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Research paper

# The affinity of transthyretin for $T_3$ or $T_4$ does not determine which form of the hormone accumulates in the choroid plexus

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ABSTRACT

Normal development of the brain is dependent on the required amounts of thyroid hormones (THs) reaching specific regions of the brain during each stage of ontogeny. Many proteins are involved with regulation of TH bioavailability in the brain: the TH distributor protein transthyretin (TTR), TH transmembrane transporters (e.g. MCT8, MCT10, LAT1, OATP1C1) and deiodinases (D1, D2 and D3) which either activate or inactivate THs. Previous studies revealed that in mammals, $T_4$, but not $T_3$, accumulated in the choroid plexus and then entered the cerebrospinal fluid. In all mammalian species studied so far, TTR binds $T_4$ with higher affinity than $T_3$, whereas TTR in non-mammalian vertebrates binds $T_3$ with higher affinity than $T_4$. We investigated if the form of TH preferentially bound by TTR influenced the form of the TH that accumulated in the choroid plexus and consequently other areas of the brain. We measured the mRNA levels corresponding to TTR, MCT8, MCT10, LAT1, OATP1C1, D1, D2 and D3 in the brains of chickens at 11 days post-hatching. TTR, D3 and OATP1C1 expression were found to be highly concentrated in the choroid plexus. D1, MCT8 and MCT10 mRNA levels were slightly greater in the choroid plexus than in other areas of the brain while D2 mRNA levels were lower. LAT1 mRNA was evenly expressed throughout the brain. Therefore, the choroid plexus appears to be a structure which exhibits sophisticated control of TH levels within the brain. We also measured the uptake of intravenously injected $^{125}$I-$T_3$ and $^{125}$I-$T_4$ into brains of chickens of the same age. $^{125}$I-$T_4$ but not $^{125}$I-$T_3$ accumulated in the choroid plexus and optic lobes. Therefore, the form of TH preferentially bound by TTR does not determine the form of TH that accumulates in the choroid plexus and other areas of the brain. As for mammals, $T_3$ present in the avian brain therefore seems mainly produced locally by conversion of $T_4$ into $T_3$ by D2.



## 1. Introduction

Thyroid hormones (THs) are signal compounds that regulate transcription of specific genes, as well as having some non-genomic effects. Many of the genes regulated by THs are involved in growth and development of vertebrates, and the most spectacular example of this is amphibian metamorphosis. However, as THs are lipophilic and partition between the aqueous and lipid phases with a ratio of 1:20,000 (Dickson et al., 1987), specific thyroid hormone distributor proteins (THDPs) are required in the blood and cerebrospinal fluid (CSF) to ensure appropriate distribution of the hormones from the site of synthesis (the thyroid gland) to the sites of action: cells throughout the body and brain (Mendel et al., 1987). In humans and many other mammals, the THDPs circulating in the blood are albumin, transthyretin (TTR) and thyroxine-binding globulin (TBG), whereas in birds the THDPs are albumin and TTR (Richardson et al., 1994) (for a review of the distribution of THDPs in various groups of vertebrates, see (Richardson, 2007)). Albumin has the lowest affinity and greatest capacity for binding THs, whereas TBG has highest affinity and lowest capacity for binding THs. TTR has intermediate affinity and capacity for TH binding, but the largest distribution volume, and is responsible for release of most THs in peripheral capillaries (see (Richardson, 2007)). There are two main forms of THs: 5′,3′,5,3-tetraiodo-l-thyr onine (thyroxine, $T_4$) and 3′,5,3-triiodo-l-thyronine, ($T_3$). $T_4$ is the predominant form found in blood and $T_3$ is the form with higher affinity for the TH nuclear receptors. In mammals, TTR has a higher

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affinity for $T_4$ than for $T_3$ (Chang et al., 1999). However, among vertebrates, this situation appears to be the exception, as in fish (Santos and Power, 1999), amphibians (Yamauchi et al., 1993), reptiles and birds (Chang et al., 1999), TTR has a higher affinity for $T_3$ than it does for $T_4$.

Precise amounts of THs are required in very specific temporal and spatial patterns for normal brain development to occur. Both deficiencies and excessive amounts of TH during development of the central nervous system (CNS) results in malformations and disease states (Braverman and Utiger, 2000a,b). It is critical that the required amounts of THs enter the brain at the requisite times during development. Furthermore, the correct amounts of THs are required by specific areas of the mature brain to maintain normal cerebral functioning during adulthood (Braverman and Utiger, 2000a,b). Thus, once the circulating THs have been carried to the brain barriers by the THDPs, they must cross the blood-brain barriers and the blood-CSF barrier to reach the brain and the CSF respectively.

The blood-CSF barrier is formed by the choroid plexi, which are located in the third, fourth and lateral ventricles of the brain, and produce most of the CSF. The protein concentration and composition of the CSF vary drastically from those of blood (Davson et al., 1987) because the blood-CSF barrier (i.e. the choroid plexus epithelial cell tight junctions) physically separates the blood from the CSF, and the epithelial cells of the choroid plexus synthesise and secrete proteins into the CSF (Cserr, 1971; Davson et al., 1987). Importantly, specialised transporters in the blood-brain barrier (BBB) selectively allow components from the blood into the brain (Saunders et al., 2013).

