Thyroxine Transport in Choroid Plexus*

(Received for publication, December 1, 1986)

Phillip W. Dickson, Angela R. Aldred, John G. T. Menting, Philip D. Marley, William H. Sawyer, and Gerhard Schreiber

From the Russell Grimwade School of Biochemistry, University of Melbourne, Parkville, Victoria 3052, Australia

The role of the choroid plexus in thyroid hormone transport between body and brain, suggested by strong synthesis and secretion of transthyretin in this tissue, was investigated in \textit{in vitro} and \textit{in vivo} systems.

Rat choroid plexus pieces incubated \textit{in vitro} were found to accumulate thyroid hormones from surrounding medium in a non-saturable process. At equilibrium, the ratio of thyroid hormone concentration in choroid plexus pieces to that in medium decreased upon increasing the concentration of transthyretin in the medium. Fluorescence quenching of fluorophores located at different depths in liposome membranes showed maximal hormone accumulation in the middle of the phospholipid bilayer. Partition coefficients of thyroxine and triiodothyronine between lipid and aqueous phase were about 20,000.

After intravenous injection of $^{125}$I-labeled thyroid hormones, choroid plexus and parts of the brain steadily accumulated $^{125}$I-thyroxine, but not $^{125}$I-triiodothyronine, for many hours. The accumulation of $^{125}$I-thyroxine in choroid plexus preceded that in brain. The amount of $^{125}$I-thyroxine in non-brain tissues and the $^{125}$I-triiodothyronine content of all tissues decreased steadily beginning immediately after injection. A model is proposed for thyroxine transport from the bloodstream into cerebrospinal fluid based on partitioning of thyroxine between choroid plexus and surrounding fluids and binding of thyroxine to transthyretin newly synthesized and secreted by choroid plexus.

The brain is one of the main target organs for thyroid hormones. From the bloodstream, thyroid hormones must cross the blood/brain barrier or the blood/cerebrospinal fluid barrier to reach the brain. The mechanism of this transport is not clear. Transthyretin (formerly called prealbumin) occurs in both serum (1) and cerebrospinal fluid (2, 3). It is thought to transport thyroid hormones in the bloodstream of both humans (4, 5) and rats (6, 7).

Recently, cDNA clones were obtained for transthyretin, providing the means to analyze the tissue distribution of transthyretin gene expression. High levels of transthyretin mRNA were observed in both rat (8, 9) and human (10) choroid plexus, localized exclusively in the epithelial cell layer (11) forming the ventricular surface of the choroid plexus. About 20% of newly synthesized and about 50% of new protein secreted by choroid plexus was found to be transthyretin (12). Apparently, choroid plexus epithelial cells are among the cells most highly specialized for the synthesis of one particular protein. The ratio of the concentration in cerebrospinal fluid to that in serum is higher for transthyretin than for all other plasma proteins, except cystatin C (formerly called gamma trace) (13, 14) and B2-microglobulin (for review see Ref. 15).

With the choroid plexus specializing in the synthesis of transthyretin and being part of the interface between the brain and the rest of the body, we addressed the question of whether the choroid plexus is involved in the transport of thyroid hormones from the blood to cerebrospinal fluid and brain by providing transthyretin. The distribution of L-thyroxine and L-triiodothyronine in rat choroid plexus pieces incubated \textit{in vitro} and in a model system consisting of liposomes of phospholipid bilayers was studied and compared with the \textit{in vivo} distribution of injected radioactive L-triiodothyronine and L-thyroxine. A model for the mechanism by which the choroid plexus may contribute to the transport of thyroxine between the bloodstream and cerebrospinal fluid by expression of the gene for a thyroid hormone transport protein is proposed.

