Inhibition by Lead of Production and Secretion of Transthyretin in the Choroid Plexus: Its Relation to Thyroxine Transport at Blood–CSF Barrier

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The choroid plexus resides within brain ventricles and separates two distinct body fluids: the blood and cerebrospinal fluid (CSF) (Davson and Segal, 1996; Johanson, 1995; Smith, 1991). Aside from its primary role in CSF production and regulation, the choroid plexus manufactures and secretes the proteins for the extracellular compartment in the central nervous system (CNS). Of the proteins in the CSF, transthyretin (TTR or prealbumin) is exclusively produced and secreted by the choroid plexus (Aldred et al., 1995; Herbert et al., 1986; Nilsson et al., 1992). TTR is a 55,000-Dalton protein consisting of four identical subunits in a tetrahedral symmetry. Per unit of weight, rat choroid plexus contains 10 times more TTR mRNA than liver, and per gram of tissue synthesizes TTR 13 times faster than the liver, which is the major organ producing serum TTR (Dickson et al., 1985; Schreiber et al., 1990). CSF TTR (~15 μg/ml) makes up 25% of total CSF protein (Aldred et al., 1995). In humans, TTR serves as a major thyroid hormone binding protein in the CNS and conveys about 60–80% of CSF thyroxine (Hagen and Elliott, 1973; Herbert et al., 1986; Larsen and DeLallo, 1989). The binding of thyroxine to CSF TTR allows a fine control of the levels of thyroid hormones in the CSF and in the extracellular space of the brain. Recent evidence has suggested that repression of TTR in CSF and/or choroid plexus may lead to an alteration of the balance of thyroid hormones in the brain (Chanoine et al., 1992; Southwell et al., 1993).

Thyroid hormones have striking effects on the CNS, particularly during the developmental period (Dussault and Ruel, 1987). Deficiency of thyroid hormones during this period produces multiple morphological, biochemical, and electrophysiological alterations of neurons and neuroglia (Dussault and Ruel, 1987; Farsetti et al., 1991; Legrand, 1984; Ruiz-Marcos et al., 1979). In children, deprivation of thyroid hormones causes irreversible mental retardation (Smith et al., 1957; Giroireux et al., 1983; Legrand, 1984). Recently, Thompson (1996) has isolated and identified the genes that are expressed in response to thyroid hormones in developing rat brain. Notably, the TTR gene in the choroid plexus is expressed early in the fetal development, a phenomenon consistent with the importance of the thyroid hormones in embryonic brain development (Thomas et al., 1989; Cavallaro et al., 1993; Schreiber et al., 1995). Thus, the choroid plexus, by manufacturing and regul-
lating CSF TTR, appears to play a crucial role in regulating and mediating the delivery of thyroid hormones to the developing brain.

Previous studies have shown that the choroid plexus seques-
ters lead (Pb) to an extraordinary degree following Pb exposure (Friedheim et al., 1983; Zheng et al., 1991, for review, see Zheng, 1996). Our recent work demonstrates that disposition of Pb in rat choroid plexus is directly associated with a significant reduction in CSF concentration of TTR (Zheng et al., 1996). The synthesis of TTR by the choroidal epithelia has been suggested to mediate the transport of thyroid hormones from the blood to CSF (Chanoine et al., 1992; Dratman et al., 1991; Schreiber et al., 1990; Southwell et al., 1993). Thus, alteration of TTR concentration in CSF and/or choroid plexus by Pb exposure may influence the transport of thyroxine at the blood-CSF barrier, leading to an altered brain economy of thyroid hormones.

The purpose of this study was to test the hypothesis that Pb exposure impairs TTR production and/or secretion in the cho-
roid plexus, which may affect the transepithelial transport of thyroxine at the blood-CSF barrier. We conducted pulse-chase experiments using [35S]methionine to label the newly synthesized proteins in the cultured choroidal epithelial cells. The effect of Pb on the production and secretion of TTR was investigated by estimating the amounts of [35S]TTR that were selectively precipitated by specific TTR antibody and separated by SDS PAGE. To investigate thyroxine transport, we adapted a two-chamber transport model which was originally developed by Southwell et al. (1993). The model allows the culture media in both chambers to be separated by a barrier of confluent epithelial cells grown on a permeable membrane. By monitoring [125I]T4 in both chambers, we examined the effect of Pb exposure on transport kinetics of thyroxine across the choroidal epithelial barrier.

