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Ulrich Schweizer, Joachim M. Weitzel, Lutz Schomburg. Think globally – act locally New insights into the local regulation of thyroid hormone availability challenge long accepted dogmas. *Molecular and Cellular Endocrinology*, 2008, 289 (1-2), pp.1. 10.1016/j.mce.2008.04.007 . hal-00532020

HAL Id: hal-00532020

<https://hal.science/hal-00532020>

Submitted on 4 Nov 2010

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Accepted Manuscript

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PII: S0303-7207(08)00128-7
DOI: doi:10.1016/j.mce.2008.04.007
Reference: MCE 6853

To appear in: *Molecular and Cellular Endocrinology*

Received date: 30-1-2008
Revised date: 20-3-2008
Accepted date: 3-4-2008

Please cite this article as: Schweizer, U., Weitzel, J.M., Schomburg, L., Think globally – act locally New insights into the local regulation of thyroid hormone availability challenge long accepted dogmas, *Molecular and Cellular Endocrinology* (2007), doi:10.1016/j.mce.2008.04.007

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Think globally – act locally
New insights into the local regulation of thyroid hormone availability
challenge long accepted dogmas

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keywords: deiodinase, Mct8, regulation, obesity, bile acids

Summary

Recent evidence derived from transgenic mouse models and findings in humans with mutations affecting thyroid hormone (TH) metabolism have convincingly supported a model of TH signalling in which regulated local adjustment of active TH concentrations is far more important than circulating plasma hormone levels. Although this theory was put forward several years ago and has been supported by significant, but inherently indirect evidence, recent insights from targeted deletion of the genes encoding deiodinase (Dio) isozymes have revived this model and greatly increased our understanding of TH metabolism. However, gene targeting proved to be a double edged sword, since the overall model was supported, but several predictions are apparently not consistent with the new experimental evidence. Human genetics further provided additional exciting data on the physiological role of Dio isozymes that need to be incorporated into any model of TH biology. The recent identification of mutations in the T3 plasma membrane transporter MCT8 has sparked new interest in the role of TH in brain function, since affected patients suffered from psychomotor retardation. Moreover, selenium (Se) and TH physiology have finally been unequivocally connected by newly identified inherited defects in a gene involved in selenoprotein biosynthesis. Finally, a link between Dio expression and energy metabolism has been delineated in mice that may hold great promise for the management of the adiposity pandemic.

The model

Traditionally, blood hormone concentrations take centre stage in the world of endocrinologists. The classical definition of a “hormone” holds that it is produced upon demand or according to biological rhythms in a specialized gland, becomes distributed via the blood stream, and acts on its distant target tissues. Target cells are characterized by their responsiveness to the hormone, depending on the expression of cognate hormone-specific receptors. In this sense, the hypothalamus-pituitary-thyroid (HPT) axis is a classical endocrine axis. It often serves as a teaching paradigm for regulatory endocrine feedback loops, since the target tissues of thyroid hormone (TH) include, among many peripheral organs, also the pituitary thyrotropes and hypothalamic neuroendocrine cells that produce thyrotropin (TSH) and TSH-releasing hormone (TRH), respectively. Determination of both circulating TH and TSH concentrations is of practical importance in the clinic for assessment of the functionality of the HPT axis. Usually, high TSH values indicate insufficient feedback activity of circulating TH, i.e. hypothyroidism, and point to thyroid gland defects. Thereby, TSH is used as an indirect biomarker of circulating TH concentrations and thyroid gland functioning e.g. in routine newborn screening protocols (Gruters et al., 2003). In reality, the HPT system is of course more complex than usually presented in textbooks and deviates from that simple scheme in several molecular aspects.

- The main product of the thyroid gland, i.e., L-3,3',5,5'-**tetraiodothyronine** (thyroxin, T4), is a pro-hormone from which a 5'-iodine atom needs to be removed in order to produce the receptor binding, active thyroid hormone, L-3,3',5-triiodothyronine (T3).
- This deiodination step can apparently be performed outside the thyroid gland by enzymes of the deiodinase (Dio) family, since thyroidectomized experimental animals or human patients lacking a functional gland can be successfully supplemented with exogenously applied T4.

- Activating and inactivating Dio isozymes are expressed in many organs and help to “customize” intracellular T3 levels according to the actual physiological demands of the tissue.
- Unlike most other hormones, circulating levels of TH are remarkably stable. Whereas T3 and T4 serum concentrations do not display circadian variations, Dio2 activity is regulated in a circadian pattern – and in some birds, by a seasonal rhythm.
- TH do not readily cross the plasma membrane, but require membrane transporters in order to gain access to their nuclear receptors. Thus, the dynamic and cell type-specific expression pattern of membrane transporters may add another level of complexity to endocrine regulation of the HPT axis.

In this review we will try to lay out the fundamental principles in local TH metabolism by deiodinase enzymes without attempting to provide a complete and balanced account of the literature. Rather, we will point out recent data that, in our eyes, do not easily fit into the accepted scheme and try to stimulate a discussion on how to revise the current model.

