Deiodinases: the balance of thyroid hormone

Local control of thyroid hormone action: role of type 2 deiodinase

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Abstract

The thyroid gland predominantly secretes the pro-hormone thyroxine (T<sub>4</sub>) that is converted to the active hormone 3,5,3'(t)-triiodothyronine (T<sub>3</sub>) in target cells. Conversion of T<sub>4</sub> to T<sub>3</sub> is catalyzed by the type 2 iodothyronine deiodinase enzyme (DIO2), and T<sub>3</sub> action in target tissues is determined by DIO2-regulated local availability of T<sub>3</sub> to its nuclear receptors, TR<sub>a</sub> and TR<sub>b</sub>. Studies of Dio2 knockout mice have revealed new and important roles for the enzyme during development and in adulthood in diverse tissues including the cochlea, skeleton, brown fat, pituitary, and hypothalamus. In this review, we discuss the molecular mechanisms by which DIO2 controls intracellular T<sub>3</sub> availability and action.

Journal of Endocrinology (2011) 209, 261–272

Introduction

Thyroid hormones are important homeostatic regulators that act via nuclear thyroid hormone receptors (TRs) in virtually all tissues during development and throughout postnatal life. 3,5,3'(t)-triiodothyronine (thyroxine, T<sub>4</sub>) is a pro-hormone that circulates at a high concentration in peripheral blood relative to the active hormone 3,5,3'(t)-triiodothyronine (T<sub>3</sub>). Concentrations of T<sub>4</sub> and T<sub>3</sub> in target tissues are controlled by metabolism; local conversion of T<sub>4</sub> to T<sub>3</sub> is catalyzed by the type 2 iodothyronine deiodinase enzyme (DIO2), while the type 3 enzyme (DIO3) prevents activation of T<sub>4</sub> and inactivates T<sub>3</sub>. This pre-receptor control of ligand availability to TRs in target cells is a crucial mechanism that regulates the timing of cellular responses to thyroid hormones in a tissue-specific manner. The physiological importance of this coordinated process has been demonstrated in several organ systems by a series of elegant in vivo studies, and in this study, we review recent developments with particular emphasis on the importance of hormone activation mediated by DIO2.

Thyroid physiology

The hypothalamic–pituitary–thyroid axis

Circulating thyroid hormone concentrations are maintained in the euthyroid range by a classical negative feedback loop (Fig. 1). Thyrotropin-releasing hormone (TRH) is synthesized in the hypothalamic para-ventricular nucleus (PVN) and stimulates synthesis and release of TSH from thyrotroph cells in the anterior pituitary gland. TSH in turn acts via the TSH receptor (TSHR) in thyroid follicular cells to stimulate cellular growth and the synthesis and release of T<sub>4</sub> and T<sub>3</sub> into the circulation (Kopp 2001). Thyroid hormone action is mediated in target tissues by TRs, but thyroid hormones also inhibit TRH and TSH synthesis and secretion in the hypothalamus and pituitary to complete a negative feedback loop (Forrest et al. 1996b, Nikrodhanond et al. 2006). This negative feedback loop maintains circulating thyroid hormones and TSH in a physiological inverse relationship that defines the hypothalamic–pituitary–thyroid (HPT) axis set-point (Bassett & Williams 2008).

Circulating thyroid hormones and uptake into target cells

The thyroid gland predominantly secretes the inactive pro-hormone T<sub>4</sub>, as well as small amounts of the physiologically...
active thyroid hormone T₃. Both T₄ and T₃ are lipophilic and poorly soluble in water, and over 95% of thyroid hormones are protein bound in the circulation. Thyroxine-binding globulin (TBG), transthyretin, albumin, and several lipoproteins function as the transport proteins for T₄ and T₃ in plasma. Free thyroid hormone levels are thus dependent upon the concentrations and saturations of these proteins. The unbound free T₃ fraction represents 0.3% of the total T₃ concentration in plasma, but because of its higher binding affinity for TBG, the free T₄ fraction represents just 0.02% of the total plasma T₄ concentration. Thus, despite the total T₄ concentration being 50-fold greater than total T₃, the circulating free T₄ concentration is only fourfold higher. T₄ is exclusively synthesized and secreted by thyroid follicular cells, whereas the majority of circulating T₃ is generated in peripheral tissues by enzymatic removal of a 5′-iodine from T₄. Due to their lipophilic nature, thyroid hormones had been likely to enter target cells by a passive process of diffusion (Friesema et al. 2005). In fact, the free hormones enter target cells via an energy-dependent, ATP-requiring, stereospecific, and saturable transport mechanism that is mediated by the monocarboxylate transporter 8 (MCT8; Friesema et al. 2003, 2006, Dumitrescu et al. 2006), MCT10, and other transporter proteins including OATP1c1, a member of the Na⁺-independent organic anion transporter protein (OATP) family (Jansen et al. 2005, Heuer 2007, van der Deure et al. 2010; Fig. 2). Transport via MCT8, for example, increases uptake of T₄ and T₃ by tenfold (Friesema et al. 2003).

