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Dissecting the role of regulators of thyroid hormone availability in early brain development: Merits and potential of the chicken embryo model

Pieter Vancamp, Veerle M. Darras^{*}^a KU Leuven, Laboratory of Comparative Endocrinology, Department of Biology, B-3000, Leuven, Belgium

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ABSTRACT

Thyroid hormones (THs) are important mediators of vertebrate central nervous system (CNS) development, thereby regulating the expression of a wide variety of genes by binding to nuclear TH receptors. TH transporters and deiodinases are both needed to ensure appropriate intracellular TH availability, but the precise function of each of these regulators and their coaction during brain development is only partially understood. Rodent knockout models already provided some crucial insights, but their *in utero* development severely hampers research regarding the role of TH regulators during early embryonic stages. The establishment of novel gain- and loss-of-function techniques has boosted the position of externally developing non-mammalian vertebrates as research models in developmental endocrinology. Here, we elaborate on the chicken as a model organism to elucidate the function of TH regulators during embryonic CNS development. The fast-developing, relatively big and accessible embryo allows easy experimental manipulation, especially at early stages of brain development. Recent data on the characterisation and spatiotemporal expression pattern of different TH regulators in embryonic chicken CNS have provided the necessary background to dissect the function of each of them in more detail. We highlight some recent advances and important strategies to investigate the role of TH transporters and deiodinases in various CNS structures like the brain barriers, the cerebellum, the retina and the hypothalamus. Exploiting the advantages of this non-classical model can greatly contribute to complete our understanding of the regulation of TH bioavailability throughout embryonic CNS development.

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1. Introduction

Thyroid hormones (THs) affect a wide variety of processes in vertebrate development, the most prominent being the development and maturation of the central nervous system (CNS) (Darras et al., 2015; Williams, 2008; Bernal et al., 2015). A severe lack of THs during human prenatal development causes irreversible brain damage, leading to pronounced mental retardation in the progeny. Moreover, subclinical hypothyroidism has also been linked to an increased risk of adverse neurological outcomes, such as attention-deficit/hyperactivity disorder and autism spectrum disorder (Morreale de Escobar and Obregon, 2004; Modesto et al., 2015). Experimental data from hypothyroid animals including chicken

suggest that THs are necessary throughout the entire period of neurodevelopment to coordinate processes like cell proliferation, migration, differentiation, myelination and synaptogenesis (Mohan et al., 2012; Verhoelst et al., 2004; Thompson and Cline, 2016). Indeed, the variation in neurological deficits depending on the exact period of TH deficiency indicates that specific TH actions occur in specific developmental time windows. Moreover, declined visual processing and motor skill function following an insufficient supply of maternal THs during the first trimester of gestation, highlights the fact that THs are already needed for early neurodevelopmental events (Zoeller and Rovet, 2004; Pop et al., 1999). Accordingly, TH and its receptor have been found to coordinate chicken neurulation (Flamant and Samarut, 1998), while in mice THs have just recently been shown to be required for neural crest cell migration (Bronchain et al., 2016). Direct evidence for TH action during early stages of CNS development however remains scarce, even though all vertebrate embryos have access to THs long before the maturation of their own hypothalamus-pituitary-thyroid (HPT)

^{*} Corresponding author. Laboratory of Comparative Endocrinology, Naamsestraat 61, P.O. Box 2464, B-3000, Leuven, Belgium.

E-mail address: Veerle.Darras@bio.kuleuven.be (V.M. Darras).

axis (Morreale de Escobar and Obregon, 2004; Prati et al., 1992; Walpita et al., 2007). *Ex utero* developing non-mammalian vertebrates such as chicken can therefore serve as valuable research tools, complementing their mammalian counterparts (Préau et al., 2015).

