Available online at www.sciencedirect.com

SCIENCE DIRECT

Developmental Brain Research 154 (2005) 121–135

DEVELOPMENTAL BRAIN RESEARCH

www.elsevier.com/locate/devbrainres

Research report

Regulation of cerebellar neuronal migration and neurite outgrowth by thyroxine and 3,3′,5′-triiodothyronine

Alan P. Farwell[a,*], Susan A. Dubord-Tomasetti[a], Andrzej Z. Pietrzykowski[a], Stanley J. Stachelek[b], Jack L. Leonard[a]

[a]*University of Massachusetts Medical School, Worcester, MA, United States*
[b]*Children's Hospital of Philadelphia, Philadelphia, PA, United States*

Accepted 16 July 2004
Available online 11 November 2004

Abstract

The timing of granule cell migration in the developing cerebellum is regulated by thyroid hormone. Granule cell migration depends on the recognition of extracellular neuronal guidance molecule(s), such as laminin, and this, in turn, requires cell surface adhesion molecules (integrins) that are anchored on the cell membrane by the actin cytoskeleton. While many of the actions of thyroid hormone, specifically 3,5,3′-triiodothyronine (T_3), are mediated by regulated gene expression, both thyroxine (T_4) and 3,3′,5′-triiodothyronine (rT_3) also exert direct, positive control of the quantity of polymerized actin in cultured astrocytes without affecting gene expression. T_4-dependent actin polymerization has been shown to (i) participate in the immobilization of laminin to the cell surface, (ii) help deposit laminin in the molecular layer of the developing cerebellum, and (iii) anchor integrin(s) that recognize laminin present in the extracellular matrix. In this study, we show that both T_4 and rT_3, but not T_3, directly regulate the F-actin content of elongating neurites of cerebellar neurons. T_4 and rT_3 also promoted extensive granule cell migration from cerebellar explants, as well as, dense cell clustering and extensive neuronal process formation when granule cells were grown on a laminin-coated surface. Both granule cell migration and neuronal process outgrowth were markedly attenuated by the addition of integrin-blocking antibodies or binding peptides, by the absence of thyroid hormone or the presence of T_3. These data suggest that the T_4-dependent actin polymerization in developing neurons is necessary for these migrating cells to recognize the laminin guidance molecule, thereby providing a novel molecular mechanism for the profound influence of thyroid hormone on brain development that is independent of regulated gene expression.

Theme: Development and regeneration
Topic: Cell differentiation and migration

Keywords: Thyroid hormone; Brain development; Neuronal migration; Actin polymerization; Microfilament; Cerebellar development; Laminin; Integrin

1. Introduction

Thyroid hormone is a key regulatory factor of the brain developmental program. The lack of thyroid hormone during the critical period of neuronal migration leads to a multitude of irreversible morphological abnormalities [7,17,41,53,64], including: (i) defects in granule cell migration, (ii) increased granule cell death [16,46,65,66], and (iii) blunted dendritic arborization of Purkinje cell [41,71,86]. Importantly, thyroid hormone replacement during the first 2 weeks of life allows the cerebellum to mature according to the euthyroid developmental program by restoring granule cell migration and Purkinje cell arborization. While the consequences of hypothyroidism on cerebellar development are well recognized, the molecular events responsible for the morphogenic actions of this hormone remain elusive.

* Corresponding author. Division of Endocrinology and Metabolism, Department of Medicine, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, Massachusetts 01655, United States. Tel.: +1 508 856 5258; fax: +1 508 856 6950.
E-mail address: alan.farwell@umassmed.edu (A.P. Farwell).

0165-3806/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.devbrainres.2004.07.016

Thyroxine (T_4) is the major secretory product of the thyroid gland and the hormone utilized to replace a failed or absent thyroid gland. T_4 is deiodinated in peripheral tissues to either 3,5,3′-triiodothyronine (T_3) [44], which is the transcriptionally active hormone, or to the transcriptionally inert 3,3′,5′-triiodothryonine (reverse T_3, rT_3), which is the most abundant iodothyronine produced in during fetal life [10,13,69,70]. Since many of the actions of thyroid hormone are mediated by T_3 binding to specific chromatin-bound receptors [67,74,88], the search for T_3-regulated candidate cerebellar gene products has been intense. Unexpectedly, few genes appear to be directly regulated by T_3 (i.e., contain an identified thyroid hormone-response element; for review, see Refs. [6,57]), raising the possibility that thyroid hormone may influence brain development by a mechanism(s) other than regulated gene expression.