**MATERIALS AND METHODS**

**Materials and animals.** Chemicals were obtained from the following sources: Pb acetate, epidermal growth factor (EGF), fibroblast growth factor (FGF), gentamycin, phenylmethyl sulfonyl fluoride (PMSF), aprotinin, leupep-
tin, laminin, prostaglandin E1 (PGE1), and cycloheximide from Sigma Chemical Co. (St. Louis, MO); Dulbecco’s modified Eagle’s medium (DMEM), Hanks’ balanced salt solution (HBSS), fetal bovine serum (FBS), and antibiotic-
antimycin from Gibco Laboratories (Grand Island, NY); pronase from Cal-
Biochem-Novabiochem (La Jolla, CA); [125I]T4 (specific activity: 4.4 Ci/μmol), SOLVABLE, and EN‘HANCE autoradiography enhancer from Du Pont (Boston, MA); L-methionine, L-glutamine, and [35S]methionine (specific activity: 1218 Ci/μmol) from ICN Biomedicals (Aurora, OH); transwell-COL culture wells from Costar (Cambridge, MA). All reagents were of analytical grade, HPLC grade, or the best available pharmaceutical grade.

**Preparation of TTR antibody.** Purified rat plasma TTR and monospecific rabbit anti-rat TTR polyclonal antibody were prepared according to the meth-
ods previously described (Navab et al., 1977; Blaner, 1990). The purified rat serum TTR used as immunogen ran as two bands on an overloaded (50 μg protein) Coomassie-stained SDS-PAGE, one at approximately 14 KDa (cor-
responding to the TTR monomer) and one at 28 KDa (corresponding to the TTR dimer). Collected rabbit antiserum was divided into aliquots and frozen at −80°C until used in the present and earlier studies (Herbert et al., 1986; Episkopou et al., 1993; Tsutsumi et al., 1992; Zheng et al., 1996, 1998). The TTR antiserum was diluted (1:300) prior to use in this study.

**Choroidal epithelial cell culture.** Choroidal epithelial cells were cultured using the method established in this laboratory (Zheng et al., 1998). In short, plexus tissues were collected from Sprague–Dawley rats (4–6 weeks old, both sexes), which were purchased from Harlan Inc. (Indianapolis, IN). The plexus were dissected, washed in DMEM, chopped with scissors, and digested in Hank’s buffer containing 0.2% pronase at 37°C for 5 min. The cells were further mechanically dissociated by 7–8 forced passages through a 20-gauge needle. The dissociated cells were washed in medium A (DMEM with 100 units/ml each of penicillin, streptomycin, and gentamycin, and 0.25 μg/ml amphotericin B) twice and resuspended in normal growth medium (medium A supplemented with 10% FBS and 10 ng/ml EGF). The cells were plated in 35-mm Petri dishes (2–3 × 105 cells per dish) and cultured in a humidified incubator with 95% air–5% CO2 at 37°C. The growth medium was replaced two days after initial seeding and every other day thereafter. The culture from 5-week-old rats showed a dominant polygonal type of epithelial cells for at least 7–10 days with a doubling time about 3–4 days. A 6–8-day culture was used in this study.

For two-chamber transport studies, permeable membranes attached to the Transwell-COL culture wells were pretreated with laminin (14 μg/ml) for 10 min and allowed to air dry for at least 45 min prior to cell seeding. Aliquots (0.5 ml) of cell suspension were plated into 12-mm laminin-coated culture wells (2 × 105 cells per well). This was designated as the inner (apical) chamber. The inner chambers were then inserted into the outer (basal) cham-
bers which contained 1 ml of culture medium. The cultures continued for 48 h and the medium was changed every 2 days thereafter. The formation of an impermeable confluent monolayer occurred within 5 days after seeding and was verified by the presence of a steady electrical resistance across the membrane (120 ± 10 SD ohm per cm2) (Southwell et al., 1993; Zheng et al., 1998).

**Pulse-chase study of TTR synthesis and secretion.** Prior to Pb exposure, the cultured epithelial cells were washed 3 times with medium A. The cells were then exposed to Pb (as Pb acetate) dissolved in medium A at a final concentration of 30 μM at 37°C for 4 h. The concentration of Pb (30 μM) was chosen because preliminary studies under this experimental condition had shown that Pb at this concentration caused the maximum inhibition of T4 transport and minimum cytotoxicity. At the end of exposure, the cells were rinsed and incubated for 40 min in medium B (serum- and methionine-free medium A) to deplete the intracellular methionine pool (Wang et al., 1994). The cultured cells were pulse-labeled for 2 h in a pulse medium (medium B supplemented with 100–150 μCi/ml [35S]methionine). Following the pulse treatment, the cells were washed 3 times with the chase medium (medium B supplemented with 10 mM L-methionine and 2 mM L-glutamine). The chase phase commenced by adding 1 ml of the chase medium to each group. At the times selected, the media were removed. The cells were washed with lysis buffer (see below) and harvested using a rubber policeman.