### Thyroid hormone metabolism

#### Deiodinase enzymes

The iodothyronine deiodinases are selenocysteine-containing enzymes that metabolize thyroid hormones to active or inactive products (Bianco et al. 2002). The type 1 deiodinase enzyme (DIO1) is rather inefficient with an apparent Michaelis constant (Kₘ) of 10⁻⁶–10⁻⁷ M and catalyzes removal of inner or outer ring iodine atoms in equimolar proportions to generate T₃, reverse T₃ (rT₃), or 3,3′-diodothyronine (T₂) depending on the substrate. Most of the circulating T₃ is derived from conversion of T₄ to T₃ by the actions of DIO1, which is localized to the plasma membrane and expressed in liver and kidney. Nevertheless, activity of the DIO2 enzyme in skeletal muscle may also contribute to circulating levels of T₃, although its role is controversial and may differ between species (Bianco et al. 2002, Maia et al. 2005, Bianco & Kim 2006, Heemstra et al. 2009b, Larsen 2009). The DIO2 enzyme is considerably more efficient than DIO1, catalyzing only the removal of an outer ring iodine atom from the pro-hormone T₄ with a Kₘ of 10⁻⁹ M to generate the physiologically active product T₃. The major role of DIO2 is to control the intracellular T₃ concentration, its availability to the nucleus, and the saturation of the nuclear T₃ receptor in target tissues. Moreover, DIO2 is likely to protect tissues from the detrimental effects of hypothyroidism because its low Kₘ continues to permit the efficient local conversion of T₄ to T₃. T₄ treatment of cells, in which MCT8 and DIO2 are co-expressed, results in increased T₃ target gene expression (Friesema et al. 2006), indicating that thyroid hormone uptake and metabolism coordinate regulation of T₃ action. By contrast, the DIO3 enzyme irreversibly inactivates T₃, or prevents T₄ being activated, by catalyzing removal of an inner ring iodine atom with a Kₘ of 10⁻⁹ M to generate T₂ or rT₃ respectively. Thus, inactivating DIO3 prevents thyroid hormone access to specific tissues at critical times and reduces TR saturation (Bianco et al. 2002, Bianco & Kim 2006).

#### Control of intracellular T₃ availability

The relative activities of DIO2 and DIO3, which have the same Kₘ values for substrate, consequently regulate intracellular concentrations of T₃ and its availability to the nuclear TR (Bianco et al. 2002, Bianco & Kim 2006, St Germain et al. 2009). In conjunction with serum-derived T₃, DIO2 and DIO3 are important local modulators of thyroid hormone responsiveness in vivo. Expression of both enzymes is regulated in a tempo-roporal and tissue-specific manner, resulting in varying levels of T₃ action in individual tissues at distinct times during development (Bates et al. 1999, St Germain et al. 2009). Acting together, DIO2 and DIO3 thus control cellular T₃ availability by a mechanism that is largely independent of serum thyroid hormone concentrations (Bianco & Kim 2006).
Thyroid hormone action

Thyroid hormone receptors

Thyroid hormone actions in target cells are ultimately determined by the availability of T₃ to its nuclear receptor (St Germain et al., 2009; Fig. 2). TRα and TRβ are members of the steroid/TR superfamily (Sap et al., 1986, Weinberger et al., 1986). The TRs are ligand-inducible transcription factors that regulate expression of hormone-responsive target genes. In mammals, the THRA gene encodes three C-terminal variants of TRα. TRα1 is a functional receptor that binds both DNA and T₃, whereas TRα2 and TRα3 fail to bind T₃ and act as antagonists in vitro (Harvey & Williams, 2002). A promoter within intron 7 of mouse Thra gives rise to two truncated variants, TRΔα1 and TRΔα2, which act as potent dominant-negative antagonists in vitro, although their physiological role is unclear (Chassande et al., 1997). The THRβ gene encodes two N-terminal TRβ variants, TRβ1 and TRβ2, both of which act as functional receptors. Two further transcripts, TRβ3 and TRΔβ3, have also been described in the rat, but their physiological role is also uncertain (Williams, 2000, Harvey et al., 2007). TRα1 and TRβ1 are expressed widely, but their relative concentrations differ during development and in adulthood due to tissue-specific and temporo-spatial regulation (Forrest et al., 1990). The current understanding is that most T₃ target tissues are either predominantly TRα1 or TRβ1 responsive or lack TR isoform specificity. Expression of TRβ2, however, is markedly restricted. In the hypothalamus and pituitary, it controls the HPT axis feedback loop by mediating the inhibitory actions of thyroid hormones on TRH and TSH expression (Fig. 1; Abel et al., 1999, 2001), while in the cochlea and retina TRβ2 is an important regulator of sensory development (Ng et al., 2001, Jones et al., 2007).