Deiodinases as local regulators of thyroid hormone signalling

Not all Dio isoenzymes are created equal (Bianco *et al.*, 2002). For example, type II 5'-deiodinase (gene symbol Dio2), an enzyme that can mediate exclusively activating 5'-deiodination (as in the activation of T4 to T3), is induced in the hypothyroid brain in order to maintain constant brain T3 levels. Conversely, type III 5-deiodinase (Dio3) is an enzyme that mediates exclusively the inactivating 5-deiodination (e.g. T4 → rT3, T3 → 3,3'-T2). This enzyme is induced in the hyperthyroid brain in order to limit the action of excessive T4 availability. In hypothyroidism decreased Dio3 activity contributes to the maintenance of adequate T3 levels. The role of type I 5'-deiodinase (Dio1) is less clear. Dio1 is able to perform both 5'- and 5-deiodination reactions with a number of iodothyronine substrates. One prominent notion that is already being put forward in several textbooks holds that Dio1

is involved in peripheral (meaning extra-thyroidal) deiodination of T4 and contributes decisively via its high expression rates in liver to the circulating T3 levels. Hence, the analysis of hepatic Dio1 expression levels and its transcriptional regulation by endocrine and immunological signals or endocrine disruptive chemicals became a central issue in basic thyroidology. Several pieces of data were taken as evidence for the central importance of hepatic Dio1 expression for the control of circulating TH concentrations:

Firstly, the liver contains very high specific Dio1 activity. Considering the size of the liver in humans and experimental rodents, hepatic Dio1 represents the deiodinating activity with the highest capacity within the body. Secondly, a concomitant fall of plasma T3 concentrations and hepatic Dio1 activity are observed in severe illness. Thirdly, PTU (6-n-propyl-2-thiouracil), a rather specific Dio1 inhibitor, decreases circulating T3 levels in T4-treated, thyroidectomized rats. Fourthly, the conversion of T4 to T3 was demonstrated with isolated perfused rat livers *ex vivo*, hepatocytes and liver microsomal membranes (Hesch et al., 1975) or with hepatic cell extracts (Visser et al., 1975). And last, but not least, the relatively high K_m value of Dio1 (0.5 μM) versus Dio2 (1-2 nM) and the considerably longer half-life of Dio1 versus Dio2 clearly point to the former as the prime candidate for conversion of thyroid-derived T4 into systemic biologically active T3 (Köhrle, 1999).

The Up-side and the Down-side of Dio1 as the regulator of TH signals

Two recent papers using novel transgenic mouse models, however, have questioned the alleged role of hepatic Dio1 for providing T3 to the circulation. One study took advantage of the fact that all three Dio isozymes are selenoproteins that contain the 21st proteinogenic amino acid selenocysteine (Sec) within their catalytic domains (Bianco *et al.*, 2002). The biosynthesis of selenoproteins depends on the Sec-specific tRNA (Trsp), which is unique

among all known tRNAs with respect to its specificity for the ambiguous OPAL stop- or Sec-codon UGA (Hatfield and Gladyshev, 2002). Conditional inactivation of *Trsp* in mouse hepatocytes resulted in loss of hepatic selenoprotein expression, including *Dio1* activity. However, TH as well as TSH levels remained unchanged in these mice (Streckfuss et al., 2005). Although the model was initially not designed to specifically reduce hepatic deiodinase activity, the mice provided first evidence that hepatic *Dio1* is not necessarily required for maintaining circulating T3 levels. Moreover, there was no upregulation of *Dio1* or *Dio2* activities in other organs, which might compensate for the lack of hepatic *Dio1* contribution to the peripheral activation of T4 to T3. Rather, a small but significant reduction was observed in the expression of hepatic T3-responsive genes, supporting at least some intracellular role of *Dio1* in regulating T3-responsive genes within the hepatocyte. This mouse model, despite its specificity for the hepatocyte, suffered from the lack of specificity for *Dio1*, leaving at least the theoretical possibility that some other hepatic selenoproteins may play a so far unidentified role in regulating the HPT axis.

Fortunately, at the same time a global knockout of *Dio1* was reported and again, loss of *Dio1* was less detrimental to the animals than one might have expected from the high expression of *Dio1* in several organs, including the liver (Schneider et al., 2006). Here, plasma T4 and rT3 levels were significantly increased in *Dio1*^{-/-} mice, while T3 and TSH remained unchanged. These changes can be interpreted as a partial resistance to respond to the elevated level of T4 at the level of the pituitary or thyroid gland and might point to an important role of *Dio1* in feedback regulation. In this context, the earlier observation of a T3-dependent induction of *Dio1* in pituitary might help to explain the derangement of the TH feedback axis in these mice (Köhrle et al., 1995). Possibly, *Dio1* in thyrotropes is able to dynamically fine-tune intracellular TH concentrations to optimally control TSH biosynthesis and secretion.

Schneider et al. concluded from their results that *Dio1* could have a role in protecting the organism from thyrotoxicosis by removing excess of the prohormone T4. The notion of *Dio1*

being involved in removal rather than activation of TH is further supported by the finding that Dio1 is induced by T3 (in the liver) and during hyperthyroidism, closing a negative feedback loop instead of driving a feed-forward reaction into thyrotoxicosis (Zavacki et al., 2005). Along these lines, the puzzling enzymatic and quantitative properties of Dio1 might finally meet an explanation: One major problem of the Dio1 enzymatic mechanism remained the dual specificity of the enzyme that allows for both 5'- and 5-deiodination. Considering that Dio1 prefers either sulfated TH (T3-S; T4-S) and their deiodination products or rT3 as substrates, a role in iodothyronine degradation appears plausible. Moreover, there is a high stoichiometric surplus of hepatic Dio1 over daily TH production rate by the thyroid gland including the production of TH metabolites (Leonard and Visser, 1984; Visser et al., 1988). If hepatic Dio1 represented a vividly catalyzing activating enzyme, minute amounts of Dio1 should suffice to dynamically activate intracellular T4 according to the actual needs of the hepatocytes. However, if the main function of hepatic Dio1 consisted in control of TH inactivation and degradation, an excess of non-cycling Dio1 for immediate inactivating adduct formation and targeted disposal would make perfect sense. Accordingly, the authors implicated in an elegant series of experiments that hepatic Dio1 is involved in the liberation of iodine from iodothyronine metabolites. For example, faecal excretion of radioactively labeled T3 and T4 increased, while urinary excretion of radioactive iodide was decreased in the Dio1^{-/-} mice (Schneider et al., 2006). Moreover, direct measurement of faecal iodothyronines revealed a strong increase of iodothyronine excretion, in particular rT3. Such findings are reminiscent of earlier findings in PTU-treated rats (Nguyen et al., 1998). Hepatic Dio1 contributes to the nuclear T3 pool only to a lesser extent, suggesting that its role does not primarily lie in local activation of T4 to T3 (van Doorn et al., 1983a; van Doorn et al., 1983b).