To exert its function, the most bioactive TH 3,5,3'-triiodothyronine (T_3) needs to bind to nuclear TH receptors (TRs), ultimately guiding TH-dependent expression of hundreds of genes (Chatonnet et al., 2015; Harvey and Williams, 2002). In vertebrates, all TR isoforms are encoded by two well-conserved genes, *THRA* and *THRB*. TR α 1 is the most abundantly expressed isoform in all vertebrate embryonic brains, while the TR β isoforms are more confined to specific areas and cell types such as developing photoreceptors in the retina and some hypothalamic neurons (Darras et al., 2011; Bernal, 2007). Transmembrane transporters facilitate cell-specific in- and efflux of THs (Visser et al., 2008). These transporters can be classified into three main families, namely the monocarboxylate transporters (MCTs), the organic anion polypeptide transporters (OATPs) and the large neutral amino acid transporters (LATs). Within these families, MCT8 is the only TH-specific transporter, while MCT10, OATP1C1, LAT1 and LAT2 also transport other substrates such as amino acids and are thereby designated as secondary TH transporters (Visser et al., 2007; Fu et al., 2013; Schweizer and Kohrle, 2013). Once inside the cell, specific seleno-enzymes called deiodinases catalyse the deiodination of THs. In the brain, mainly type II (DIO2) ensures activation of 3,5,3',5'-tetraiodothyronine (T_4) into T_3 by outer ring deiodination, while type III (DIO3) inactivates T_3 and T_4 into 3,5-diiodothyronine (T_2) and reverse T_3 respectively by inner ring deiodination. Type I deiodinase (DIO1) can activate as well as inactivate THs, but has a rather low affinity (Bianco and Kim, 2006), and with the exception of late embryonic cerebellum, no DIO1 activity has been found in the chicken nor the rodent or human prenatal brain (Barez-Lopez et al., 2014; Bates et al., 1999; Van Herck et al., 2012). The concerted expression of TH transporters in combination with the DIO2/DIO3 ratio defines the intracellular T_3 availability, which impacts the cellular gene expression profile (Chatonnet et al., 2015; Schweizer and Kohrle, 2013). As TH-regulated gene expression changes during embryonic development, the dynamic spatiotemporal expression pattern of TH regulators paves the way for a strictly orchestrated development of substructures in the brain.

2. The chicken embryo as a developmental model

As neurological deficits may already arise during earlier stages, a focus shift towards the more early stages of embryonic development is needed to understand the mechanisms regulating TH availability. With regard to this special issue on non-classical vertebrate models, we elaborate on the chicken which, as a matter of fact, has been the model of choice for decades to unravel the fundamentals of vertebrate development (for an overview, see (Davey and Tickle, 2007)). The introduction of the genomics era in the 1990s reconsolidated the chicken as a leading model in the discovery of the molecular basis of fate mapping in the developing brain (Simeone et al., 1992; Rubenstein et al., 1994; Figdor and Stern, 1993).

The chicken embryo develops quite rapidly, only taking 21 days from fertilisation till hatching (Stern, 2005). The developmental stages from the first day of incubation (embryonic day 0 or E0) till E20 have been described in detail by Hamburger and Hamilton (1951). The *in ovo* development makes the embryo easily accessible for manipulation at any developmental stage (Murphy and Clark, 1990; De Groef et al., 2008) and since the stock of maternal THs is deposited in the egg yolk before fertilisation, it is possible to interfere with TH metabolism in the absence of maternal

compensatory mechanisms (Jenkins and Porter, 2004; Wilson and McNabb, 1997). In addition, the chicken has a functional HPT axis around mid-embryogenesis, similar to the human foetus around midgestation (Thommès, 1987; Van Herck et al., 2013a), while this occurs much later in rodents (De Felice and Di Lauro, 2004). Finally, the relatively large brain size allows easy discrimination and targeting of various brain regions, especially for studying early stages of development (Van Herck et al., 2013a; Bennett and Harvey, 1985).