Developmental control of T₃ action

Unliganded TRs compete with T₃-bound TRs for DNA response elements. They act as potent transcriptional repressors and have been shown to have critical regulatory roles in the development of a number of key tissues (Hashimoto et al., 2001, Chassande, 2003, Venero et al., 2005, Wallis et al., 2008). Unoccupied TRs interact with co-repressor proteins, including nuclear receptor co-repressor (NCoR) and the silencing mediator for retinoid and TR (SMRT), which recruit histone deacetylases and maintain a non-permissive closed chromatin structure to inhibit gene transcription. Ligand-bound TRs, however, interact with steroid receptor co-activator 1 (SRC1) and other related co-activators in a hormone-dependent fashion. These co-activator proteins function as histone acetyl transferases, thereby promoting an open nucleosome structure leading to target gene activation. The contrasting chromatin-modifying effects of liganded and unliganded TRs, thus, greatly enhance the magnitude of the transcriptional response to T₃ (Harvey & Williams, 2002, Chassande, 2003). In addition to the positive stimulatory effects on target gene expression, T₃ also mediates transcriptional repression to inhibit the expression of certain key target genes, including TSH. Although such negative regulatory effects are physiologically critical, the
responsible underlying molecular mechanisms have not been fully characterized (Cheng et al. 2010). Although expression of both TRα1 and TRβ1 is widespread, their relative levels of expression differ between tissues during embryogenesis and in postnatal life. Differential control of TRα1 and TRβ1, therefore, provides another mechanism to regulate tissue-specific T3 responses during development and growth (O’Shea et al. 2006). Although the free T4 concentration is approximately fourfold greater than free T3, the TR-binding affinity for T3 is 15-fold lower than its affinity for T4 (Lin et al. 1990). Thus, T4 acts as a pro-hormone, which must be metabolized to T3 for the mediation of thyroid hormone actions (Bianco & Kim 2006). Taken together, the temporospatial and tissue-specific regulated expression of both the DIO2 and the DIO3 enzymes (Bates et al. 1999) and the TRα1 and TRβ1 nuclear receptors (Forrest et al. 1990) combine to provide a complex but co-ordinated system for fine control of T3 availability and action in individual cell types during development. DIO3 is expressed in fetal tissues and the utero-placental unit where it acts as a barrier that prevents maternal thyroid hormone access to the developing fetus (Wasco et al. 2003).

Unliganded TRs are key factors that prevent premature cell differentiation and maintain cell proliferation in order to allow organogenesis to proceed in the developing fetus (Plateroti et al. 2001, Flamant et al. 2002, Chassande 2003). In mammals, the reciprocally regulated decrease in DIO3 activity and increase in DIO2 activity at birth results in a rapid rise in T3 production (Bates et al. 1999). Similar changes have been observed during metamorphosis and hatching in amphibians and birds respectively (Huang et al. 2001). An increase in DIO2 expression in target tissues together with a decrease in DIO3 expression results in an increased intracellular T3 concentration, binding of T3 to its nuclear receptor, and initiation of cell differentiation (Campos-Barros et al. 2000, Sachs et al. 2000, Huang et al. 2001, Plateroti et al. 2001, Flamant et al. 2002, Mai et al. 2004, Ng et al. 2004). Thus, TRs function as developmental switches that are dependent on the activities of the deiodinases and which regulate the onset of T3 target tissue differentiation during embryogenesis. For example, in the developing embryo, DIO2 has been shown to regulate the pace of endochondral ossification and bone formation (Dentice et al. 2005), while activity of DIO2 in developing cartilage is regulated by the morphogen Indian hedgehog and the ubiquitin ligase WSB-1 (Dentice et al. 2005). In addition, ubiquitin-mediated degradation of DIO2 has been shown to regulate thyroid hormone activation in several other tissues (Bianco & Larsen 2005, Fekete et al. 2007, Sagar et al. 2007). In this situation, ubiquitin-mediated proteasomal degradation of DIO2 is increased following exposure to substrate (T4), and this mechanism thus represents a rapid and sensitive posttranslational mechanism to control and limit the DIO2 activity and T3 production (Gereben et al. 2000, Steinsapir et al. 2000, Zavacki et al. 2009).