\textbf{EXPERIMENTAL PROCEDURES}$^1$

\textbf{RESULTS}

\textit{L-Thyroxine and L-Triiodothyronine Accumulate by a Nonsaturable Mechanism within Choroid Plexus Pieces Incubated in Vitro}—Choroid plexus obtained from the brains of rats were incubated with radioactive thyroxine or triiodothyronine \textit{in vitro} for different lengths of time as described under "Experimental Procedures." After separation of choroid plexus pieces and medium, the distribution of radioactivity was determined (Fig. 1). The rates of uptake of both thyroid hormones were highest at time 0 and decreased thereafter. Eventually, an equilibrium was reached with 61% of total thyroxine and 56% of total triiodothyronine found associated with the choroid plexus pieces. The final ratio of thyroxine or triiodothyronine concentration in choroid plexus over that in medium was about 240 and 190, respectively. For this comparison, a homogeneous distribution of thyroxine or triiodothyronine is assumed throughout choroid plexus tissue. Most of choroid plexus tissue is an aqueous phase. As is shown below, thyroxine and triiodothyronine accumulate very strongly in the lipid phase of a two-phase aqueous/lipid sys-

---

$^*$This work was made possible by grants from the National Health and Medical Research Council of Australia and the Australian Research Grants Committee. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

$^1$Supported by a Queen Elizabeth II Fellowship.

---

1 Portions of this paper (including "Experimental Procedures," part of "Results," Figs. 5, 6, 8, 10, and 12, and Table II) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M-4066, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
plexus incubated in vitro. Choroid plexus from male rats weighing between 260 and 360 g were washed by shaking for 30 min at 37 °C in incubation medium and, after collection by short centrifugation at low speed, incubated in the presence of 125I-thyroxine (final concentration 144 pM) (top) or 125I-triiodothyronine (final concentration 79 pM) (bottom), as described under "Experimental Procedures." After incubation for the time indicated on the abscissa, choroid plexus (4 mg) and incubation medium (0.8 ml) were separated by centrifugation and washed once, and wash fluid was combined with the supernatant from the previous centrifugation. Choroid plexus were homogenized in incubation medium (1.2 ml) and radioactivity was determined in a gamma counter as described under "Experimental Procedures." The initial rate of uptake of thyroxine or triiodothyronine into choroid plexus incubated in vitro was calculated from the equations. These rate constants, k_in and k_out, and initial reaction rates, dX_in/dt and dX_out/dt, were calculated from the equations. These rate constants are independent of concentrations, X_med, X_con, and X_tot (concentration in medium, in choroid plexus, and total, respectively). The initial reaction rates are proportional to X_con or X_med at time 0 and to the rate constants. The values obtained are summarized in Table I. Although total concentrations of hormones at the beginning of the "release experiments" were only about 60% of those in the "uptake experiment," the values for k_in and k_out were identical within experimental variation. The initial rate of uptake of thyroxine into choroid plexus, i.e. 10 pmol s⁻¹ liter⁻¹, gives an uptake of 350 fmol min⁻¹/choroid plexus.

Partitioning between Aqueous and Lipid Phases Leads to Accumulation of Thyroid Hormones in Lipid Bilayer Membranes—Both thyroxine and triiodothyronine were found to quench the fluorescence of 9-anthroyloxy fatty acids in arti-

**Fig. 1.** Uptake of radioactive thyroxine (top) and triiodothyronine (bottom) into choroid plexus incubated in vitro. Choroid plexus from male rats weighing between 260 and 360 g were washed by shaking for 30 min at 37 °C in incubation medium and, after collection by short centrifugation at low speed, incubated in the presence of 125I-thyroxine (final concentration 144 pM) (top) or 125I-triiodothyronine (final concentration 79 pM) (bottom), as described under "Experimental Procedures." After incubation for the time indicated on the abscissa, choroid plexus (4 mg) and incubation medium (0.8 ml) were separated by centrifugation and washed once, and wash fluid was combined with the supernatant from the previous centrifugation. Choroid plexus were homogenized in incubation medium (1.2 ml) and radioactivity was determined in a gamma counter as described under "Experimental Procedures." The initial rate of uptake of thyroxine or triiodothyronine into choroid plexus incubated in vitro was calculated from the equations. These rate constants, k_in and k_out, and initial reaction rates, dX_in/dt and dX_out/dt, were calculated from the equations. These rate constants are independent of concentrations, X_med, X_con, and X_tot (concentration in medium, in choroid plexus, and total, respectively). The initial reaction rates are proportional to X_con or X_med at time 0 and to the rate constants. The values obtained are summarized in Table I. Although total concentrations of hormones at the beginning of the "release experiments" were only about 60% of those in the "uptake experiment," the values for k_in and k_out were identical within experimental variation. The initial rate of uptake of thyroxine into choroid plexus, i.e. 10 pmol s⁻¹ liter⁻¹, gives an uptake of 350 fmol min⁻¹/choroid plexus.