In search of the source of circulating T3

Since (hepatic) Dio1 is apparently dispensable for maintaining normal plasma T3 levels, one may conclude that Dio2 represents the isozyme that must be responsible for production of circulating T3 from thyroid-derived T4. Indeed, since Dio3 is only involved in 5-deiodination, there is no other candidate enzyme for this biosynthetic step.

Therefore, several authors have tried to determine the relative contributions of Dio1 and Dio2 to circulating T3 levels. Depending on the methodology used, the estimates for the contribution of peripherally expressed Dio2 for circulating T3 concentrations range from 20%-63% in experimentally manipulated rodents (Nguyen et al., 1998) to 80% in humans as derived from in vitro properties of cloned enzymes in HEK cells (Maia et al., 2005). Clearly, there are species differences that must be taken into account. Still, the analysis of TH concentrations in Dio2^{-/-} mice is in sharp contrast to these estimates. In contrast, hypophyseal TH feedback is disturbed and T4 and TSH levels are strongly increased in Dio2^{-/-} mice (Schneider et al., 2001), while peripheral T3 production and circulating T3 concentrations appear largely unaffected. Rather, adaptive thermogenesis is impaired in Dio2^{-/-} mice (de Jesus et al., 2001). In addition, developmental processes, in particular in the cochlea and eye (Ng et al., 2004), turned out to be dependent on cell-specific Dio2 expression. Together, these findings rather support a local intracellular function of Dio2 in certain TH target cells than a general function for circulating TH production.

Interestingly, substantial experimental data had been presented long ago implying that the thyroid gland itself represents a major source of circulating T3 (Chanoine et al., 1993a). It was suggested that the T3 released from the thyroid is a product from local T4 deiodination within the gland (Chanoine et al., 1993b). Accordingly, using Se deficiency as a means to reduce hepatic Dio1 activity, a role for thyroidal T3 production was proposed in the rat, although in that study thyroidal Dio activity was not measured (Chanoine et al., 1992). In

another study, thyroidal Dio1 activity was reported resistant to Se deficiency (Bates et al., 2000). It seems conceivable that changing local Dio activity at the level of the thyroid gland could fine-tune TH release. For example, iodine deficiency efficiently increases thyroidal Dio1 activity (Mitchell et al., 1998). In addition, we know now that thyroidal Dio responds to TSH and that its expression is altered in pathological settings (Murakami et al., 2001; Kanou et al., 2007). Although this hypothesis of TH deiodination within the thyroid has never gained general support, the new data from the aforementioned knockout mouse models may help to revive the idea. Clearly, further experiments using novel tools are now needed to re-evaluate this hypothesis.

Dio2: Control of central TH signals

The brain is quite sensitive to deviations from required TH levels. The debilitating consequences of cretinism or congenital hypothyroidism are textbook examples of developmental deficiencies and do not need more explanation at this place. In particular during brain development, TH is needed to regulate neuronal migration, dendritic arborization, myelination and e.g. the formation of the auditory system (Koibuchi and Chin, 2000; Zoeller et al., 2002). Moreover, TH is also required for precursor cell proliferation in the hippocampus and is associated with fundamental behavioural programs. The role of TH in psychiatric disease is just increasingly being recognized and surprising and as yet enigmatic results are observed in severe bipolar-depression where some patients profit from treatment with supraphysiological dosages of TH without apparent cardiac side-effects (Bauer et al., 2005).

Our current model states that T4 is preferentially taken up into the brain and local deiodination, via Dio2 in astrocytes, provides the required amount of T3 to neurons. This model puts cerebral Dio2 at centre stage for regulating TH-dependent developmental programs (Galton, 2005; Bernal, 2005). Accordingly, it is conceivable that Dio2 is up-

regulated in the brain during hypothyroidism in order to meet the constant need of neurons for adequate T3 levels in face of reduced circulating T4 (Bianco *et al.*, 2002).

However, it came as a complete surprise that *Dio2*^{-/-} mice are not suffering from cretinism as predicted by the current model. While cerebellar development is disturbed in hypothyroid mice or rats, *Dio2*^{-/-} mice do not display obvious cerebellar defects or a strong neurological phenotype (Galton *et al.*, 2007). These findings clearly challenge the current dogma that puts much emphasis on local T3 production via *Dio2* in astrocytes. Mice that are devoid of all thyroid hormone receptors display a hypothyroid phenotype (Gothe *et al.*, 1999). However, the neurological damage in these mice was less severe than in *Pax8*^{-/-} mice which are lacking a thyroid gland (Ng *et al.*, 2004; Christ *et al.*, 2004). It was concluded that TRa1 needs to be activated (in cerebellar neurons) in order to avoid adverse effects by the unliganded aporeceptor, while lack of TRa1 signalling as such did not produce much of a brain phenotype (Morte *et al.*, 2002).