These considerations are important physiologically and in thyroid disease. In thyroid hormone deficiency, DIO2 expression and activity are increased, whereas its expression and activity are reduced in thyrotoxicosis. By contrast, DIO3 expression is regulated in a reciprocal manner at extremes of thyroid dysfunction. Thus, the ratio of DIO2 and DIO3 activity determines homeostatic control of T3 availability to the nuclear receptor even in thyroid disease. In the brain, the DIO2 activity is increased in response to hypothyroidism (Burmeister et al. 1997), whereas activity of DIO3 is markedly reduced (Friedrichsen et al. 2003). This response is considered to protect the developing brain from changes in circulating thyroid hormones and to mitigate the severe and detrimental effects of hypothyroidism (Calvo et al. 1990, Guadano-Ferraz et al. 1999, Heuer 2007). Thus, maintenance of thyroid hormone availability in specific brain regions is critically regulated by reciprocal expression of DIO2 and DIO3 (Tu et al. 1997, 1999, Bianco et al. 2002, Kester et al. 2004).

**DIO2 knockout mice**

In order to investigate the function of DIO2 in vivo, knockout mice deficient in the enzyme were generated and found to have normal fertility (Schneider et al. 2001). DIO2 knockout (D2KO) mice exhibit isolated hypothyroidism in critical tissues that depend on DIO2-catalyzed T4 to T3 conversion to regulate cellular thyroid status. Thus, D2KO mice have an elevated circulating TSH concentration, are unable to sustain a normal body temperature following cold exposure despite a normal circulating T3 concentration, are deaf, and have brittle bones (de Jesus et al. 2001, Schneider et al. 2001, Ng et al. 2004, Bassett et al. 2010), suggesting important physiological roles for DIO2 in the pituitary gland and brain, in brown adipose tissue, in the cochlea, and in the skeleton.

**Role of DIO2 in regulation of the HPT axis**

Analysis of the HPT axis in D2KO mice demonstrated a two- to threefold increase in the circulating TSH concentration, a 27–40% increase in the T4 level accompanied by reduced clearance of T4 from plasma, but a normal T3 (Schneider et al. 2001, Christoffolete et al. 2007, Galton et al. 2007). Serum TSH was suppressed following treatment with T3 but not in response to T4, indicating that D2KO mice have pituitary resistance to feedback regulation by T4 (Schneider et al. 2001). Surprisingly, TRH mRNA levels in the PVN were not increased in D2KO mice despite their elevated TSH level, suggesting that resistance to T4 suppression results mainly from Dio2 deficiency in the pituitary rather than the hypothalamus (Rosene et al. 2010). Thus, DIO2 is essential for regulation of the HPT axis and enables the pituitary to respond to changes in the circulating T4 level (Fig. 1). In addition, it is important to note that the inactivating DIO3 enzyme is also a key regulator of the HPT axis. Analysis of DIO3-deficient (D3KO) mice indicates that DIO3 plays a key role in the development of the HPT axis set-point. Neonatal D3KO mutants have elevated.
cifically in the brain despite low circulating T4 levels. However, they also have increased DIO2 activity as a result of the low circulating T4 levels, and this is reflected by an increase in the cellular T3 concentration (Hernandez et al. 2006). These observations demonstrate a vital role for DIO3 in the determination of thyroid status in peripheral target tissues and indicate the close inverse relationship between DIO2 and DIO3 during maturation of the HPT axis.