**Fig. 2.** Uptake of radioactive thyroid hormones into choroid plexus incubated in vitro under the presence of different concentrations of unlabeled hormone. Isolation and washing of choroid plexus was performed as described under "Experimental Procedures." Choroid plexus was then incubated in the presence of either 125I-thyroxine (10 pM) or 125I-triiodothyronine (10 pM) and different concentrations of the corresponding unlabeled hormone (100 pM to 1 μM). The choroid plexus pieces were incubated for 80 min at 37 °C. Choroid plexus and supernatant were then separated by centrifugation and radioactivity was determined in choroid plexus homogenates and supernatants as described under "Experimental Procedures." Ordinate, amount (fmol) of hormone in 600 μl of medium; abscissa, amount (fmol) of hormone in cells.
FIG. 3. Release of radioactive thyroxine and triiodothyronine from choroid plexus incubated in vitro. Isolation and washing of choroid plexus, as described under "Experimental Procedures," was followed by incubation in the presence of [125I]-thyroxine (top, final concentration 144 pm) or [125I]triiodothyronine (bottom, final concentration 78 pm) for 1 h at 37°C. Choroid plexus were then collected by centrifugation, washed once as before, resuspended in incubation medium, and incubated for various times as indicated on the abscissa. Choroid plexus and supernatant were separated by centrifugation and radioactivity was determined in choroid plexus homogenates and supernatants as described under "Experimental Procedures." The best fitting curve for thyroxine had the equation \( y = 46 - 37e^{-0.046x} \) or \( y = 32 - 30e^{-0.097x} \). The amount of thyroxine or triiodothyronine from incubated choroid plexus. One choroid plexus per time point.

TABLE I

Rate constants and initial reaction rates for uptake and release of thyroxine and triiodothyronine into and from choroid plexus incubated in vitro

| Type of experiment | Initial rate of reaction | \( k_a \) | \( k_r \) |
|--------------------|--------------------------|---------|---------|
|                     | \( \text{pMol s}^{-1} \text{liter}^{-1} \) | \( s^{-1} \) | \( s^{-1} \) |
| Uptake of thyroxine from medium into choroid plexus | 10 | 0.07 ± 0.02 | 0.04 ± 0.04 |
| Release of thyroxine from choroid plexus into medium | 2 | 0.06 ± 0.04 | 0.02 ± 0.01 |
| Uptake of triiodothyronine from medium into choroid plexus | 4 | 0.04 ± 0.01 | 0.03 ± 0.03 |
| Release of triiodothyronine from choroid plexus into medium | 2 | 0.06 ± 0.04 | 0.04 ± 0.01 |

The light emitted by the fluorescent group positioned near the middle of the bilayer was quenched the most, and that of the fluorescent group positioned near the bilayer surface was quenched the least, indicating the existence of higher local concentrations of the quenching moiety (i.e. the iodinated thyroxine rings) near the middle of the membrane. The partition coefficient between lipid and aqueous phases in multilamellar liposomes was calculated to be \((17.5 ± 1.4) \times 10^2\) for thyroxine (mean ± S.E.) and \((23.5 ± 2.7) \times 10^2\) for triiodothyronine (mean ± S.E., 0.025 < \( p < 0.05\) for the difference.
between thyroxine and triiodothyronine. Therefore, thyroid hormones accumulate greatly in phospholipid bilayers suspended in an aqueous medium.

Above pH 7 the quenching of the fluorescence of 16-(9-anthroyloxy) palmitic acid (i.e. a compound with the fluorophore group located near the middle of the bilayer) by either thyroxine or triiodothyronine became strongly dependent on the pH (Fig. 5, Miniprint).