Why is the neurological phenotype of mice lacking all receptors different from the phenotype of mice lacking the established ligands? Are there more ligands? Are there more receptors? In fact, effects of T4 and rT3 opposing the effects of T3 were described in cerebellar granule neurons and astrocytes (Farwell *et al.*, 2005; Farwell *et al.*, 2006) and appear to be mediated by the binding of iodothyronines to integrins (Davis *et al.*, 2007). Moreover, the role of the recently characterized decarboxylation products of TH, i.e. the different thyronamines, is largely unexplored in brain, but might turn out to be of fundamental importance as just demonstrated for onset and maintenance of hibernation or neuroprotection (Doyle *et al.*, 2007). However, the exact mechanisms of action exerted by these compounds and the receptors and signalling pathways involved still need to be worked out.

Local thyroid hormone transport systems in the brain

In older texts, TH are often regarded as hydrophobic substances that passively cross the plasma membrane owing to their two aromatic rings. This notion can no longer be supported, since it completely neglects the zwitterionic amino acid nature of TH. In addition, thanks to the persistent work of Friesema and Visser, it should be clear now that TH need protein transporters in order to cross the plasma membrane (Visser et al., 2007). The recent identification of patients with mutations in the gene encoding the monocarboxylate transporter 8 (MCT8), a neuronal and pituitary cell-specific TH transport protein, nicely fits into the model of local thyroid hormone regulation at first sight (Dumitrescu et al., 2004; Friesema et al., 2004; Schwartz et al., 2005). These patients suffer from a very severe form of X-linked mental retardation. In addition, high levels of circulating T3 coexist with borderline low T4 and high/normal TSH indicating some pituitary resistance to TH (Refetoff and Dumitrescu, 2007). In the current working model, MCT8 is required for T3 entry into thyrotrophs and mutations that abrogate the ability of MCT8 to mediate this import deprive the thyrotroph of its T3 sensor. Similarly, neurons in the brain may suffer from local hypothyroidism due to an impaired T3 transport into the neuron. In fact, brain T3 levels of Mct8-deficient mice are only half of wildtype concentrations and Dio2 is greatly induced in these mice (Dumitrescu et al., 2006). The mice are good models for the endocrine derangements seen in MCT8-deficient patients, i.e., high T3 in the face of low/normal T4 and low/normal TSH. Upon injection of radiolabelled T3 or T4, only T4 is taken up into the brain (at wildtype rates), while T3 is not taken up – suggesting a predominant role of Mct8 in T3 import via the blood brain barrier (Dumitrescu *et al.*, 2006; Trajkovic et al., 2007). If deletion of Mct8 massively reduced the cerebral T3 levels, uptake of T3 at the blood-brain-barrier (BBB) must be reconsidered in a revised model. However, MCT8 expression is not exclusively neuronal, but also exceptionally strong in circumventricular organs, in particular in hypothalamic tanycytes, specialized cells lining the ventral part of the third ventricle (Heuer et al., 2005). These cells project processes to blood vessels in the arcuate nucleus and

contact the portal system in the median eminence, possibly regulating T3 exposure to regulatory components in the hypothalamic-pituitary axis. Since tanycytes also express the highest Dio2 activities in the brain, MCT8 may convey T3 produced locally from T4 into the portal blood vessels, the hypothalamus, or the cerebrospinal fluid. Apart from tanycytes, the choroid plexus expresses high levels of MCT8 and thus, choroid plexus and tanycytes may represent the main entrance routes of T3 into the brain.

Unfortunately, Mct8-deficient mice raised more conceptual problems: The mice do not display neurological symptoms of hypothyroidism, although their T3 import at the BBB is impaired and T3 levels are reduced. In fact, Mct8-deficient neurons can take up T3, albeit at a reduced rate (U. Schweizer et al., unpublished) and thus, at least in mice, there may exist additional T3 transporters in neurons – the presence of which may help to explain the lacking neurological phenotype of Mct8-deficient mice. For whatever reasons, Mct8-deficient mice are apparently no good models for the neurological aspects of the Allen-Herndon-Dudley syndrome in humans, at least not under regular breeding conditions.

Unraveling the function of type III deiodinase

Fewer predictions have been made in the canonical model regarding the role of Dio3. As mentioned above, this enzyme exclusively catalyses TH inactivating reactions. The highest Dio3 activities are found in placenta, skin, and brain. During development, Dio3 is predicted to protect the brain during critical periods from excess TH (Galton, 2005). In fact, this proposed function is compatible with the results obtained with Dio3-deficient mice (Hernandez et al., 2006). Hypothalamic control of the HPT axis apparently needs protection from high postnatal T3 exposure in order to ensure correct adjustment of the TSH setpoint. In Dio3 mutant mice, this protection is lost leading to hypothalamic repression of the HPT axis and consecutive central hypothyroidism. Interestingly, T4 levels are more profoundly reduced than T3 in these mice. More recently, the profound derangements of the HPT axis have been

analysed in more detail (Hernandez et al., 2007). Strikingly, neither the hypothalamus nor the pituitary responds normally to TRH and TSH, respectively. Moreover, despite increased TSH levels, the thyroid is smaller than in the wildtype and shows only a blunted T4 response to TSH stimulation. When hypothyroidism was induced in *Dio3*^{-/-} mice, TSH was only marginally induced, again pointing to persistent central deregulation of the HPT axis and its setpoints. Similarly, *Dio3*^{-/-} mice were hypersensitive to hyperthyroidism induced by T3 administration and thereby convincingly verify the notion that *Dio3* is crucial for protection from TH surplus during critical periods of time during which the HPT setpoint becomes fine-tuned.