Role of DIO2 in the brain

In the brain, DIO2 is expressed in glial cells, third ventricle tanyocytes, astrocytes, and some sensory neurons including nuclei within the trigeminal and auditory pathways (Guadano-Ferraz et al. 1997, 1999, Tu et al. 1997). As discussed earlier, studies in D2KO mice indicated that DIO2 is required for local T3 generation in the pituitary and essential for normal control of the HPT axis (Schneider et al. 2001). In addition, neonatal D2KO mice have a 25–50% reduction in tissue T3 concentrations throughout the brain, which is similar to that seen in hypothyroid wild-type littermate mice. The reduced tissue T3 concentration in neonatal D2KO mice does not result from increased T3 degradation, as activity of the inactivating Dio3 enzyme is not altered in any brain regions (Galton et al. 2007). Thus, deficiency of DIO2 results in reduced local T3 generation throughout the developing brain. Nevertheless, expressions of the T3-responsive genes Hairless, TrkB, Rca3, and Sre1 are less susceptible to change in D2KO mice compared with the altered expression observed in thyroid-deficient mice (Galton et al. 2007). Thus, despite the markedly increased expression of Dio2 in the newborn brain (Bates et al. 1999), D2KO mice have a mild neurological phenotype in comparison with the severe consequences of systemic hypothyroidism. Although T3 availability in neurons is dependent on the Dio2 activity in adjacent glial cells, these surprising data reveal that compensatory mechanisms can mitigate the neurological damage resulting from Dio2 deficiency and also show that alternative sources of T3 can access the brain during development (Galton et al. 2007). These compensatory sources are not likely to involve increased transport mediated by MCT8 because the levels of MCT8 expression in the brain are similar in euthyroid, hypothyroid, and D2KO mice (Galton et al. 2007). Although Dio2 expression peaks at important times during development of the brain and is required to generate adequate intracellular concentrations of T3 throughout the brain, it seems that the consequences of Dio2 deficiency during central nervous system development are mitigated by other sources of T3 such as the cerebrospinal fluid (CSF) or serum (Galton et al. 2007).

The physiological efficiency of these compensatory sources of T3 was demonstrated following assessment of neurobehavioral function, which revealed only minimal differences between D2KO and wild-type mice following testing of reflexes, locomotion and agility, learning and memory, olfaction, anxiety, and exploration (Galton et al. 2007). Interestingly, a recent study was performed in which T3 target gene expression in cerebral cortex was compared between hypothyroid wild-type D2KO and MCT8KO mice (Morte et al. 2010). The aim was to investigate whether the source of tissue T3 in brain via local T3 generation (disrupted in D2KO mice) or via transport across the blood–brain barrier (disrupted in MCT8KO mice) elicited differing target gene responses. Little effect on T3 target gene response was seen in MCT8KO mice because a compensatory increase in Dio2 expression was identified which was proposed to mitigate local T3 deficiency. By contrast, in D2KO mice, there was increased expression of T3 target genes normally inhibited by T3, but no effect was seen on genes that are normally positively regulated by T3. In hypothyroid wild-type mice, however, expression of both negatively and positively regulated T3 target genes was affected (Morte et al. 2010). Taken together, these intriguing observations suggest that the source of T3 in the brain (locally generated T3 versus T3 transported from serum and CSF) may influence the T3 response elicited.

Role of DIO2 in brown adipose tissue and adaptive thermogenesis

D2KO mice exposed to cold are unable to maintain their body temperature despite the presence of a normal circulating T3 concentration. The mild hypothermia following cold exposure results from impaired energy expenditure in brown adipose tissue that is mitigated by a compensatory shivering response associated with acute weight loss (de Jesus et al. 2001). Isolated brown adipocytes from D2KO mice fail to respond normally to adrenalin cyclase activators or noradrenaline resulting in impaired cAMP, oxygen consumption, and mitochondrial uncoupling protein 1 mRNA responses to adrenergic stimulation. These defects are similar to observations in hypothyroidism but are not seen in brown adipocytes obtained from D2KO mice treated with T3 (de Jesus et al. 2001). The findings indicate that the cAMP-dependent Dio2 enzyme is required for adrenergic responsiveness and adaptive thermogenesis in brown adipocytes. Further studies, however, revealed a large compensatory increase in brown fat sympathetic stimulation that bypasses the reduced adrenergic responsiveness of D2KO brown adipocytes. The increased sympathetic tone in brown fat induced a marked lipolytic response, which depletes fatty acid stores and results in the defective adaptive thermogenesis and hypothermia observed in D2KO mice (Christofoleto et al. 2004). Subsequent studies also showed that bile acids activate Dio2 in brown fat via a cAMP-dependent mechanism involving the G-protein-coupled receptor TGR5, thus identifying a new role for Dio2 in diet-induced thermogenesis as bile acids were also shown to protect mice from diet-induced obesity (Watanabe et al. 2006). Accordingly, D2KO mice have greater...
susceptibility to diet-induced obesity that may result in part from impaired brown adipose tissue development during embryogenesis (Hall et al. 2010) as well as impaired diet-induced thermogenesis.

