, immune and reproductive systems. In developing mammals, the thyroid regulates growth and metabolism and plays a critical role in

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tissue development and differentiation (Choksi et al., 2003; Harvey and Williams, 2002).

Development of the central nervous system (CNS) in mammals proceeds through precisely determined, well-defined events in time and location. Most of these events are determined by genetic factors. Nevertheless, epigenetic factors, such as hormones and growth factors, are also important because they act to control the timing and coordination of mechanistically unrelated processes (Bernal et al., 2003). Deficiency of thyroid hormones during a critical period of development of the CNS in mammals leads to profound and potentially irreversible defects in brain maturation, and clinical syndromes arising from thyroid hormone deficiency during fetal and postnatal periods are well recognized in humans and animals (Porterfield and Hendrich, 1993; Delange, 1997).

In the rat, thyroid hormone deficiency causes a series of abnormalities in the CNS, where alteration of cell migration, neuronal differentiation and demyelination are principal findings (Bernal et al., 2003; Porterfield and Hendrich, 1993; Delange, 1997; Lucio et al., 1997; Oppenheimer and Schwartz, 1997). Thyroid hormones appear to regulate those processes associated with terminal brain differentiation such as neuronal migration, dendritic and axonal growth, synaptogenesis and myelination (Bernal, 2002). The distinguishing feature of the cerebral cortex and hippocampus in hypothyroid rats is the retarded development of the neuropil, which is characterized by smaller and more tightly packed peripheral and central neuronal cell bodies. In addition, there is a reduced synaptogenesis manifested by diminished axonal growth and dendritic outgrowth, elongation and branching, and distribution of dendritic spines. In the cerebellum, thyroid hormone deficiency results in delayed proliferation and migration of granule cells from the external germinal layer, stunted dendritic arborization and ectopic localization of the Purkinje cells, and diminished axonal myelination. Faivre et al. attributed the cause of delayed cellular migration and neuronal differentiation to a decrease in the microtubule number in Purkinje cells (Faivre et al., 1984). Developmental retardation of the rat brain can be prevented if administration of thyroid hormone is started before the end of the second week after birth (Legrand, 1986; Eayrs, 1971).

Temporal patterns of thyroid hormone-dependent gene expression in the brain suggest that the critical period of thyroid hormone sensitivity for cerebellum development is limited to the first 2–3 postnatal weeks in the rat (Bernal et al., 2003). Studies of thyroid hormone's effects on brain development have employed several mammalian species, and the most extensive and detailed investigations have focused on the neonatal rat. In general, hypothyroidism in rats was induced in pregnant mothers or neonatal rats by administering various chemicals such as propylthiouracil and methimazole in drinking water, food or through intraperitoneal (IP) injection, which will block the oxidation of iodide to iodine and consequently block the formation of T3 and T4 (Bernal et al., 2003; Porterfield and Hendrich, 1993; Delange, 1997; Lucio et al., 1997).

Our work presents an inherited autosomal recessive thyroid-deficient neurological disorder affecting a colony of Sprague–Dawley rats (Harlan Inc. IN) maintained at Texas A&M University, Lab Animal Facility. In this rat model, the thyroid hormonal (TH) assay consistently showed a significant decrease in thyrotropin releasing hormone (TRH) mRNA and protein, thyroid stimulating hormone (TSH), T3 and T4 levels in the affected rats when compared to their control littermates. These TH findings suggest a tertiary type of hypothyroidism in this animal model. On histopathological examination the CNS demonstrated progressive widespread neuronal changes, demyelinization and astrogliosis. Our present investigation of this inherited hypothyroid condition in the Sprague–Dawley (SD) rat confirmed previously reported findings of chemically-induced hypothyroidism. In addition, our study reveals new morphological and biochemical findings not previously reported, such as severe multisystem neuronal post-synaptic changes and abnormal differentiation in the adrenal medulla. To our best knowledge, there is no previous report of spontaneously inherited tertiary hypothyroidism in the rat.

2. Results

2.1. *CNS pathology in thyroid hormone deficient rats*

This neurological disorder caused by hypothyroidism, affects young rats during the first month of life. The clinical manifestation was characterized by progressive development of ataxia, spasticity, weight loss and hypercholesterolemia which are noticed at 14 day's postnatal (dpn). The severity of the disease increased gradually and rats died at 30 dpn due to severe neurological deficits. On necropsy examination, there is no visible gross morphological alteration of the central nervous system (CNS). The body weight at 30 dpn was 58% less than normal littermates (control 77.8±7.2 g, hypothyroid 32.3±2.6 g). There was a significant decrease of pituitary and thyroid weight in hypothyroid, ataxic rats when compared with normal littermates (thyroid gland weight: control 16.0±2.7 mg, hypothyroid 7.3±2.0 mg; pituitary gland weight: control 7.1±0.8 mg, hypothyroid 3.6±1.2 mg). The *P* value for both thyroid gland weight and pituitary gland weight was <0.01. Histopathology was performed at 14, 25 and 30 dpn on CNS tissues that were perfused *in vivo* with 4% paraformaldehyde or 10% buffered formaldehyde. Microscopic examination demonstrated progressive and widespread neuronal/glial changes in the CNS of ataxic rats. The most striking morphopathological finding on hematoxylin and eosin (H&E) stained slides was in the cerebellum and was characterized as a hypoplastic appearance with increased cytoplasmic eosinophilia of cerebellar Purkinje cells, which increase in severity from 14 to 30 dpn. In addition, ectopic localization of Purkinje cells in the cerebellum of hypothyroid rats was consistently observed. There was also a persistent external germinal cell layer in the cerebellum (Fig. 1B). There was a consistent ischemic appearance of neurons primarily in the cortical pyramidal cell layer, brain stem, hippocampus, and olfactory bulb. An example of neuronal ischemic change from the cortex of ataxic rats sacrificed at 30 dpn is illustrated (Figs. 1C and E). A distorted development of the hippocampus dentate gyrus, marked ischemic appearance of the pyramidal neurons, and hypercellularity was also consistently observed in hypothyroid rats (Figs. 2B and D). There was an individual variation in the degree of hippocampus alterative changes in the ataxic rats on histological examination. The altered neuronal