A role for *Dio3* during brain development has already been suggested (Verhoelst et al., 2002). Especially during the massive sonic hedgehog(Shh)-dependent proliferation, *Dio3* activity is high in cerebellar granule cell precursors during their. Recently, it has been shown that *Dio3* is a Shh-dependent gene, positively regulated by *Gli2* in hair follicle development (Dentice et al., 2007). Interestingly, Shh also mediated the degradation of *Dio2* protein via expression of a E3-conjugating enzyme, WSB-1 (Dentice et al., 2005), thus acting directly on the balance of local T3 activation and inactivation. Taken together, a picture unfolds in which the differentiating activity of T3 can be locally suppressed in order to promote cell proliferation.

Importance of selenoprotein biology for TH feedback control

All three *Dio* isozymes are Se-containing proteins and need sufficient supply of Se in order to be synthesized. Therefore, during Se deficiency, selenoprotein biosynthesis decreases. In order to distribute scarce amounts of Se to the places where it is needed most, there exists a hierarchy among selenoproteins in which, e.g., *Dio1* ranks higher than *Gpx1* (Gross et al., 1995). Nevertheless, it has been observed early on that *Dio1* activity decreases in the liver of Se-deficient rodents and might impact directly on circulating T4 and T3 concentrations

(Arthur et al., 1990;Behne et al., 1990;Bates et al., 2000). Admittedly, the influence of Se status on the regulation of Dio1 expression and circulating TH is often masked and only becomes rate-limiting and obvious during states of severe Se deficiency or in pathological settings. It appears as if TH metabolism is largely independent from the Se status in a healthy individual. Thus it came as a complete surprise that it was in an endocrine unit where the first cases of inherited mutations in a gene crucial for selenoprotein biosynthesis, i.e. the Sec-insertion element sequence Binding Protein 2 (SBP2), were identified. In children or adolescent patients with slight growth defects and an unusual pattern of TH and TSH levels, hypomorphic mutations in their SBP2 genes were found (Dumitrescu et al., 2005). SBP2 is a binding protein for a hairpin structure within the 3'-UTR of selenoprotein mRNAs that is essential for selenoprotein translation (Hatfield *et al.*, 2002). Due to the clinical picture, it was hypothesised that the patients may carry mutations in genes encoding their Dio isozymes. By linkage analyses, however, these genes were excluded as were also other factors involved in selenoprotein biosynthesis. In suppression tests, the increased TSH serum levels were readily suppressed by T3 but not by T4, suggesting that hypophyseal Dio2 expression was impaired. Since for obvious reasons pituitary Dio2 activity cannot be tested directly, other selenoproteins from accessible sources were sampled. When selenoprotein-related parameters like serum Se concentration, serum selenoprotein P (SePP) and glutathione peroxidase 3 (GPx3) levels or GPx1 and Dio2 activities from cultured fibroblasts were assessed, a generalized impairment of selenoprotein biosynthesis was diagnosed in these patients. These findings proved that SBP2 was in fact a rate-limiting component for selenoprotein biosynthesis, and the identified mutations altered SBP2 activity during biosynthesis of certain selenoproteins as recently verified in a set of elegant in vitro studies (Bubenik and Driscoll, 2007). Moreover, it became apparent that in humans, the thyroid axis is exquisitely dependent on appropriate Dio expression and sensitive to impaired selenoprotein biosynthesis. Obviously, vitally important selenoenzymes like GPx-4 or thioredoxin reductases 1 and 2

were not similarly affected in these patients; otherwise they would not have survived until birth as seen in respective mouse models (Yant et al., 2003; Conrad et al., 2004; Jakupoglu et al., 2005).

It is tempting to speculate that the phenotype of patients with SBP2 mutations represents a combined Dio1 and Dio2 deficiency as suggested from the in vitro analyses (Bubenik *et al.*, 2007). These patients exhibit high rT3 levels as in Dio1^{-/-} mice and impaired conversion of T4 to T3 as well as impaired pituitary feedback regulation as in Dio2^{-/-} mice. However, it has not been reported whether SBP2 mutant patients have developmental defects in pituitary or cochlea or display an unusual temperature tolerance.

Thyroid hormone, deiodinases, and energy expenditure

TH are among the most potent stimuli of the basic metabolic rate. Whereas hyperthyroid patients (e.g. in Graves'-/ Basedow disease) as well as hyperthyroid animals show increased heart rate and body temperature, a decreased concentration of TH (e.g. in hypothyroidism, such as in Hashimoto's disease) is associated with reduced heart rates and reduced body temperature (Silva, 2006). It is an actively discussed question, whether the reduction of circulating TH levels in critically ill patients, that goes hand in hand with induction of Dio3 in many tissues, represents a medical emergency requiring hormone replacement therapy or whether this is a physiological adaptation ultimately reducing metabolic rate and conserving energy (Huang and Bianco, 2008).

