Role of transthyretin in thyroxine transfer from cerebrospinal fluid to brain and choroid plexus

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Kassem, Nouhad A., Rashid Deane, Malcolm B. Segal, and Jane E. Preston. Role of transthyretin in thyroxine transfer from cerebrospinal fluid to brain and choroid plexus. Am J Physiol Regul Integr Comp Physiol 291: R1310–R1315, 2006. First published July 6, 2006; doi:10.1152/ajpregu.00789.2005.—The transport of $^{125}\text{I}$-labeled thyroxine (T$_4$) from the cerebrospinal fluid (CSF) into brain and choroid plexus (CP) was measured in anesthetized rabbit [0.5 mg/kg medetomidine (Domitor) and 10 mg/kg pentobarbitonal sodium (Sagatal) iv] using the ventriculocisternal (V-C) perfusion technique. $^{125}\text{I}$-labeled T$_4$ contained in artificial CSF was continually perfused into the lateral ventricles for up to 4 h and recovered from the cisterna magna. The %recovery of $^{125}\text{I}$-labeled T$_4$ from the aCSF was 47.2 ± 5.6% (n = 10), indicating removal of $^{125}\text{I}$-labeled T$_4$ from the CSF. The recovery increased to 53.2 ± 6.3% (n = 4) and 57.8 ± 14.8% (n = 3), in the presence of 100 and 200 μM unlabeled T$_4$, respectively (P < 0.05), indicating a saturable component to T$_4$ removal from CSF. There was a large accumulation of $^{125}\text{I}$-labeled T$_4$ in the CP, and this was reduced by 80% in the presence of 200 μM unlabeled T$_4$, showing saturation. In the presence of the thyroid-binding protein transthyretin (TTR), more $^{125}\text{I}$-labeled T$_4$ was recovered from CSF, indicating that the binding protein is critical to the supply of CNS T$_4$. However, $^{125}\text{I}$-labeled T$_4$ uptake into the ependymal region (ER) of the frontal cortex also increased by 13 times compared with control conditions. Elevation was also seen in the hippocampus (HC) and brain stem. Uptake was significantly inhibited by the presence of endocytosis inhibitors nocodazole and monensin by > 50%. These data suggest that the distribution of T$_4$ from CSF into brain and CP is carrier mediated, TTR dependent, and via RME. These results support a role for TTR in the distribution of T$_4$ from CSF into brain sites around the ventricular system, indicating those areas involved in neurogenesis (ER and HC).

hippocampus; ependymal region; blood-brain barrier


In the cerebrospinal fluid (CSF), the main binding proteins are TTR and ALB, but in lower concentrations compared with plasma. Free T$_4$ concentration in CSF is 70 pM in dogs (16), and the total T$_4$ in humans is ~644 pM (0.5 ng/ml) (33), and the levels depend on CSF proteins. However, the specific role of some T$_4$-binding proteins in the CSF, such as TTR, which is secreted by the choroid plexuses (CP) remains controversial.

It has been suggested that TTR assists T$_4$ transport from blood to brain across the blood-CSF barrier (BCSFB) (8, 28), and therefore TTR synthesis is critical to the supply of CNS T$_4$. However, research using the TTR null mouse (26) has shown that lack of TTR has little effect on brain T$_4$, despite low CSF levels, thus rejecting a role for TTR in control of T$_4$ entry to the brain.

Once in the CSF, T$_4$ can be accumulated by the CP, as it has been shown in in vitro studies of isolated choroid plexus (8), as well as in in vivo ventriculocisternal (V-C) perfusion studies (18). In addition, T$_4$ may be lost by drainage with CSF from the ventricle or efflux to blood across the BCSFB and blood-brain barrier (BBB).

Little is known about what happens to T$_4$ once it enters the CSF or the role of TTR in its distribution. This study aimed to investigate whether CSF-borne T$_4$ was subsequently taken up by the brain and the role of TTR in this process, by using the rabbit in vivo V-C perfusion technique.

MATERIALS AND METHODS

V-C Perfusion

All procedures were within the current guidelines of the Animals (Scientific Procedures) Act, 1986, United Kingdom. This technique is based on the method previously described (7). New Zealand White rabbits of either sex weighing 1.5–4 kg were anesthetized by intravenous injection with a mixture of medetomidine hydrochloride (Domitor) 0.5 mg/kg and pentobarbitonal sodium (Sagatal) 10 mg/kg. A catheter inflow needle was placed in each lateral ventricle as described (7). A single outflow needle was positioned in the cisterna magna. Artificial CSF (aCSF) was infused into the ventricles at a total rate of 60 μl/min, containing $^{125}\text{I}$-labeled T$_4$ (0.37 MBq/40 ml) and the extracellular marker $^{14}$C-mannitol (0.148 MBq/40 ml; from New England Nuclear).