The programmed migration of granule cells from the external granular layer to the inner granular layer is one of the key thyroid hormone-regulated events in cerebellar development [15,34,49,68,78,82]. Both neuron pathfinding and guided neurite elongation rely on adhesion molecules, such as integrins, that project from the leading edge of the neuronal growth cone and bind to guidance cues in the extracellular matrix (ECM). These adhesion molecules are anchored in place by interactions between their cytoplasmic tail and filamentous actin cytoskeleton [11,22,47,85]. For example, chemical disruption of the actin cytoskeleton in cultured neurons markedly impairs neuronal growth cone motility and pathfinding ability [5,28,52,68]. Similarly, blocking antibodies raised against the β1 integrin subunit disrupt neural crest migration [9] as does disruption by targeted mutation of the α3β1 integrin, a receptor for the ECM neuronal guidance molecule laminin, which causes abnormal layering in the cerebral cortex [4]. These findings suggest that cell migration depends, in part, on interactions between the ECM and the extracellular domain(s) of integrins that are stabilized by anchoring to the intracellular filamentous actin cytoskeleton.

We have previously shown that the organization of the filamentous actin cytoskeleton in cultured astrocytes is regulated by thyroid hormone without affecting either transcription or translation [22,23,43,77]. Two transcriptionally inactive hormones, T_4 and rT_3, initiate this novel nongenomic action; T_3 is at least 100-fold less potent than either effector hormone. Astrocytes grown for only 24 h in the absence of thyroid hormone show a disorganized actin cytoskeleton and their F-actin content is 40–60% lower than cells grown in T_4 containing medium. Both the normal organization of the actin cytoskeleton and the cell F-actin content are restored rapidly (10–20 min) by treatment with physiological levels of either T_4 or rT_3. The effect(s) of T_4 and rT_3 on actin polymerization in astrocytes is both dose dependent and saturable, with a ED_{50} of ~0.4 nM for both hormones while the transcriptionally active T_3 fails to alter cytoskeletal organization [21–24,43,77]. One consequence of the thyroid-hormone-dependent organization of the actin cytoskeleton that plays a key role in cerebellar development is the secretion, deposition, and patterning of laminin on the astrocyte surface. In particular, when astrocytes are grown in the presence of T_4 or rT_3 and have intact microfilaments, secreted laminin is anchored to the cell surface in specific arrays. In contrast, the disassembled microfilaments present in the hormone-deficient or in T_3-treated astrocytes result in the complete loss of newly secreted laminin from the astrocyte surface [21,24]. These findings in cultured astrocytes are also observed in the developing cerebellum in vivo where the timing of appearance and quantity of laminin found in the molecular layer of the developing rat cerebellum is both delayed and diminished as compared to that in the euthyroid cerebellum [20].

In this study, we show that the F-actin content and the organization of the actin cytoskeleton in cultured neurons are thyroid hormone dependent. As expected, thyroid-hormone-dependent modulation of the granule cell actin cytoskeleton, in turn, promotes granule cell migration from explants onto a laminin substrate and extensive neurite outgrowth from neuronal cultures. These thyroid-hormone-dependent developmental events are blocked by synthetic peptides that compete for the integrin recognition sequence (RGD, arginine–glycine–aspartic acid) and by β1 integrin-blocking antibodies. These data suggest that the thyroid-hormone-dependent regulation of interactions between the actin cytoskeleton, integrins, and laminin exerts a profound influence on neuronal migration during brain development.

2. Materials and methods

2.1. Animals and reagents

Pregnant (16–17 days gestation) rats were obtained from Charles-River Labs (Kingston, NY). Care of animals conformed to the guidelines of the University of Massachusetts Animal Care and Use Committee and appropriate measures were taken to minimize pain and discomfort. T_4, laminin (from Engelbreth–Holm–Swarm cells), anti-200 kDa neurofilament IgG, antiactin IgG, anti-β1 integrin IgG, and BSA were purchased from Sigma (St. Louis, MO). T_3 and rT_3 (>99% pure) were obtained from Henning (Berlin, Germany). Anti-GFAP IgG was obtained from BTI (Stoughton, MA), antirabbit IgG–Texas Red conjugate was obtained from Amersham (Arlington Heights, IL) and Alexa Fluor® 488 phalloidin was obtained from Molecular Probes (Eugene, OR). Basal Media Eagle (BME) with Earle's salts media, antibiotics, Hank's solution, synthetic peptides -GRGDSP- and -GRGESP- and 0.25% (w/v) trypsin were obtained from Life Technologies (Gaithersburg, MD) and defined bovine calf serum (heat inactivated) from HyClone, (Logan UT). [^{3}H]-methyl-thymidine was obtained from NEN Lifesciences (Boston, MA). Culture flasks were obtained from Nunc (Copenhagen, Denmark) and 6-well tissue culture plates were obtained from Falcon