Interestingly, Dio1- and Dio2-knockout mice showed surprisingly little influence on body weight or body composition (see above), although Dio2-deficient mice have clearly impaired adaptive thermogenesis and Dio1^{-/-} mice are slightly heavier than their wildtype littermates. Adaptive thermogenesis is mediated by uncoupling proteins (UCPs), which short-circuit the mitochondrial proton gradient. UCP1 is a major target of T3-action and has been suggested to be the central mediator of increased heat production in hyperthyroid animals. Surprisingly,

knockout mice devoid of UCP1 have been shown to be lean instead of obese as was initially expected (Liu et al., 2003). However, UCP1 knockout mice displayed an increased Dio2 activity potentially central to body weight reduction. These and other data suggested that targeting the thyroid hormone – Dio system may be a route to effectively control body weight (Aronne and Thornton-Jones, 2007). Accordingly, overexpression of UCP1 in transgenic mice increased energy expenditure and made the mice resistant to diet induced obesity (Kopecky et al., 1995). Another, classical example for a massively T3-regulated gene in liver is the mitochondrial glycerol phosphate dehydrogenase (mGPD), the key enzyme of the glycerol-phosphate shuttle. This shuttle transfers reducing equivalents from the cytosol into the mitochondrion by converting one cytosolic NADH molecule into one mitochondrial FADH₂ molecule. The difference in energy between these two molecules is used to transport the reduction equivalents against the concentration gradient into the mitochondrion and is finally dissipated as heat. Triggering the glycerol phosphate shuttle therefore results in a net reduction of respiratory efficiency, which ultimately increases heat production.

Major interest in this issue was renewed when diet-induced obesity was prevented – and even reversed – by dietary supplementation with bile acids (BA) (Watanabe et al., 2006). Remarkably, this effect appears not to be regulated via the nuclear receptor for BA, but rather via the G-protein coupled receptor TGR5. The authors showed that activation of TGR5 with chenodeoxycholic acid stimulated cAMP production and subsequently induced the cAMP-sensitive Dio2 gene in brown adipose tissue (BAT). Of note, the effect of BA is completely abrogated in Dio2^{-/-} animals, demonstrating that local T3 production is sufficient to resist diet-induced obesity. This finding is reminiscent of the impaired cold tolerance in Dio2^{-/-} mice, in which cAMP is increased via autonomic innervation of the BAT, but cannot be translated into heat production (De Jesus et al., 2001). In humans, other than in rodents, skeletal muscle is probably the major thermogenic tissue, although recent studies suggested that there is more BAT in adult humans than classically assumed (Cinti, 2006). Thus, this

pathway holds great potential for therapeutic intervention in cases of diet induced obesity, although much has still to be learned about the pharmacology of BA (Houten et al., 2006) and their cognate receptor molecules in man.

Apart from classical thermogenic tissues, the liver may be another site where enhancement of TH signalling may increase body energy expenditure. Specifically, targeting the TRb-specific agonist Mb07344 to the liver was sufficient to positively influence blood cholesterol and triglyceride concentrations (Erion et al., 2007). In addition to lowering blood cholesterol, another TRb agonist, i.e. KB2115, also stimulated bile acid synthesis (Berkenstam et al., 2008). Along these lines, a synthetic TRb-specific ligand, GC-1, is being developed as a potential anti-obesity drug (Grover et al., 2004; Villicev et al., 2007).

The role of rarely considered iodothyronine derivatives

Triiodothyronine is by far not the only highly active compound derived from iodinated thyroglobulin; Increased mitochondrial energy expenditure is also afforded by pharmacological application of 3,5-T₂, a naturally occurring metabolite of TH produced by Dio-dependent deiodination (Lanni et al., 2005). Again, there is a compound out of the big family of iodothyronines that can prevent diet-induced obesity. Unfortunately, impressing as the effects are, its mode of action is not completely understood and safety concerns regarding the use as a drug exist, although to date no adverse side effects have been observed.

Very recently, decarboxylated derivatives of iodothyronines, i.e. thyronamines (TAM, in particular T1-AM) have been identified as highly potent modulators of metabolic rate (Scanlan et al., 2004). T1-AM occurs in vivo at concentrations comparable with T₃ in blood and tissues, however, its site of biosynthesis is not known. TAM are likely biosynthetic derivatives of iodothyronines that sustained sequential deiodination and decarboxylation (Piehl et al., 2008). In general, the effects of TAM are antagonizing T₃ actions, i.e. they

lower metabolic rate and body temperature. Interestingly, T1-AM can profoundly change the balance between glucose and lipid utilisation (Brulke et al., 2007). Moreover, T1-AM has a negative inotropic and a negative chronotropic effect on the heart (Chiellini et al., 2007). TAM may signal via G-protein-coupled trace amine associated receptors (Regard et al., 2007). Recently, it has been suggested that TAM modulate monoamine transmitter metabolism in brain (Snead et al., 2007).

Perspectives

Several years ago, it may have seemed obvious to most researchers and clinicians that the HPT axis is largely understood and targeted pharmacological manipulation of this axis in congenital hypothyroidism or other thyroid-related disorders has become clinical routine. Then, with the stringent application of gene targeting methodology to the Dio gene family, the unequivocal demonstration of specific transporters for TH across cell membranes, and the surprising identification of other endogenously circulating biologically active TH derivatives, results were obtained that challenged many facets of the already widely accepted picture. Human genetics provided even more clues and additional molecules with unexpected properties now need to be incorporated into a new model. Perspicuous dependency on regular selenoprotein biosynthesis was finally documented during the analyses of thyroid hormone patients. Besides, less expected intensive cross talk to fat and cholesterol metabolism became obvious when BA were identified as key regulators of Dio2 expression in thermogenic BAT. At present, we are unable to provide a novel, consistent model that already incorporates all of these findings. Still, we need to realize that TH metabolism and signalling is far from understood in its full complexity. It appears to us as if we were currently witnessing tremendous progress in our understanding of this all-too familiar paradigmatic feedback axis. Now, pharmacological manipulation of elements of the HPT axis is becoming more

fashionable, even in formerly remote areas of internal medicine like obesity and diabetes that traditionally seemed at a safe distance to classical thyroidologists.