3. Methods to study the local function of TH regulators in the chicken embryo

As in all vertebrates, a correct TH supply is essential for proper chicken embryonic brain development. Using mostly quantitative PCR and *in situ* hybridisation, a detailed description of the spatiotemporal expression patterns of TH transporters and deiodinases has been described in most structures of the developing chicken embryonic CNS over the past years (Delbaere et al., 2015; Van Herck et al., 2015; Bourgeois et al., 2016; Geysens et al., 2012; Gereben et al., 2004). The cloning of chicken DIO1, DIO2 and DIO3 revealed a highly conserved protein structure and function compared to mammalian orthologues (Van der Geyten et al., 1997; Gereben et al., 1999). Furthermore, chicken OATP1C1 was characterised as a TH transporter in 2006 (Nakao et al., 2006), while our group more recently cloned MCT8, MCT10 and LAT1 and showed they all facilitate TH transport in a similar fashion as their mammalian orthologues. One notable difference with mammals as well as other vertebrates is that LAT2 seems to be missing in all avian genomes studied so far (Bourgeois et al., 2016).

The above information can be used to study local TH regulator function during chicken embryonic CNS development. Studies using thyroid-disrupting agents like methimazole (MMI) and amiodarone create an overall hypothyroid state and have contributed to determine the overall responsiveness of TH regulator expression/activity, and changes in serum and brain TH levels. The last decade, a number of innovative techniques have become available (Stern, 2005) that allow a more detailed analysis of TH regulator function. In particular for embryonic development, novel gain- and loss-of-function techniques allow to transiently silence or overexpress genes (for an overview, see (Sauka-Spengler and Barembaum, 2008)). Among the plethora of tools available, one favoured method is the transfection of cells with a short hairpin (sh)RNA-expressing RNAi construct, resulting in transient mRNA degradation or inhibition of mRNA translation. *In vivo* electroporation is thereby often used to deliver the construct into neural precursor cells of a target brain region, resulting in gene silencing in a restricted time- and tissue-specific way (Nakamura et al., 2004; Das et al., 2006; Baeriswyl et al., 2008; Rao et al., 2004). Alternatively, retroviral-mediated gene transfer using replication competent avian sarcoma (RCAS) viral vectors can be used for more prolonged and dispersed transgene expression (Das et al., 2006; Hughes, 2004). Below, we will discuss how some of these novel techniques can be used to investigate the contribution of TH regulators and their interaction during chicken embryonic development. More specifically, we will elaborate on four well-studied CNS regions namely the brain barriers, the cerebellum, the retina and the hypothalamus.

4. The concerted action of TH transporters and deiodinases at the embryonic brain barriers

In order to reach target cells within the CNS, THs first need to be transported across the blood-brain barrier (BBB) and/or the blood-cerebrospinal fluid barrier (BCSFB). The dynamic, regional

expression of TH transporters facilitates local TH uptake and reflects the varying need for TH amongst brain regions (Bernal et al., 2015). In the chicken embryonic brain, LAT1 is presently the only TH transporter found to be strongly expressed in the endothelial cells (ECs) of the BBB. In addition, mRNA expression of *DIO2* strongly overlaps with LAT1 expression (Fig. 1) (Van Herck et al., 2015; Bourgeois et al., 2016), while it is not found in astrocytes which start to differentiate between E11 and E15 (Gereben et al., 2004). Consequently, THs would be predominantly transported across the BBB by LAT1 and T_4 can be activated by *DIO2* within ECs themselves, producing active T_3 that directly enters brain tissue (Van Herck et al., 2015; Verhoelst et al., 2011). In the posthatch chicken, *DIO2* expression is markedly decreased, but remains primarily associated with ECs (Gereben et al., 2004).

The above mechanism differs from that in peri- and postnatal rodents, where MCT8 and OATP1C1 are the main TH transporters expressed at the BBB (Roberts et al., 2008; Sugiyama et al., 2003), and *DIO2* seems to be primarily localised in astrocytes (Guadano-Ferraz et al., 1997). In rodents, THs are thus most likely transported by MCT8 and/or OATP1C1 across the BBB, and T_4 can be subsequently activated by astrocyte *DIO2*, whereafter T_3 can be transported to neurons or exert a function in the astrocytes themselves. It remains to be investigated whether *DIO2* expression in the rodent embryonic brain is more similar to the pattern observed in chicken or not. So far, the data suggest some differences exist in the TH uptake and activation pathway among vertebrates, impeding generalisation of TH regulatory mechanisms deduced from a single species. The chicken can therefore serve as a complementary model to unravel the function of TH transport and activation at the level of the BBB.