The main protein synthesised and secreted by the choroid plexus of mammals, birds and reptiles is TTR (Harms et al., 1991), whereas the main protein synthesised and secreted by the choroid plexus of amphibians is a lipocalin, prostaglandin D synthetase, also known as Cpl1 and β-trace (Achen et al., 1992; Beuckmann et al., 2000; Lepperdinger, 2000). In mammals, TTR synthesis by the choroid plexus and secretion into the CSF is involved with the transport of $T_4$, but not $T_3$, from the blood into the CSF (Chanoine et al., 1992; Dickson et al., 1987; Schreiber et al., 1990; Soprano et al., 1985; Southwell et al., 1993; Stauder et al., 1986). In rats, $^{125}$I-$T_4$ that had been injected into the blood rapidly accumulated firstly into the choroid plexus then subsequently moved to striatum, cortex then cerebellum (Dickson et al., 1987). Studies of TTR null mice showed there was no accumulation of $^{125}$I-$T_4$ into the choroid plexus and the choroid plexus had only 14% $T_4$ content when compared to wild type mice (Palha et al., 2000). Moreover, $T_4$ delivery to the subventricular zone of the brain is reduced in TTR null mice (Richardson et al., 2007). Thus, TTR synthesised by the choroid plexus is likely involved in the delivery of $T_4$ to the CSF and the brain. Humans can lack either of the other TH distributor proteins, albumin (see (Peters, 1992) or TBG (Bennhold, 1954) without an overtly different phenotype, but no description of a human lacking TTR is known. Thus, it was concluded that as TTR is the only THDP also synthesised in the brain (i.e. in the choroid plexus), it may be essential for human life in its role in the movement of $T_4$ from the blood into the CSF (Harms et al., 1991).

At least three classes of solute carrier transporters actively participate in the influx and efflux of THs into and out of cells: monocarboxylate transporters (MCTs) such as MCT8 and MCT10, organic anion transporting polypeptides (OATPs) such as OATP1C1 and l-type amino acid transporters (LATs) such as LAT1 and LAT2 (Visser et al., 2011). Representatives of these TH transmembrane transporters are found in both the blood-brain barrier and in the blood-CSF barrier (e.g. (Mayerl et al., 2012; Van Herck et al., 2015) and the distribution of specific transmembrane transporters differs between species (Darras et al., 2015; Wirth et al., 2014). For example, LAT2 is found in the choroid plexus of mice but not in the choroid plexus of humans (Wirth et al., 2009) and appears to be completely absent in chickens (Darras et al., 2015).

Once inside the cell, THs can be activated or inactivated by a family of deiodinase enzymes (D1, D2 and D3). These activities include activation of $T_4$ to $T_3$ (which has higher affinity for the nuclear receptors) and inactivation of $T_4$ to $rT_3$ and of $T_3$ to $T_2$ (for review see (Darras et al., 2015)). Thus, the combination of THDPs in blood, TTR synthesized by the choroid plexus, THTTs in cell membranes, and intracellular deiodinases all contribute to determining the absolute amounts of $T_4$ and $T_3$ and ratios of $T_4$ to $T_3$ within specific regions of the brain. To the best of our knowledge, the proportional contribution of THs entering the brain via choroid plexus-derived TTR compared with entry via THTTs at the choroid plexus and at the blood-brain barrier is unknown.

In order to investigate whether the preference of ligand affinity (for $T_4$ or $T_3$) of TTR influences the form of TH that accumulates in the choroid plexus and then subsequently moves into other areas of the brain, we analysed the uptake of each of the two main forms of THs ($T_4$ and $T_3$) into the choroid plexus and various brain regions. Initially, we confirmed data generated by others in rats (Dickson et al., 1987) in a second mammal: mice. Then we performed a similar experiment in 1.5 week old chickens. We chose birds, as TTR from birds has higher affinity for $T_3$ than for $T_4$ (Chang et al., 1999), whereas TTRs from mammals has higher affinity for $T_4$, and because Van Herck et al. (2015) hypothesised that chicken brains could have enhanced $T_3$ uptake compared to that seen for mammalian brains. The ontogenetic profiles of TTR, THTTs and deiodinases mRNAs in the choroid plexus and blood-brain barrier of chickens from embryonic until early post-natal developmental stages are known, as are the $T_3$ and $T_4$ content of various brain regions (Van Herck et al., 2015). However, the factors that could be involved in the regulation of TH availability and accumulation in the choroid plexus are not known. Thus, we analysed the mRNA expression pattern of TTR, the known TH transmembrane transporters (MCT8, MCT10, LAT1 and OATP1C1) and the deiodinases (D1, D2 and D3) in the choroid plexus and compared this with expression in the rest of the brain (telencephalon, diencephalon, mesencephalon, cerebellum and brain stem). We also tracked the uptake of $^{125}$I-$T_3$ and $^{125}$I-$T_4$ into the choroid plexus and other brain regions.

## 2. Materials and methods

### 2.1. Animals

#### 2.1.1. Animals used for TH uptake assays

Adult BalbC mice weighing 23–30 g, adult Wistar rats weighing 325–366 g and Ross (broiler) chickens (from a commercial hatchery: Belgabroed, Merksplas Belgium) between 7 and 10 days post-hatch weighing 160–250 g were used. All animals were kept at room temperature in species appropriate cages and nesting materials with *ad libitum* access to food and water.

#### 2.1.2. Animals used for qPCR analyses and TH extractions

Chicken eggs were obtained from a commercial hatchery (Belgabroed, Merksplas Belgium) and for each chick, the day of hatching was recorded. Chickens were euthanized on post-hatch day 11 (C11) as per KU Leuven ethics protocols. Blood samples were collected by carotid artery bleeding after sedation. After centrifugation, plasma was stored at −20 °C until analysis. The choroid plexus was collected from the lateral ventricles in the telencephalon and the remaining brain was divided into 5 parts: telencephalon (forebrain), diencephalon, optic lobes and rhombencephalon which was then subdivided into cerebellum

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