**Transnathyretin Shifts Partitioning Equilibrium between Choroid Plexus and Medium toward the Aqueous Phase**—The average ratio of the concentration of thyroid hormone inside the choroid plexus to that in the medium, established when about 4 mg of choroid plexus was incubated for 40 min in 0.6 ml of medium, was 292 for thyroxine and 208 for triiodothyronine. The medium used in this incubation did not contain transthyretin. However, when pieces of choroid plexus were loaded with radioactive thyroid hormones and then incubated in transthyretin containing medium, the equilibrium distribution was shifted to a higher proportion of hormones being present in the medium. The distribution of thyroxine, or triiodothyronine, between choroid plexus and medium and the concentration of transthyretin in the incubation medium were related (shown for thyroxine in Fig. 6, Miniprint). The ratio of the concentration inside the choroid plexus to that in the medium decreased to 76 for either thyroxine or triiodothyronine for a concentration of 40 µg of transthyretin/ml of medium, and to 20 for thyroxine in the presence of 160 µg of transthyretin/ml of medium, measured after 40 min.

When transthyretin (40 µg/ml) to be added to the release incubation medium was saturated first by an excess amount of thyroxine (10 µM), the shift in the distribution ratio for radioactive thyroxine was prevented. The concentrations of transthyretin used in the experiments were comparable to those reported by others for cerebrospinal fluid (27).

**Tissue Distribution of I25I-Thyroxine and Triiodothyronine 10 Min after Intravenous Injection**—The distribution of intravenously injected I25I-labeled thyroxine and triiodothyronine in various tissues after 10 min was determined as described under “Experimental Procedures.” After correction for radioactivity contributed by thyroxine or triiodothyronine in blood, a typical pattern was obtained for the accumulation of thyroxine (Fig. 7) and triiodothyronine (Fig. 8, Miniprint) in various tissues. Choroid plexus was found to be the tissue with the strongest accumulation of thyroxine. After subtracting the amount of thyroxine contributed by the blood, choroid plexus contained about 2.7 times more thyroxine in 1 mg of tissue than in 1 µl of blood. The only other organ in which thyroxine accumulated over the concentration in blood was the liver, with about 1.9 times the level in blood. Both choroid plexus and liver are the only two of the tissues shown in Fig. 7 which synthesize transthyretin.

**Kinetics of Uptake of Thyroxine and Triiodothyronine into Choroid Plexus and Other Tissues in Vivo**—The distribution of radiolabeled thyroxine in tissues was determined at various times after an intravenous injection of the labeled hormone. The results are presented in Fig. 9. In choroid plexus the level of thyroxine increased with time, beginning with an initial rate of uptake of 38 fmol min⁻¹/choroid plexus, reaching a half-maximal value after about 1 h and leveling off after about 5.5 h. Five hours after injection of I25I-thyroxine the amount of labeled hormone in 1 mg of choroid plexus was about 20 times that in 1 µl of blood. In contrast, the level of radiolabeled thyroxine in the liver increased only very slightly between 10 and 20 min and decreased thereafter. Radioactive material recovered from liver and choroid plexus was shown to be thyroxine by thin layer chromatography and comparison with authentic thyroxine (Fig. 10, Miniprint). The level of labeled thyroxine in kidney and pituitary did not change between 10 min and 5.5 h of incubation. In contrast to choroid plexus, uptake of I25I-thyroxine into striatum, cortex, and cerebellum was initially relatively slow, and half-maximal values were reached only after 3 h or later (Fig. 11).

The kinetics of uptake of I25I-triiodothyronine into tissues differed characteristically from those for I25I-thyroxine (Fig. 12, Miniprint). In contrast to the high rate of uptake of I25I-thyroxine, the level of labeled triiodothyronine in the choroid plexus decreased with time beginning immediately after injection. This decrease was not specific for the choroid plexus, as

---

**Fig. 7. Tissue distribution of injected I25I-thyroxine in vivo after 10 min.** The distribution of I25I-labeled thyroxine 10 min after injection of the labeled hormone into the caval vein was determined as described under "Experimental Procedures." The results are given as the mean for three animals ± S.E.