For instance, understanding the role of *DIO2* could be very interesting as genetic polymorphisms in the human *DIO2* gene have been correlated with increased risks for clinical conditions such as bipolar mood disorder and mental retardation, and demand more research to elucidate the potential role as therapeutic target in a clinical setting (Verloop et al., 2014). The strong *DIO2* expression in the vertebrate embryonic brain from very early stages supports the idea it could already serve an important function in neurodevelopment. Importantly, while MMI-treated hypothyroid hens exhibited a strongly increased compensatory *DIO2* activity in brain, this was not the case in their offspring at an early embryonic stage (E6) despite low brain T_3 content (Van Herck et al., 2013b). In another study, injection of a mixture of T_4 and T_3 into the yolk of chicken embryos 24 h prior to sampling decreased brain *DIO2* activity at E8, but again a response was absent at E4 (Van Herck et al., 2012). These observations emphasize the vulnerability of the early embryonic brain to TH alterations.

One possibility to unravel the cellular function of *DIO2* would be to transduce developing ECs of the chicken BBB with an RCAS vector that introduces shRNAs, i.e. RCAS-RNAi (Das et al., 2006), to silence its expression. The early-generated and easy accessible chorioallantoic circulation (van Golde et al., 1996) allows efficient

transduction of the ECs following intravenous injection of viral particles (Bourgeois et al., unpublished results). Furthermore, a combined knockdown of *DIO2* and LAT1 could be performed to get further insight in the interaction of both TH regulators at the BBB. This could be accomplished by using two viral RNAi vectors with different envelopes or by cloning both micro RNA sequences into one vector, keeping in mind the impossibility of superinfection (Das et al., 2006; Vergara and Canto-Soler, 2012). Other non-classical models like zebrafish can also aid in unravelling the function of these TH regulators. Recently a *Dio2*-deficient zebrafish line was generated which displays a clear phenotype, and has down-regulated expression of several genes coding for regulators of TH action (*dio3*, *thra* and *thrb*) in brain tissue (Houbrechts et al., 2016a).

To date, there remains a controversy about the importance of the BCSFB relative to the BBB. The BCSFB, located in the choroid plexus and formed by tight junctions of epithelial cells, could be an underestimated and/or understudied alternative gateway for distribution of endocrine substances throughout the brain (Banks, 2012), especially in early development, when blood vessels start to protrude the brain and the BBB has not yet functionally matured (Ek et al., 2012). Indeed, T_3 and T_4 can be detected in the chicken brain from the earliest included stage E4 (Prati et al., 1992), as well as TH transporters and deiodinases (Van Herck et al., 2012). Not surprisingly, strong mRNA expression of *MCT8*, *MCT10*, *OATP1C1*, *LAT1* and *DIO3* at the BCSFB was observed from E6 onwards using *in situ* hybridisation (Van Herck et al., 2015). *MCT8* and *OATP1C1* are also expressed in the human and rodent prenatal choroid plexus (Roberts et al., 2008; Grijota-Martinez et al., 2011), making it reasonable to assume that at least part of the TH provision in brain is facilitated via this barrier system. Moreover, the predominant TH distributor protein transthyretin, which is highly expressed in the choroid plexus in both mammals and birds, could serve as a way to fuel the brain with THs since it is maximally expressed just prior to the period of extensive brain growth (Chang et al., 1999; Richardson et al., 2015). As the BBB functionally matures and strongly increases its contact surface with target cells, the BCSFB probably becomes subordinate in its role of TH supply to the brain (Abbott et al., 2010; Saunders et al., 2013). Transfection or transduction of the choroid plexus can be performed by electroporation or intraventricular/intravenous injection of viral vectors, as was recently done in mice (Iwayama et al., 2016; Gibson et al., 2010). This technique could easily be adopted to the chicken embryo.