**Role of DIO2 in muscle**

Muscle is an important T₃ target tissue, and euthyroidism is required for its efficient function and regeneration. Recent studies in mice have demonstrated that Dio2 mRNA and activity are expressed in skeletal muscle. Type I slow-twitch fibers displayed fivefold greater Dio2 activity than type II fast-twitch fibers, and hypothyroidism resulted in a threefold induction of activity without changes in mRNA levels (Marsili et al. 2010). MyoD is a master regulator of myogenic differentiation and muscle regeneration, and new studies have established that Dio2-mediated generation of T₃ is essential for efficient transcription of MyoD (Dentice et al. 2010). Furthermore, the Dio2 activity is present in muscle stem cells and increases during myogenic differentiation. Accordingly, in Dio2−/− mice, myocytes exhibit a hypothyroid phenotype despite normal circulating T₃ levels, the expression of T₃-responsive genes including MyoD is markedly reduced, and muscle regeneration is delayed following injury. In primary myoblasts, a forkhead box transcription factor, FoxO3, has been shown to induce Dio2 expression and mediate the surge in Dio2 activity necessary to increase the local intracellular T₃ concentration and thereby ensure normal muscle formation and regeneration (Dentice et al. 2010). Thus, Dio2 is essential for skeletal muscle development, function, and repair.

**Role of DIO2 in the cochlea**

In the cochlea, Dio2 is expressed in periosteal connective tissue surrounding the internal sensory tissues, with enzyme activity peaking at postnatal day P7, a few days prior to the onset of hearing around P14. TR expression, however, is localized to the cochlea sensory epithelium, suggesting that peristiole Dio2 provides a temporospatially regulated paracrine supply of T₃ to the sensory epithelium that is necessary for correct timing of cochlea development and maturation (Campos-Barros et al. 2000). This hypothesis was supported by findings in Dio2−/− mice, which exhibit delayed differentiation of the auditory sensory epithelium and delayed cochlea development with abnormal formation of the tectorial membrane. The resulting deafness in Dio2−/− mice is similar to that seen in systemic hypothyroidism or in TRβ knockout mice (Forrest et al. 1996a, Rusch et al. 1998, 2001) but occurs despite circulating levels of thyroid hormones that are normally permissive for development of hearing. Treatment of Dio2−/− mice with T₃ ameliorated the phenotype, indicating that Dio2-dependent local generation of T₃ in the surrounding bony labyrinth is essential for development of the cochlea and subsequent auditory function (Ng et al. 2004). In this case, the activating Dio2 enzyme functions as a local paracrine amplifier of T₃ action to regulate sensory development. More recently, Dio3 was also found to be expressed in the cochlea, and Dio3-deficient mice were shown to be deaf and have advanced cochlear maturation (Ng et al. 2009), indicating that Dio3 normally protects the cochlea from premature T₃-induced differentiation. Thus, development of the cochlea and the onset of normal auditory function require tightly controlled and correctly timed availability of T₃ that is achieved by co-ordinated reciprocal alterations in the expression and activities of Dio2 and Dio3.

**Role of DIO2 in the skeleton**

It is well known that hypothyroidism causes delayed bone formation and linear growth retardation. Possible roles for Dio1 and Dio2 in bone were first studied in the context of growth. A minor and transient impairment of weight gain was initially reported in male D2KO mice, although linear growth was not determined (Schneider et al. 2001). Weight gain and growth, however, were normal in Dio1−/− and in Dio1−/−/− Dio2−/− mice and in combined C3H/HeJ Dio1−/−/− Dio2−/− mice (Berry et al. 1993, Schoenmakers et al. 1993, Schneider et al. 2006, Christofolfolete et al. 2007). We and others showed that Dio1 is not expressed in bone and cartilage (LeBron et al. 1989, Dreher et al. 1998, Gouveia et al. 2005, Williams et al. 2008), indicating that Dio1 does not directly influence T₃ action in bone.