**Fig. 9. Kinetics of uptake of intravenously injected I25I-thyroxine into choroid plexus, liver, kidney, and pituitary.** The level of I25I-thyroxine in tissues at various times after an intravenous injection of I25I-thyroxine was determined as described under "Experimental Procedures." CP, choroid plexus; L, liver; B, blood; K, kidney; P, pituitary. Each point is the mean for three animals ± S.E.
The choroid plexus takes up thyroxine, but not the more biologically active triiodothyronine. However, thyroxine taken up into the brain can be deiodinated to triiodothyronine. Dratman and Crutchfield (33) observed the uptake of $^{125}$I-thyroxine into synaptosomes in rat brain and its conversion to triiodothyronine.

The importance of the choroid plexus for providing transthyretin to the cerebrospinal fluid is supported by the following estimate of the rate of production of transthyretin in rat cerebrospinal fluid and the rate of synthesis of transthyretin in rat choroid plexus. The rate of production of cerebrospinal fluid in rats weighing between 200 and 300 g is 2.2 $\mu$l/min (34). Assuming a transthyretin concentration of 17.3 ng/$\mu$l (for a review of protein concentrations in cerebrospinal fluid see Ref. 15), this corresponds to 38.1 ng of transthyretin/min. For a number of plasma proteins, synthesis rates in liver have been shown to be proportional to intracellular concentrations of mRNA (35). Assuming this would also be the case for synthesis rates in choroid plexus and taking into account the 25 times larger concentration of transthyretin mRNA in rat choroid plexus than in liver (9), a rat choroid plexus of 3-4 mg would produce about 49-66 ng of transthyretin/min. That is, the synthetic capacity of choroid plexus, estimated from protein production rates and mRNA concentrations, could be sufficient to account for providing the transthyretin in cerebrospinal fluid.

The maximum thyroxine binding capacity of 1 ng of transthyretin is 0.018 pmol of thyroxine, assuming a molecular weight for rat transthyretin of 54,510 (9) and an occupancy rate of one molecule of thyroxine per molecule of transthyretin. Therefore, transthyretin synthesized and secreted by one choroid plexus could carry a maximum of 0.90-1.2 pmol of thyroxine/min into the cerebrospinal fluid. The initial rate for the uptake of thyroxine in vivo into choroid plexus was 0.04 pmol/min, corresponding to about 3% of the maximum transport capacity for thyroxine by transthyretin synthesized in the choroid plexus.

Based on our data, we would like to propose the following hypothesis for the contribution of the choroid plexus to maintaining appropriate concentrations of thyroxine in the extra-
Thyroxine Transport in Choroid Plexus

**Fig. 13. Hypothetical model for the transport of thyroxine from blood to cerebrospinal fluid via the choroid plexus.** The direction of migration of thyroxine is from the bloodstream at the left side of the figure to the central nervous system at the right side of the figure. TTR, transthyretin; T₄, thyroxine; CSF, cerebrospinal fluid.

Cellular compartment of the central nervous system (Fig. 13).

Partitioning of thyroxine occurs between blood plasma and choroid plexus. Newly synthesized transthyretin is secreted by the choroid plexus into cerebrospinal fluid. This transthyretin would bind thyroxine. The precise site for this binding (intracellular, near membranes, or extracellular) has still to be identified. The transthyretin-thyroxine complex is swept away from the choroid plexus surface by the unidirectional flow of cerebrospinal fluid from the ventricles to the spinal canal and the subarachnoidal space. The proposed hypothesis does not postulate that the choroid plexus is the exclusive site for the transport of thyroxine from the blood to all parts of the brain. Thyroxine would also partition between lipid and aqueous phases elsewhere at the blood/brain barrier. However, in the absence of strong local synthesis and secretion of apotransthyretin, the very large partition coefficient would mean that much of the thyroxine remains in the lipid phase.

Thyroxine in the cerebrospinal fluid would not interfere with the regulatory feedback of free thyroid hormones on the stimulation of thyrotropin-releasing hormone on thyrotropin release by the pituitary gland because this gland is located outside the blood/brain barrier.

Partitioning between two phases is not a very specific feature. However, the expression of the transthyretin gene in the epithelial cells of the choroid plexus is a very specific phenomenon. The combination of partitioning and expression of the gene for a secreted, specific transport protein could be an alternative system to a receptor-mediated transport system. The driving forces for the transfer of thyroxine from blood to brain through choroid plexus could be the synthesis and secretion of transthyretin and the unidirectional flow of the cerebrospinal fluid.