5. Unravelling the function of TH regulators in the developing cerebellum

The cerebellum is probably the most intensively studied TH-sensitive brain structure in vertebrates (Koibuchi, 2013; Faustino and Ortiga-Carvalho, 2014). Induction of hypothyroidism in neonatal rats led to a significant reduction in cerebellar weight, delayed cell proliferation and migration, retarded cell differentiation and reduced cell numbers (Nicholson and Altman, 1972a,

Fig. 1. Detection of LAT1 and *DIO2* at the chicken embryonic BBB at E18 via immunolabeling and *in situ* hybridisation, respectively. The LAT1 transporter protein is found at both the luminal and abluminal membrane of endothelial cells, which also express *DIO2* mRNA.

1972b, 1972c). Similarly, cell migration was delayed in the cerebellum of MMI-treated chicken embryos (Verhoelst et al., 2004), and especially early Purkinje cell differentiation was shown to be highly susceptible to hypothyroidism (Bouvet et al., 1987). The expression of various TH regulator genes throughout the cerebellar anlage as early as E4 (Van Herck et al., 2012; Delbaere et al., 2016a) further indicates the necessity of a strictly controlled TH availability starting from early development.

As such, a noticeable observation in the embryonic chicken cerebellum is the strong *MCT8* mRNA expression in differentiating Purkinje cells (PCs), the sole output neuron of the cerebellum, during the first two weeks of development. This is followed by a spatial shift towards the dendritic tree in what are probably the spots of synaptogenesis during the week prior to hatching (Delbaere et al., 2016a). Elucidating the cellular function of *MCT8* has been the subject of numerous studies, as inactivating mutations in the human *SLC16A2* gene coding for *MCT8* have been linked to a syndrome of severe psychomotor retardation, called the Allan-Herndon-Dudley Syndrome (AHDS) (Schwartz et al., 2005; Dumitrescu et al., 2004; Friesema et al., 2004). The pronounced locomotor phenotype may be linked to defects in the extrapyramidal system (Tonduti et al., 2013; Fuchs et al., 2009; Kakinuma et al., 2005) as well as abnormal cerebellar structure and impaired biochemical PC differentiation (Lopez-Espindola et al., 2014).

Mct8 knockout and *Mct8/Oatp1c1* double knockout mice have already proven the essential role of these transporters for TH uptake at the level of the BBB (Trajkovic et al., 2007; Wirth et al., 2014; Dumitrescu et al., 2006; Mayerl et al., 2014), but it is also important to assess the need for *MCT8* at the level of the neurons themselves. Our research group therefore recently set up a strategy to investigate the role of *MCT8* in PCs in chicken, and investigated how local *MCT8* knockdown could affect cerebellar development. Because PC precursors are born in the ventricular zone around E3 (Luo and Redies, 2004), targeted electroporation with an *MCT8*-RNAi construct at this stage allowed to efficiently knock down *MCT8* in this cell type. This resulted in reduced PC differentiation and expression of Retinoid-related orphan receptor alpha (*RORα*) in the first week of development, and markedly impaired PC dendritic arborisation at later embryonic stages (Delbaere et al., 2016b). Hence, PC precursor cells rely on *MCT8* for intracellular T_3 uptake, which is in line with chicken PCs showing T_3 dependency for normal dendritic arborisation (Bouvet et al., 1987), and *MCT8*- and hence T_3 deficiency mimics the situation of mice expressing a dominant negative form of *TRα1* in PCs (Fauquier et al., 2014). Furthermore, *MCT8* deficiency in PCs disrupted granule cell proliferation and migration, most likely because of dysregulated intercellular PC-driven cross-talk (Delbaere et al., 2016b). This study clearly illustrates how deficiency of a TH regulator in one cell type can affect autonomous as well as non-autonomous cell processes.