The composition of aCSF (in mmol/l) was 153 Na$^+$, 2.81 K$^+$, 1.7 Mg$^{2+}$, 2.81 Ca$^{2+}$, 131 Cl$^-$, 1.7 SO$_4^{2-}$, 1.48 PO$_4^{3-}$, 27.4 HCO$_3^-$, and 5.3 glucose, and gassed with 95% O$_2$–5% CO$_2$. Blue dextran was added at a concentration of 192 mg/100 ml aCSF. The perfusion continued for 2–4 h, and outflow samples were collected every 10 min.

Blue dextran is a large marker molecule (mol wt 2 $\times$ 10$^6$) (Sigma), confined to the CSF and was used to determine the secretion rate (K$_s$) of the newly synthesized CSF (7). K$_s$ (μl/min) = (C$_{in}$ – C$_{out}$/C$_{out}$) × P, where P is the rate of perfusion (μl/min) and C$_{in}$ and C$_{out}$ are the

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concentration of the blue dextran (arbitrary units) entering and leaving the ventricles measured on a Unicam (UV-1) spectrophotometer with absorbance at 625 nm.

Sampling of Brain Tissue

At the end of the perfusion time, the animal was humanely killed by anesthesia overdose and the brain was rapidly removed. The ventricles were then opened and rinsed with 0.9% saline. The lateral and fourth ventricle CPs were removed, and the ventricular ependymal region (ER) in the frontal cortex region was dissected. The tissue containing the ependymal layer in contact with CSF, was dissected as a 1.0-mm-thick layer from the area next to the midline (septal side, see Ref. 6), covering the midbrain toward the floor of the lateral ventricle. A scalpel was used horizontally to gently tease and peel back the white ER from the ventricular wall. Any gross contamination with brain parenchyma, especially gray matter, could be clearly seen at postsisolation. For these purposes, the samples were not further microdissected to avoid all contamination with underlying cells; however, a uptake in this tissue was compared with samples of tissue immediately below the dissected region (again, 1-mm thick) designated the sub-ER (SER).

The brain was separated into different sections of frontal cortex, hippocampus (HC), cerebellum, brain stem (BS), caudate nucleus, and ependyma, and the remaining brain sections were also homogenized by using a brain homogenizer. CSF inflow and outflow samples (100 μl in triplicate), and tissue samples were solubilized for 48 h in 0.5 ml Solusol (National Diagnostic) in liquid scintillation vials, and 3.5 ml of scintillation liquid added (Ultima Gold, Packard, UK). Samples were counted (LKB Wallac 1219 Beta liquid scintillation counter), with 1.0 mg/ml TTR. In addition, uptake of 125I-labeled T4 activities expressed as dpm (disintegrations per minute).

Preparation of TTR

One milligram of human TTR (Biodesign International) was dissolved in 10 ml of sterile saline and kept frozen at −25°C until needed. The complex was prepared by adding 100 μl 125I-labeled T4 (18.58 pmol) and 1 ml TTR (0.1 mg/ml) to 0.5 ml saline, and allowed to bind for 20 min at room temperature. The solution was dialyzed overnight at 4°C in seamless cellulose tubing (with a cutoff of 12,000 M) or monensin (100 μM, 200 μM) was also used.

Expression of Results

Brain uptake. At the end of 2-h V-C perfusion, the regional brain uptake (R_B) of 125I-labeled T4 was expressed as R_B (ml/g) = 100× tissue (dpm/g)/C_m (dpm/ml), where C_m is the radioactivity per unit volume of entering perfusion fluid (aCSF).

The net cellular uptake was calculated after correction for 14C-mannitol uptake.

125I-Labeled T4 Recovery in CSF

The 125I remaining in outflow CSF in each 10-min sample was measured. The %recovery for each sample was calculated from (C_oaf/C_o) × 100.

The steady-state ratio of 125I-labeled T4 in recovered aCSF was calculated from the mean value of %recovery derived from the last four samples of perfusion fluid.
perfusion at a final concentration of 1.52 μg/ml TTR. The %recovery of 125I-labeled T4 was significantly increased from 41.1 ± 3.4 to 75.3 ± 7.0% (P < 0.01), with a 79% change (Fig. 4; Table 2).