Figure 1: (left) A simplified textbook model of the HPT axis illustrates the hierarchical regulation of the thyroid gland depending on hypothalamic and hypophyseal control signals. The liver is thought to contribute significantly to the circulating T3 levels by Dio1-dependent activation of T4. (right) In the revised model, the role of local T3 production by the thyroid gland – as opposed to hepatic T3 release - becomes emphasised. In addition, deiodinase (Dio) activities in the hypothalamus and in the pituitary are required to fine-tune the biological responses of these tissues. Thyroid hormone target tissues may either depend completely on circulating T3 or are able to increase intracellular T3 levels by local Dio2 expression. During development or under (patho)physiological conditions, intracellular TH levels may become decisively reduced by induction of local Dio3 expression.

Figure 2: Novel compounds involved in metabolic regulation related to thyroid hormones. While direct derivatives of T4 are potent physiological modulators of many metabolic processes, synthetic TR agonists are being developed in order to minimize side-effects. Bile acids are another class of natural compounds acting on the thyroid hormone axis, but their pharmacological potential is still under investigation.

Acknowledgements: Research in our groups was supported by grants from the Deutsche Forschungsgemeinschaft (Scho 849/2-1; We 2458/3-2) and SFB665, Deutsche Krebshilfe (10-1792 Schol), EnForCé, and EFRE TSB Zukunftsfonds Berlin (#10182000).

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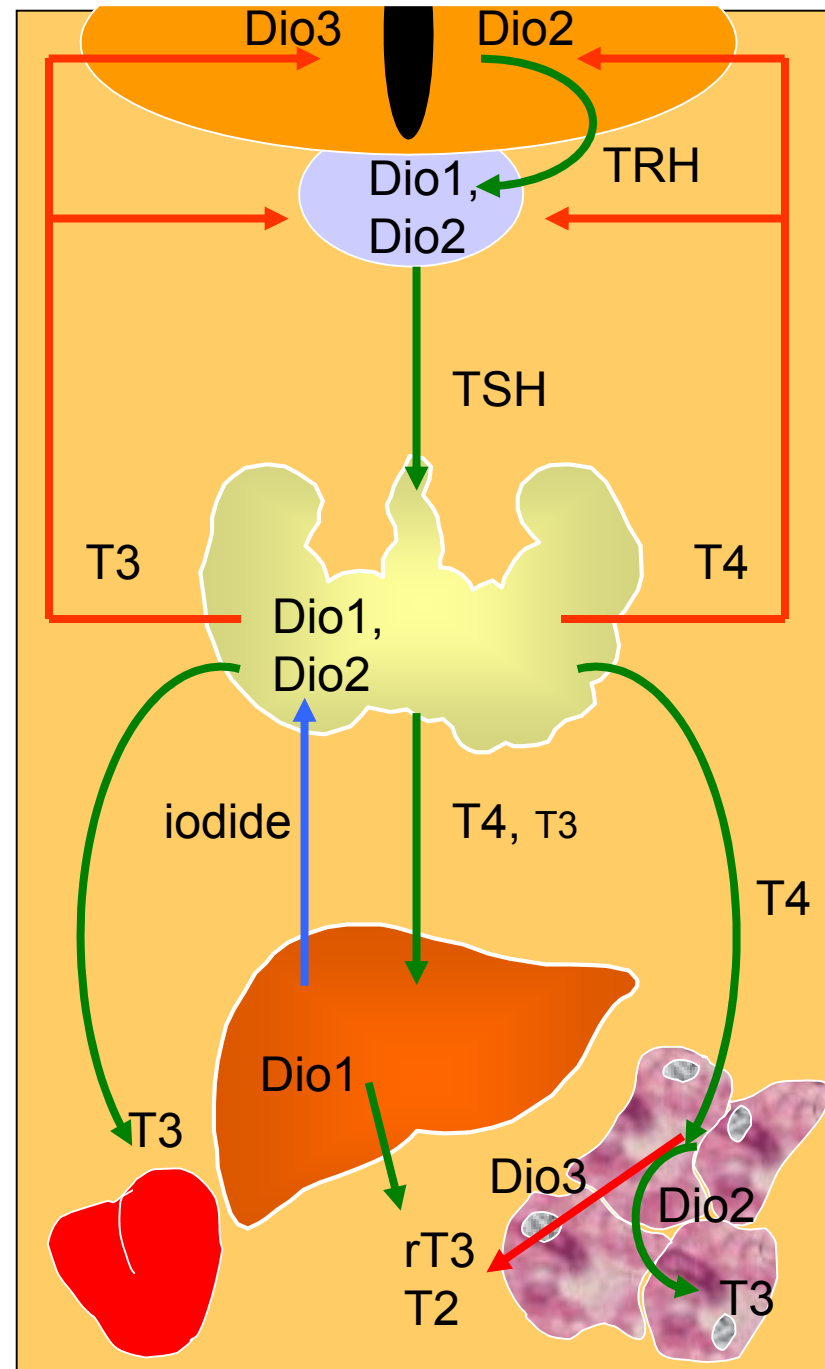
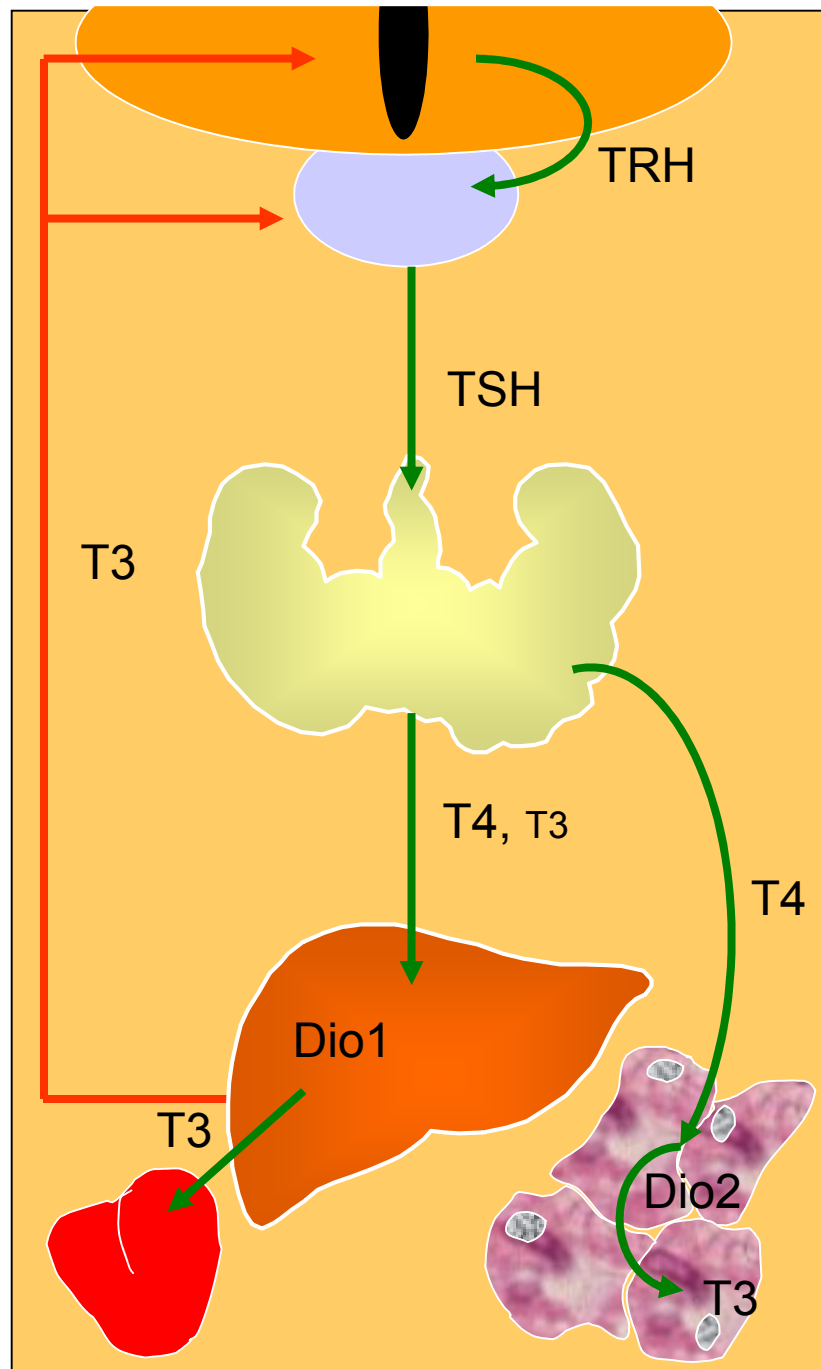
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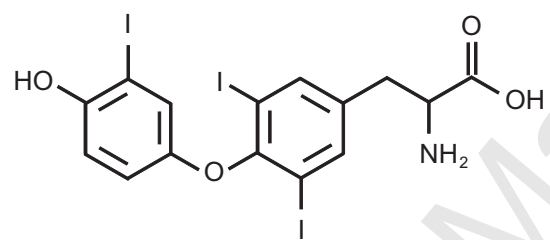
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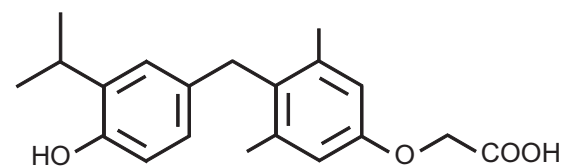
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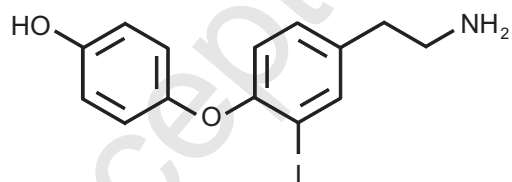