Simply changing the stage of electroporation and adapting the position of the electrodes would allow targeting other cell types such as the granule cells (Funahashi and Nakamura, 2008). As *DIO2* (Verhoelst et al., 2005), *TRα1* and *TRβ2* mRNA expression (Forrest et al., 1991) is present throughout the embryonic cerebellum, and *DIO3* is localised in PCs (Verhoelst et al., 2002), silencing of these other TH regulator genes could aid in unravelling their function and interaction at the cell- and tissue-specific level.

6. Unravelling the function of TH regulators during retinal development

The retina is a layered photosensitive structure consisting of seven basic neural cell types with strongly conserved morphology and function (Bassett and Wallace, 2012). Its unique peripheral and

thus easily accessible location makes it an attractive model to study the role of THs in neurodevelopment, as it actually originates as an extension of the developing diencephalon (Harpavat and Cepko, 2003). The functional involvement of THs in retinal development has first been established in amphibians (Hoskins, 1990), followed by chicken (Sjoberg et al., 1992), mammals (Sevilla-Romero et al., 2002) and more recently fish (Allison et al., 2006; Reider and Connaughton, 2014).

Our research group has documented the mRNA expression patterns of *MCT8*, *LAT1*, *DIO2* and *DIO3* during the embryonic development of the chicken retina, starting from E4. The expression of these TH transporters and deiodinases varies in a temporal manner and gets rapidly confined to specific cell layers (Bourgeois et al., 2016; Geysens et al., 2012). In addition, Trimarchi and colleagues demonstrated that the mRNA expression of *DIO2*, *DIO3* and a number of TH-responsive genes coincide during three developmental waves (Allison et al., 2006; Reider and Connaughton, 2014). In relation to the TRs, it has been shown earlier that *THRA* is universally expressed throughout the developing chicken retina, while *THRB* is most likely only expressed in the photoreceptor layer (Sjoberg et al., 1992; Trimarchi et al., 2008). These findings correlate with evidence that THs stimulate cone photoreceptor development in the avian retina through *TRβ*-mediated TH action (Fischer et al., 2011). The dynamic expression patterns imply that TH availability and action needs to be strictly regulated in the developing retina, starting at the most early stages.

Nevertheless, only the deiodinases and *TRβ* have been shown to possess a specific function in the developing vertebrate retina (Ng et al., 2001, 2010; Houbrechts et al., 2016b; Galton et al., 2009), and no reports exist on the effect of TH transporter deficiency so far. In addition to their relatively large brain size, chicken embryos have large and easily accessible eyes compared to mammals (Vergara and Canto-Soler, 2012), advantages that can be exploited to further investigate the role of TH transporters and deiodinases in retinal development. Via injection of a GFP-expressing construct into the subretinal space followed by electroporation into the retinal neuro-epithelium, Doh et al. optimized a method to target the chicken retina as early as E3 (Doh et al., 2010; Islam et al., 2012). The RCAS-RNAi system was also used as an efficient loss-of-function method for *DIO2*, but the authors unfortunately did not further study the phenotypic effects (Harpavat and Cepko, 2006). We have recently successfully knocked down *MCT8* in the early developing retina (Fig. 2) (Vancamp and Darras, 2016), and already found evidence for cell proliferation and migration defects (Vancamp & Bourgeois, unpublished results).

7. The interaction of TH regulators to fine-tune the hypothalamic HPT set point

The exceptionally high expression of TH regulators in the hypothalamic region from early stages onwards emphasizes the need to precisely control TH availability for its development and function (for an extensive review, see (Fekete and Lechan, 2014)). However, as for the other regions mentioned above, their exact impact on prenatal development is only partly understood. This could be very important, as it is believed that embryonic TH signalling fine-tunes the adult set point of the HPT axis by affecting hypothalamic neuroanatomical development (Alkemade, 2015; Friesema et al., 2012).