**Uptake Into Brain Regions Is Increased With TTR**

**T**4 uptake into ependymal and SERs. Under control conditions the highest uptake of 125I-labeled T4 into the ER of the frontal cortex (0.04 ± 0.01 ml/g) was found to be significantly higher than the SER (0.01 ± 0.01 ml/g; P < 0.01, Fig. 4). TTR resulted in a very large increase from 0.034 ± 0.01 ml/g (P < 0.01) and 0.24 ± 0.07 ml/g (P < 0.05), respectively (Fig. 5).

**Uptake into HC, BS, and CP.** Uptake into the HC without ER (HC with no ER), was also significantly increased from 0.02 ± 0.01 to 0.19 ± 0.05 ml/g, (P < 0.01; Fig. 5D). When T4 uptake was measured in the HC, including the ER (HC with ER), TTR resulted in a very large increase from 0.034 ± 0.01 ml/g to 0.64 ± 0.21 ml/g, (P < 0.01; Fig. 5C).

TTR caused a marked increase in the uptake of 125I-labeled T4 into BS by >50 times compared with control (Fig. 5E). Under control conditions the highest uptake of 125I-labeled T4 in the brain was found in the CPs, and this was significantly increased by greater than two times in the presence of TTR-125I-labeled T4 complex (Fig. 5F).

**DISCUSSION**

The present study has shown that T4 is taken up from CSF into CP and various brain regions. This uptake is partially carrier mediated, enhanced in the presence of TTR, and likely to involve RME.

The finding that T4 entry from CSF into brain is via a carrier-mediated process, is consistent with previous studies with CP (8). From the current study, the data indicate that this process favors a transport mechanism for T4 in the direction from CSF across CP to the blood. Early observations support...
this suggestion since in mice, transport of T4 from brain toward the blood is saturable (1). In addition, various CNS cells show saturable TH uptake including human glioma (15), rat glia (12), and astrocytes (2).

**TTR Role in T4 Transport From CSF Into CP and Brain Tissues**

Over the last decade, there has been a debate about the role of TTR in T4 transport, focusing primarily on uptake from plasma to CNS (25–28) and whether TTR is essential for this process at either BBB or BCSFB. The present data confirm a role for TTR in T4 distribution once the hormone has entered the CSF. This study suggests that binding of TTR to T4 in the CSF compartment prevents T4 removal from CSF. It is not easy to identify in vivo the exact removal pathway, but both BBBS and BCSFBs could be involved. Indeed, a recent study from our laboratory (5) showed that TTR reduced T4 transport from CSF to blood across the isolated perfused sheep CP. The availability of TTR in CSF is also correlated with increased T4 uptake into CP and brain. The reason for elevated tissue uptake may be a simple consequence of elevated T4 in CSF, providing a concentration gradient favoring tissue uptake. Additionally, we have evidence for RME of TTR/T4 since RME inhibitors reduced uptake. RME of TTR/T4 would be favored in a high TTR environment and allow more T4 uptake than by diffusion alone.

TTR-dependent T4 uptake in ependyma is consistent with previous studies showing TTR endocytosis by ependyma (20), suggesting that the presence of TTR results in a uniform distribution of T4 within the brain parenchyma similar to the effect in perfused liver lobules (23). After exchange between the CSF and extracellular fluid, free T4 within the extracellular

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**Table 2. %Recovery of 125I-labeled T4 after 2-h V-C perfusion in the presence of TTR-125I-labeled T4 complex with or without endocytosis inhibitors monensin (100 µM) or nocodazole (50 µM)**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>125I-labeled T4 %Recovery in CSF</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 125I-labeled T4</td>
<td>12</td>
<td>46.6 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>TTR/T4 complex</td>
<td>6</td>
<td>75.3 ± 7.0†</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TTR/T4 + monensin</td>
<td>3</td>
<td>72.1 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>TTR/T4 + nocodazole</td>
<td>3</td>
<td>66.2 ± 6.7*</td>
<td>&lt;0.05</td>
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</table>

*Values are means ± SE; n is the number of experiments. †P < 0.05, significant difference from complex, ††P < 0.01, significant difference from control.