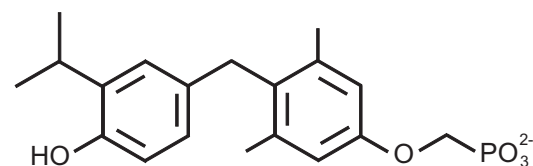
triiodothyronine (T3)



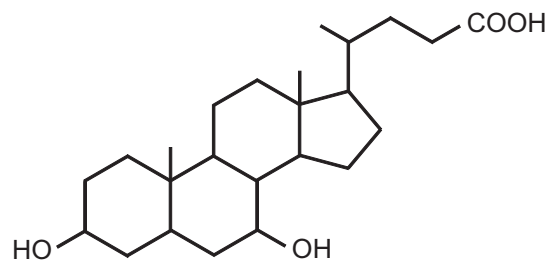
GC-1



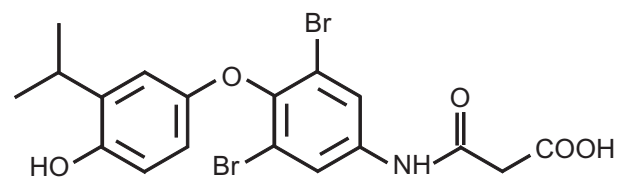
3-iodothyronamine (T1AM)



MB07344



chenodeoxycholic acid



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