The harvested cells were immediately mixed with 200 μl of warm lysis buffer that consisted of 50 mM Tris (pH 8.0), 150 mM NaCl, 1 mM DTT, 1 mM EDTA, 1 mM PMSF, 20 μg/ml leupeptin, 20 μg/ml aprotinin, 1% Triton X-100, 1% SDS, and 0.1% BSA. The mixture was incubated at 70°C for 15 min to ensure complete lysis. The culture medium was centrifuged at 10,000 g for 10 min to remove cell debris, after which aliquots (300 μl) of medium were mixed with 100 μl of 4X lysis buffer and incubated at 70°C for 15 min. Both cell and medium lysates were then diluted 10-fold with 150 mM NaCl and 0.1% BSA in 10 mM Tris buffer (pH 8.0) to achieve a 0.1% SDS final concentration. The diluted solutions were used for separation and quantitation of [35S]TTR described below. Another aliquot (50 μl) of cell and medium lysates were diluted with distilled, deionized water and used for protein determination.

**Immunoprecipitation and SDS-PAGE.** Aliquots (300 μl) of diluted cell lystate and culture medium were incubated with rabbit anti-rat TTR antiserum (1:300) at room temperature for 2 h. The TTR-antibody complexes were
precipitated by adding 100 μl of Protein A agarose beads (Bio-Rad) in 50 mM Tris (pH 8.0), 150 mM NaCl, 1 mM EDTA, and 0.05% Triton X-100 and incubating at 4°C overnight with constant shaking. Following centrifugation at 10,000g for 10 min, the pellets were dissolved and incubated in eluting buffer containing 0.1 M Tris (pH 6.8), 5% SDS, 5% -mercaptoethanol, 10 mM DTT, and 10% glycerol at 95°C for 5 min. The mixture was further centrifuged at 13,000g for 10 min to separate the eluted proteins from the beads. The supernatant containing primarily [35S]TTR underwent further purification.

Aliquots (20 μl) of the supernatant, purified rat TTR standard, and protein molecular weight markers were applied to two identical 8.5% SDS-polyacrylamide gels and electrophoresized under constant current of 14 KDa were excised and dissolved in 0.5 ml of SOLVABLE solution at 50°C for 3 h. Incorporation of [35S]methionine into newly synthesized TTR was then determined by scintillation counting in a Packard Tri-Carb Model 2100TB liquid scintillation counter. The other gel was immersed in 100 ml of EN'HANCE solution for 1 h for enhancement and completely dried using a Model Gel Dryer (Bio-Rad) at 70°C for 2 h. The dried gels were then exposed to Kodak Biomax MR film for 4–7 days using an intensifying screen.

**Transport of [125I]-T4 at two-chamber culture system.** Choroidal epithelial cells were plated onto a laminin-coated permeable membrane, which was on the bottom of the inner chamber. The inner chamber was immersed in the medium of the outer chamber. The formation of a confluent monolayer on the bottom of the inner chamber was immersed in the medium of the outer chamber. The outer chamber was immersed in the medium of the outer chamber. The inner chamber was immersed in the medium of the inner chamber. The inner chamber was immersed in the medium of the inner chamber.

The formation of a confluent monolayer occurred normally within 5 days after initial plating. The cells under this condition displayed a similar morphology to that observed in plastic culture dishes and survived for at least 2 weeks (Southwell et al., 1993; Zheng et al., 1998). Upon the formation of an impermeable monolayer at Day 7–8, the cells corresponding to TTR standard and molecular weight at 14 KDa were excised and dissolved in 0.5 ml of SOLVABLE solution at 50°C for 3 h. Incorporation of [125I]-T4 at two-chamber culture system.

The cells were cultured on the Transwell-COL wells were washed twice with medium C (serum-free DMEM supplemented with 5 μg/ml each of insulin and transferrin, 5 ng/ml each of sodium selenite and FGF, 10 ng/ml EDF, and 25 μg/ml PE_1). The cells were then exposed to 30 μM Pb in medium C of both chambers for 4 h. At the end of Pb exposure, [125I]-T4 was added into the outer chamber to the final concentration of 40 pM (0.18 μCi/ml). A volume (5 μl) of media in both chambers was removed at various times and determined for radioactivity using a Packard model Cobra-II gamma counter.

The method of Bradford (1976), using bovine serum albumin as the reference, was used for all protein determinations.

**Statistics.** Concentrations of TTR and proteins in cells and culture media as affected by time and Pb treatment were analyzed by two-way analysis of variance (ANOVA). When ANOVA revealed an overall treatment effect, contrast analyses were performed at individual time points using Scheffe’s multiple comparison test (Scheffe, 1967).