Nevertheless, important roles for Dio2 during skeletogenesis and in adult bone are emerging. The Dio2 activity was demonstrated in the perichondrium surrounding the embryonic chick growth plate where its activity is regulated by the skeletal morphogen SHH (Dentice et al. 2005). SHH is secreted by perichondrial cells and acts in growth plate chondrocytes to stimulate ubiquitin-mediated degradation of Dio2. The resulting modulation of thyroid hormone signaling in the growth plate is accompanied by increased PTHrP signaling, which is also seen in hypothyroidism (Stevens et al. 2000) and which regulates the pace of chondrocyte differentiation during early skeletogenesis (Dentice et al. 2005). Analysis of developing bone from mice at embryonic days E14.5/E18.5 revealed the presence of Dio2 activity (Capelo et al. 2008), suggesting that a similar regulatory role for Dio2 in cartilage during early skeletogenesis may occur in the mouse as well as the developing chick (Dentice et al. 2005).

There have been conflicting reports regarding the expression and activity of Dio2 in whole bone tissue extracts and in skeletal cells (LeBron et al. 1989, Bohme et al. 1992, Ballock & Reddi 1994, Dreher et al. 1998, Wakita et al. 1998, Gouveia et al. 2005, Morimura et al. 2005, Capelo et al. 2008). Using a sensitive and highly specific HPLC-based assay, we demonstrated that specific Dio2 activity is restricted to differentiated osteoblasts but is undetectable in chondrocytes and osteoclasts (Williams et al. 2008). The significance of this finding was investigated in Dio2−/− mice (Bassett et al. 2010).
There were no differences in linear growth and bone formation between D2KO and wild-type mice, although adult D2KO mice had brittle bones with impaired resistance to fracture. The phenotype was due to reduced osteoblastic bone formation without impairment of osteoclastic bone resorption, which caused a reduced rate of mineral apposition and prolongation of the formation phase of the bone remodeling cycle, thus facilitating an increase in secondary mineralization that resulted in a generalized increase in bone mineralization density (Bassett et al. 2010). The T₃ target gene analysis demonstrated cellular T₃ deficiency restricted to osteoblasts, indicating that maintenance of adult bone mineralization and optimal bone strength requires local DIO2-mediated production of T₃ in osteoblasts (Bassett et al. 2010).

These findings suggest that the restricted expression of DIO2 in adult bone is necessary to maintain a higher intracellular T₃ concentration in osteoblasts relative to other skeletal cells. As in other tissues, the DIO2 activity in osteoblasts is increased in hypothyroidism and inhibited in hyperthyroidism (Gouveia et al. 2005). Thus, DIO2 acts to buffer the effects of altered serum thyroid hormone levels on the skeleton; the adverse effects of T₃ deficiency on bone mineralization may be mitigated by increased DIO2 activity in osteoblasts, while inhibition of DIO2 activity in hyperthyroidism limits the detrimental effects of thyroid hormone excess (Bassett et al. 2010). This hypothesis suggests that optimal bone mineralization and strength are maintained over the physiological range of systemic thyroid hormone concentrations by the regulated activity of DIO2 in osteoblasts. Escape from this local feedback mechanism in osteoblasts may account in part for the increased susceptibility to fracture observed in hypothyroidism and thyrotoxicosis (Vestergaard & Mosekilde 2002, Vestergaard et al. 2005), suggesting the possibility of DIO2 as a therapeutic target for the treatment of osteoporosis.

A recent human population study has also suggested that DIO2 may influence susceptibility to osteoarthritis. A genome-wide linkage analysis identified an association between the DIO2 polymorphism rs225014 and the generalized symptomatic osteoarthritis (Meulenbelt et al. 2008), although the association was not replicated in a subsequent association study and meta-analysis (Kerkhof et al. 2010). Nevertheless, a recent meta-analysis has also identified a possible role for DIO3 in osteoarthritis susceptibility (Meulenbelt et al. 2010). Taken together, these new and preliminary findings suggest that deiodinase-regulated T₃ availability in chondrocytes may play an important role in the regulation of cartilage renewal and repair.