The general model states that tanycytes, a special type of ependymal cells lining the third ventricle (Rodriguez et al., 2005), take up THs primarily via *MCT8* and to a lesser extent *OATP1C1*, while *DIO2* co-expression facilitates conversion of T_4 into T_3 . Active T_3 is then transported to thyrotropin-releasing hormone (TRH)-producing neurons in the paraventricular nucleus (PVN), where it

Fig. 2. MCT8 knockdown following electroporation of an MCT8-RNAi vector in the early chicken embryonic retina. First, the RNAi construct which also expresses RFP is injected into the subretinal space at E4 using a glass capillary connected to a pressure micro-injector, after which it is electroporated into the retinal neuro-epithelium. One day later, transfected, RFP-expressing cells can be detected on coronal sections of the retina. Detailed pictures show the absence of *MCT8* mRNA (black arrows) in RFP-expressing areas (white arrows), confirming efficient *MCT8* knockdown. RPE: retinal pigment epithelium. Image of the chicken embryo modified from (Bellairs, Osmond).

stimulates or inhibits TRH release by binding to TR α 1 or TR β 2, respectively (Fekete and Lechan, 2014).

In chicken, *MCT8* and *OATP1C1* are expressed in tanycytes of the mediobasal hypothalamus starting at E14 (Gereben et al., 2004; Mohacsik et al., 2016), and an increase in *DIO2* activity towards the end of embryonic development has been linked to the onset of T₃-dependent negative feedback signalling (Mohacsik et al., 2016). However, dynamic expression of *MCT8* and *OATP1C1* as well as *DIO2* is already observed in the hypothalamic region during the first half of embryonic development (Geysens et al., 2012), long before the maturation of the HPT axis, and could therefore function to control local intracellular T₃ availability during early hypothalamic neuro-development. As the tanycytes line the third ventricle, injection of an RNAi vector into the ventricular space followed by electroporation allows easy targeting of these cells in chicken, as was already done at very early stages (Das et al., 2006). Therefore, by interfering with a TH regulator in tanycytes in early development, one could gain additional insight in their complex interplay and unravel some of the underlying mechanisms that determine the set point of HPT activity during adult life.

8. Conclusion and future perspectives

Increasing evidence for the significance of correct regulation of TH availability from the earliest stages of development, as well as important interspecies differences, have forced scientists to introduce multiple vertebrate models to unravel the mechanisms regulating TH availability in the developing CNS. The recent advances in genomics have provided a plethora of tools to

conditionally overexpress or silence genes in a tissue- and time-restricted manner. The easily accessible and large chicken embryo has thereby been rediscovered as an outstanding developmental model. Our recent understanding of the spatiotemporal expression pattern of TH regulators allows to specifically target a developing brain region or cell type in order to elucidate their local function and interaction. For the purpose of this review, we highlighted some regions of interest such as the brain barriers, the cerebellum, the retina and the hypothalamus as convenient models to study. Over the next years, we should be able to deduce fundamental aspects of TH regulator function by systematically targeting these regions, especially during early developmental stages when the brain is extremely vulnerable for alterations in TH supply as observed in maternal hypothyroidism and in AHDS patients. Furthermore, the chicken embryo may provide additional answers regarding the rising concern on harmful effects of thyroid-disrupting pollutants during prenatal brain development. Chicken embryos are one of the established models for testing developmental toxicity and several organic pollutants have already been shown to interact with thyroid function (e.g. Beck et al., 2006; Pretorius et al., 2006; Darras, 2008). The increased knowledge on early developmental stages and the methodologies available today allow to determine with more precision at which level they interfere with the regulation of TH availability and action. Hence, exploiting the strengths of the chicken and other vertebrate models in thyroid research can complement results obtained in mammals. This paradigm shift to a more Evo-Devo approach will be inevitable to reveal fundamental aspects of TH regulator action, as well as their contribution to various pathological settings.

Declaration of interest

The authors have nothing to disclose.

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