The fact that predominantly thyroxine but not triiodothyronine is transported through choroid plexus has two explanations. First, the molar concentration of free thyroxine in plasma is much higher than that of free triiodothyronine (7-fold in the rat (36)). Second, the affinity of transthyretin for thyroxine is much greater than that for triiodothyronine (by a factor of 12.5 in the human; data for the rat are not known).

In the bloodstream, the levels of free thyroid hormones are kept relatively constant, whereas the levels of total (free plus protein-bound) hormone changes can vary considerably (the so-called "free thyroid hypothesis"). Previously, transcellular transport involving vesicles has been discussed for the transfer of serum proteins from blood to cerebrospinal fluid (37, 38). Such a transport mechanism would supply thyroid hormones to the cerebrospinal fluid in amounts varying with total hormone levels in blood. The mechanism proposed in this paper would lead to establishing a pool of thyroid hormones in choroid plexus related in amount to the more stable level of free hormones in the bloodstream. The amount of hormones provided to the brain would then depend predominantly on the rate of transthyretin synthesis and secretion by choroid plexus. This has been found to be regulated independently from the expression of the transthyretin gene elsewhere in the body (12).

Acknowledgments—We are very grateful to E. Gill for word processing, A. Schreiber for computer curve fitting, and the Even Downie Metabolic Unit of the Alfred Hospital, Melbourne, for radioimmunoassays.

REFERENCES

1. Seibert, F. B., and Nelson, J. W. (1942) J. Biol. Chem. 143, 29-38
2. Kabat, E. A., Landow, H., and Moore, D. H. (1942) Proc. Soc. Exp. Biol. Med. 49, 260-263
3. Kabat, E. A., Moore, D. H., and Landow, H. (1942) J. Clin. Invest. 21, 571-577
4. Logan, S. H. (1965) J. Clin. Invest. 42, 143-160
5. Oppenheimer, J. H. (1988) N. Engl. J. Med. 278, 1153-1162
6. Davis, P. J., Spaulding, S. W., and Gregerman, R. I. (1970) Endocrinology 87, 978-986
7. Sutherland, R. L., and Brandon, M. R. (1976) Endocrinology 98, 91-98
8. Dickson, P. W., Aldred, A. R., Marley, P. D., Guo-Fen, T., Howlett, G. J., and Schreiber, G. (1985) Biochem. Biophys. Res. Commun. 127, 890-895
9. Dickson, P. W., Howlett, G. J., and Schreiber, G. (1985) J. Biol. Chem. 260, 8214-8219
10. Dickson, P. W., and Schreiber, G. (1986) Neurosci. Lett. 66, 311-315
11. Stauder, A. J., Dickson, P. W., Aldred, A. R., Schreiber, G., Mendelsohn, F. A. O., and Hudson, P. (1986) J. Histochim. Cytochem. 34, 949-953
12. Dickson, P. W., Aldred, A. R., Marley, P. D., Bannister, D., and Schreiber, G. (1986) J. Biol. Chem. 261, 3475-3478
13. Lofberg, H., and Grubb, A. O. (1979) Scand. J. Clin. Lab. Invest. 39, 619-626
14. Grubb, A. O., and Lofberg, H. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3024-3027
15. Schreiber, G. (1987) in The Plasma Proteins: Structure, Function and Genetic Control (Putnam, F. W., ed) 2nd Ed., Vol. V, pp. 293-363, Academic Press, Orlando, FL
16. Dickson, P. W., Howlett, G. J., and Schreiber, G. (1982) Eur. J. Biochem. 129, 289-293
17. Marley, P. D., Rehfeld, J. F., and Emson, P. C. (1984) J. Neurochem. 42, 1523-1535
18. Edwards, K., Schreiber, G., Dryburgh, H., Urban, J., and Inglis, A. S. (1976) Eur. J. Biochem. 63, 303-311
19. Schreiber, G., and Schreiber, M. (1978) Subcell. Biochem. 2, 307-353
20. Thulborn, K. R., and Sawyer, W. H. (1978) Biochim. Biophys. Acta 511, 125-140
21. Rotzsch, Von W., Köhler, H., and Martin, H. (1967) Hoppe-Seyler's Z. Physiol. Chem. 348, 839-840
22. Hayta, K., and Ul, M. (1966) Clin. Chim. Acta 14, 361-366
23. Tagerad, S. E. O., and Cuello, A. C. (1979) Neuroendoc. 4, 2021-2029
24. Schreiber, G., Howlett, G., Nakagahema, M., Millership, A., Martin, H., Urban, J., and Kotler, L. (1982) J. Biol. Chem. 257, 10271-10277
25. Pardridge, W. M., and Mietus, L. J. (1980) J. Clin. Invest. 66, 367-374
26. Stern, V. O., and Volmer, M. (1919) Physik. Zeitschr. 20, 183-188
Thyroxine Transport in Choroid Plexus