Conserved and pivotal role of DIO2 in the control of seasonal reproduction

A series of recent elegant studies, initially in the Japanese quail (Coturnix japonica; Yoshimura et al. 2003) but also in mammals, have identified a major role for DIO2 in the seasonal control of reproduction. Seasonal time measurement is achieved by sensing of the changing photoperiod in temperate zones. Regulatory sensing of the changing photoperiod and the subsequent gonadal response are localized to the medio-basal hypothalamus (MBH). In subtraction hybridization studies in the Japanese quail, DIO2 expression was found to be induced by light and the MBH tissue T₃ concentration was increased tenfold following long-day exposure compared with short-day exposure (Yoshimura et al. 2003). Furthermore, i.c.v. infusion of T₃, like exposure to long-day conditions, stimulated gonadal growth while infusion of long-day-exposed quails with iopanoic acid (a DIO2 inhibitor) prevented testicular growth. These findings demonstrated that DIO2-mediated local conversion of T₄ to T₃ in the MBH in response to light is a key pathway mediating the photoperiodic seasonal reproduction response (Yoshimura et al. 2003). Further studies revealed that the photoperiod response is triggered by light-induced expression of TSH in the pars tuberalis, which subsequently stimulates DIO2 expression in ependymal cells of the MBH via a TSHR-mediated pathway coupled to cAMP that results in light-induced LH secretion (Nakao et al. 2008). Additional studies have also revealed that reciprocal changes in DIO2 and DIO3 expression are induced in the MBH in response to changes in the photoperiod (Yasuo et al. 2005), and thus coordinated regulation of DIO2 and DIO3 expression has the capacity to mediate sensitive and rapid responses to changes in the photoperiod, thereby highlighting the importance of local control of tissue T₃ availability in the MBH for seasonal reproduction. Nevertheless, the precise downstream molecular consequences of increased T₃ production in the MBH still remain to be elucidated. A melatonin–responsive photoperiodic reproduction system in various mammals has also been shown to involve TSH and DIO2 (Watanabe et al. 2004, Revel et al. 2006, Yasuo et al. 2006, 2007, Hanon et al. 2008, Nakao et al. 2008, Ono et al. 2008), suggesting that seasonal reproduction in mammals and birds is regulated by similar conserved pathways that lie downstream of the initial light or melatonin photoperiod stimulus (Yoshimura 2010).

Conclusions

In recent years, the importance of controlled intracellular availability of T₃ in target tissues has been appreciated. The vital roles played by DIO2 in development, during the establishment of the HPT axis and in specific tissues including the pituitary gland, brain, brown adipose tissue, cochlea, and bone, have been documented in considerable detail. Yet, much remains and exciting and important discoveries are inevitable. For example, recent studies are identifying new roles for DIO2 in the heart (Wang et al. 2010), in skeletal muscle (Grozovsky et al. 2009), during inflammation (Kwakkel et al. 2009), and in the pituitary in response to specific drug challenges (Rosene et al. 2010). Given the
broadness of expression of DIO2 and its response to cellular stress (Gereben et al. 2008), it is likely that the functional repertoire for DIO2 will expand. The immediate challenges will be to identify these new roles and to determine whether functions ascribed to DIO2 from animal studies and genetic manipulation have physiological or pathological importance in man. Of importance in this context, a common Thr92Ala polymorphism has been identified in DIO2 (Peeters et al. 2003). Although in vitro biochemical studies indicated no difference in the enzymatic properties of the DIO2 Thr and Ala variants (Peeters et al. 2003, Canani et al. 2005), thyroid and skeletal muscle tissue extracts from Ala/Ala individuals displayed reduced DIO2 activities (Canani et al. 2005). The mechanism responsible for reduced tissue activity is not known but may result from linkage disequilibrium between the Thr92Ala polymorphism and a second functional variant elsewhere (Canani et al. 2005). Nevertheless, in addition to osteoarthritis (Meulenbelt et al. 2008, Kerkhof et al. 2010), the Thr92Ala polymorphism has also been associated with variation in the HPT axis (Peeters et al. 2005, Butler et al. 2010), altered bone turnover (Heemstra et al. 2010), variable and contradictory effects on cognitive parameters, and the response to thyroid hormone replacement (Appelhof et al. 2005, Torlontano et al. 2008, Heemstra et al. 2009a, Panicker et al. 2009), as well as having an inconsistent relationship to hypertension, insulin resistance, and the metabolic syndrome (Mentuccia et al. 2002, 2005, Canani et al. 2005, 2007, Grarup et al. 2007, Gunnietaik & Williams 2007, Gunnietaik et al. 2007, Peeters et al. 2007, van der Deure et al. 2009, Dora et al. 2010, Estvalex et al. 2010).

Ultimately, an important challenge will be to exploit DIO2 as a drug target to manipulate tissue thyroid status, perhaps in the treatment of metabolic disorders including obesity or skeletal disorders such as osteoporosis and osteoarthritis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by the Medical Research Council (grant numbers G0501486, G0800251) and Biotechnology and Biological Sciences Research Council (BBF02170/4).

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Received in final form 20 January 2011
Accepted 3 February 2011
Made available online as an Accepted Preprint 3 February 2011

Journal of Endocrinology (2011) 209, 261–272

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