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**Fig. 5. The uptake of 125I-labeled T4 into the ependyma region (ER) of the frontal cortex (A), sub-ER (SER, n = 6) (B), hippocampus including ER (HC with ER, n = 6) (C), HC excluding ER (HC with no ER, n = 6) (D), brain stem (BS) (n = 6) (E), and choroid plexus (CP, n = 9) (F) after 2-h V-C perfusion. The uptake of 125I-labeled T4 was measured under control condition, in the presence of 1.52 µg/ml TTR as TTR-125I-labeled T4 complex, n = 5, (+TTR), and with either of two endocytosis inhibitors, 50 µM monensin (TTR+N, n = 3) and 100 µM monensin (TTR+M, n = 3). Values are means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001, difference from +TTR. †125I-labeled T4 uptake was measured under control condition, in the presence of 1.52 µg/ml TTR as TTR-125I-labeled T4 complex, n = 5, (+TTR), and with either of two endocytosis inhibitors, 50 µM monensin (TTR+N, n = 3) and 100 µM monensin (TTR+M, n = 3). Values are means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001, difference from +TTR. **P < 0.05, significantly different from control.**
space could be subject to rapid uptake by cells, and conversion to T3 by type II deiodinase (29). Elevated T3 uptake in the presence of TTR suggests that the even distribution of T4 within this tissue is not only dependent on the free fraction of T4 in CSF, but also on the T4 bound to TTR.

Ependyma as Reservoir for Brain Uptake of T4

To reach its targets within the brain, T4 from CSF must first cross the ER of the ventricles. The ER is a gateway between CSF and brain, and it appears that TTR mediates transfer of T4 from CSF and facilitates uptake into the ER, to a greater extent than into the SER. Increased ER uptake of T4 is likely to involve both passive diffusion of T4 and a TTR-T4 receptor, allowing for greater uptake than can be achieved by diffusion alone, similar to that found in human ependymoma cells (19, 20). The ER could, therefore, act as a reservoir for brain T3, relevant under conditions of increased hormone demand by various regions. Previous studies have demonstrated receptor-mediated uptake and internalization of TTR in cultured human astrocytoma cells (9). The HC also accumulated T4 in the presence of TTR. Interestingly, TTR is abundantly expressed in BS, HC, and cerebral cortex (4, 22), as well as CP (8), which may act to enhance T4 uptake and retention by cells. Since THs may facilitate neuronal regeneration after CNS injury (32), it is interesting that the ER, which had high TTR-125I-labeled T4 uptake in this study, is also an area of active neurogenesis in the adult, which increases after brain injury (24).

RME

The inhibitory effect of the endocytosis inhibitor monensin on the CP, ER, SER, and HC uptake of TTR-125I-labeled T4, suggests the presence of RME, as discussed above. Monensin blocks protein degradation and trafficking out of the endosome (13), but the drug’s effect on the intracellular organelle was not investigated in our study. The effect also appeared to be similar to that obtained in in vitro studies in which these drugs reduced the uptake of T4 in cultured anterior pituitary cells by 40% (11). It should be noted that monensin also acts as a sodium ionophore that rapidly exchanges Na and H ions (31), and so it is likely that Na/H exchange is increased in brain tissues in our study. From the work of others on the kidney (14), a link between Na/H exchange (via NHE3) and receptor-mediated endocytosis has been made, in which the presence of Na/H exchange is essential for RME. We did not study Na/H exchange directly; however, from previous work we would presume that any elevation of Na/H exchange induced by monensin would encourage uptake by RME. In contrast, we saw inhibition of T4 uptake, which suggests that the effect of monensin on T4/TTR uptake is as an RME inhibitor, although changes in intracellular pH as a regulatory mechanism cannot be ruled out, and warrant further investigation.

Early findings (17) established that both drugs, monensin and nocodazole, inhibited endocytosis of ALB by the alveolar epithelium in rabbit. Because it is known as a microtubule toxin (3), nocodazole may cause disruption to the endosomal recycling of transporter proteins, and therefore it can be proposed that the TTR-125I-labeled-T4 complex cycles between the cell surface and intracellular stores by a nocodazole-sensitive mechanism in the brain tissue and to a lesser extent in the CP. Despite inhibition of tissue uptake by RME inhibitors, recovery of T4 in CSF did not increase. This is perhaps not surprising, given the very large pool of T4 in bulk CSF relative to the potential small pool in the tissue surrounding the ventricles.

The data presented in the current study permitted us to conclude that the high level of T4 found in the CSF, CP, ER, and other brain regions may reflect the presence of TTR in the CSF compartment. In its turn, TTR may represent a major contributing factor in keeping high concentration of free T4 in CSF, as well as brain. In addition, both brain barriers may also play an essential role in the homeostasis of CNS T4. Carrier-mediated and RME may represent a mode for T4 transfer from the CSF into brain regions. These studies provide new insight into T4 transport systems within the CNS and form the basis for further investigations to elucidate the importance of these factors and the molecular mechanisms that control the biology of thyroid hormone transport and its delivery into the brain.

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