27. Hagen, G. A., and Elliott, W. J. (1973) J. Clin. Endocrinol. Metab. 37, 415-422.
28. Brightman, M. W. (1977) Exp. Eye Res. 25, (suppl.) 1-25.
29. Hagen, G. A., and Solberg, L. A. Jr. (1974) Endocrinology 95, 1398-1410.
30. Pandit, W. M. (1979) Endocrinology 105, 605-612.
31. Herbert, J., Wilcox, J. N., Pham, K. T.-C., Freema, R. T., Jr., Zeviani, D., Wark, A., Soprano, A., Goodman, D. W., Siecker, Z., Allen, E. R., and Schon, E. A. (1986) Neurosci. 36, 900-911.
32. Crandall, H. F. (1977) Physiol. Rev. 51, 273-311.
33. Dratman, M. B., and Crutchfield, F. L. (1978) J. Am. Physiol. 235, E638-E647.

SUPPLEMENTARY MATERIAL TO:
Thyroxine Transport in Choroid Plexus

Phillip U. Ockson, Angela P. Aldred, John G. T. Henning, Philip Aldred, William H. Sawyer and Gerhard Schreiber

EXPERIMENTAL PROCEDURES

Materials and Methods

In vitro Incubations of Choroid Plexus

Histological, rats from an inbred colony kept in the laboratory were used in all experiments. Specific activity 32P I[32P] and 125I-[125I]Thyroidal Hormones (specific activity 32P 2000 Ci/mmol) were obtained from American, cytoskeleton from Biochrome, Ltd., London, U.K. Lymphocyte activation was performed by collagen and cross-linked antigen particles from Mycosett, Inc. and nylon fiber from Molecular Probes. Rat plasma transferrin was purified as previously described (16).

Methods

In vitro Incubations of Choroid Plexus

Male rats (one per time point) weighing between 250 and 360 g were anaesthetized with ether and decapitated. Choroid plexus was dissected from the brain, frozen and stored at -80°C as previously described (16) and placed immediately into 600 ml of incubation medium. The incubation medium (10) and concentrations of amino acids in the incubation medium (10) were as described previously. Choroid plexus pieces were incubated in the medium at 97°C for 45 min in a shaking water bath. After incubation, choroid plexus pieces were homogenized in 1.2 ml of 125I- or 32P-labeled hormone solutions. Radioactivity in choroid plexus homogenates and pooled cellular media with supernatants was determined in a gamma counter.

For experiments analyzing the release of thyroid hormones from choroid plexus ("release incubations"), incubation of choroid plexus pieces for loading of the tissue with radioactive thyroid hormones was for 3 h, as described above. After collection by centrifugation, the choroid plexus pieces were washed once, as before, and resuspended in medium containing 100 μM of 125I- or 32P-labeled hormone solutions. Cytoskeleton (final concentration 30 μM) was added to the choroid plexus incubations. At various times of incubation, samples were centrifuged for 30 s at 800 g and the supernatants were used for analysis. The choroid plexus pieces were resuspended in 50 μl of medium and centrifuged at 800 g for 30 s. After decanting, the supernatants were pooled with those from the previous step. Choroid plexus pieces were homogenized in 1.2 ml of 125I- or 32P-labeled hormone solutions. Radioactivity in choroid plexus homogenates and pooled cellular media with supernatants was determined in a gamma counter.

For experiments in the presence of transfectin, incubation of choroid plexus for uptake of radioactive thyroid hormone was for 3 h as follows: release incubation for 30 min, as described above. At the start of the release incubation, choroid plexus pieces were washed once, as above, and resuspended in medium containing 100 μM of 125I- or 32P-labeled hormone solutions. Cytoskeleton (final concentration 30 μM) was added to the choroid plexus incubations. After 3 h, the medium was removed and replaced with a fresh medium containing 100 μM of 125I- or 32P-labeled hormone solutions. The change in fluorescence is shown in Fig. 5. The curves describing the quantitative relationship between pH and quenching caused by one-proton transition but not by two-proton transition was calculated as follows for the example of thyroglobulin (Tg). Kinetic studies of Tg uptake were performed by adding Tg to a solution of Tg at pH 7.4 at 0.1 ml per minute. The Tg uptake was determined as described under "Experimental Procedures" and the radiolabeled Tg was considered to be the tracer for Tg uptake. The pH dependence of the velocity of Tg uptake was determined at pH 5.5-7.4. The Tg uptake was determined at various times of incubation. The Tg uptake was determined as described under "Experimental Procedures" and the radiolabeled Tg was considered to be the tracer for Tg uptake. The pH dependence of the velocity of Tg uptake was determined at pH 5.5-7.4. The Tg uptake was determined at various times of incubation.
Thyroxine Transport in Choroid Plexus

FIG. 1. pH dependence of the quenching of 125I-(3-mercapto-4-hydroxy)-benzoic acid by thyroxine (top panel) and triiodothyronine (bottom panel) in unmodified [125I]thyroxine. The absence of quencher, A, intensity of 125I-(3-mercapto-4-hydroxy)-benzoic acid in the presence of quencher, B, intensity of 125I-(3-mercapto-4-hydroxy)-benzoic acid in the absence of quencher.

Transport in Choroid Plexus of Thyroxine and Triiodothyronine

The distribution of I-labeled triiodothyronine was determined as described under "Experimental Procedures". The results are given as the radioactivity in mg tissue as a percent of the radioactivity in 1 ml blood (mean for three animals ± S.E.). The radioactivity in each tissue has been corrected for blood contamination as described in "Experimental Procedures".

TABLE II

| Incubation time (min) | 125I-Thyroxine | 125I-Triiodothyronine |
|-----------------------|----------------|----------------------|
| 0                     | 90%            | 93%                  |
| 2                     | 91%            | 91%                  |
| 20                    | 91%            | 73%                  |
| 60                    | 91%            | 73%                  |

Characterization of Radiolabeled Material in Choroid Plexus and Liver

FIG. 10. Characterization of radiolabeled material in choroid plexus and liver. The radiolabeled material in liver and choroid plexus, 3 h after injection of [125I]-labeled thyroxine, was recovered and characterized using thin layer chromatography (Fig. 10). The system used separated thyroxine, triiodothyronine and iodine. The radiolabeled species found in the liver and choroid plexus with thyroxine. Each lane in the thin layer chromatography was cut into segments and the radioactivity in each lane was determined by liquid scintillation counting. The results were 91% and 28% in the rest of the lane. For the liver the results were 92%, 69% and 38%, respectively.

In order to understand the data on the distribution of radiolabeled hormones it is important to have an estimate of the rate at which the injected hormone equilibrates with the plasma binding proteins. The rate of binding of radiolabeled thyroxine and triiodothyronine was determined as described under "Experimental Procedures". The results in Table II show that the percent of thyroxine bound to plasma did not change with increasing times of incubation. The determination of the rate of binding was tested by the time used to separate free and bound material. Essentially all the labeled thyroxine was bound in less than 1.5 h after injection.

Front-ORIGIN

Liv CP T4 T3

Characterization of radiolabeled material in choroid plexus and liver.
**Thyroxine Transport in Choroid Plexus**

![Graph](image)

**FIG. 7.** Kinetics of distribution of intravenously injected [125I]iodothyronine to various tissues. The levels of [125I]iodothyronine in tissues at various times after an intravenous injection of [125I]iodothyronine was determined as described in Experimental Procedures. P, pituitary; K, kidney; L, liver; CP, choroid plexus; B, blood. Each point is the mean for three animals ± S.E.