## RESULTS

### Expression of TTR by Cultured Epithelial Cells

When the newly synthesized proteins were pulse-labeled with [35S]methionine, 35S-labeled species that were selectively immunoprecipitated showed two major bands on SDS-PAGE gels with the molecular weight equivalent to 14,000 and 43,000 Dalton (Fig. 1). Since TTR is a 55,000-Da protein consisting of four identical subunits in a tetrahedral symmetry, the bands at 14,000 Da in fact represent a single dissociated chain of TTR subunits. As the bands at 43,000 Da were usually poorly displayed in the medium preparations, the bands may represent a nonspecific species which was present in, but not secreted by, the cells. Therefore, we chose 14,000-Da bands to monitor [35S]TTR in the subsequent studies. As shown in Fig. 1, after the pulse treatment, the newly synthesized [35S]TTR molecules increased in the culture media, while decreased in the cultured cells, suggesting that the choroidal epithelial cells in our primary culture possessed the ability to synthesize and release TTR.

### Effect of Pb on Protein and Total TTR Synthesis

When cell lysates and culture media were assayed for protein concentrations, Pb treatment had no significant effect on the concentrations of total proteins presented in either the cell lysates or culture media (Table 1).

<table>
<thead>
<tr>
<th>Chasing time (hour)</th>
<th>Control (μg/ml)</th>
<th>Pb-treated (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>839.4 ± 228.4</td>
<td>588.1 ± 69.4</td>
</tr>
<tr>
<td>0.5</td>
<td>573.0 ± 62.3</td>
<td>550.9 ± 43.6</td>
</tr>
<tr>
<td>1.0</td>
<td>603.1 ± 67.0</td>
<td>557.3 ± 75.5</td>
</tr>
<tr>
<td>2.0</td>
<td>630.5 ± 110.8</td>
<td>553.2 ± 72.9</td>
</tr>
<tr>
<td>3.0</td>
<td>485.0 ± 30.5</td>
<td>443.4 ± 53.6</td>
</tr>
</tbody>
</table>

**TABLE 1**

**Effect of Pb Exposure on Protein Concentrations in Cultures of Choroidal Epithelia in the Pulse-Chase Studies**

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Note. Protein concentrations in cell lysate or culture medium were determined by BioRad assay kit using BSA as standard. Data represent means ± SE (n = 5). Pb treatment did not significantly alter protein concentrations in cell lysate or in culture medium by two-way ANOVA.

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### Effect of Pb on Protein and Total TTR Synthesis

When cell lysates and culture media were assayed for protein concentrations, Pb treatment had no significant effect on the concentrations of total proteins presented in either the cell lysates or culture media (Table 1).

The total [35S]TTR production was estimated by summation of radioactivity in 14,000-Da bands in both cell lysates and media, and was normalized for protein concentration. Two-
way ANOVA of the data presented in Fig. 2 revealed a marginal overall inhibitory effect of Pb on total TTR ($p = 0.074$). However, Pb significantly inhibited total [35 S]TTR by 37% ($p < 0.01$) at 2 h and 34% ($p < 0.01$) at 3 h, respectively, following the chase (Fig. 2). It is noteworthy that the onset of Pb action appeared in a delayed temporal pattern. There was no significant change in total [35 S]TTR until the cells were chased for more than 1 h (Fig. 2).

**Effect of Pb on Intracellular TTR**

Figure 3A delineates the time courses of intracellular newly synthesized [35 S]-TTR in cell lysates. Intracellular [35 S]TTR in the control group appeared to decline over the 3-h chase period, indicating either a continuous secretion of [35 S]TTR molecules from the intracellular space to extracellular media or the destruction of [35 S]TTR. By linear regression of the terminal phase (0.5–3 h) of TTR concentration-time curve, the intracellular half-life ($t_{1/2}$) of the newly synthesized [35 S]TTR approximated 2.6 h in the control group (Fig. 3A). In contrast, the concentrations of cellular [35 S]TTR in the Pb-treated group were maintained at a relatively stable level. While Pb exposure resulted in an increase in overall cellular concentrations of [35 S]TTR in comparison to the controls, this was not statistically significant by two-way ANOVA ($p = 0.0929$). However, the cellular concentrations of [35 S]TTR at the later times of the chase phase were significantly higher in the Pb-treated group than those in the controls ($p < 0.01$) (Fig. 3A). Autoradiography of TTR bands on the gel also shows that Pb treatment caused an intracellular retention of [35 S]TTR (Fig. 3B).

**Inhibition of TTR Secretion by Pb**

Pb exposure suppressed the secretion of newly synthesized [35 S]TTR from epithelial cells to the culture media. Two-way ANOVA revealed that there was an overall significant statistical difference in [35 S]TTR of culture media between the Pb-treated group and the control group ($p < 0.05$, Fig. 4A). The difference was greatest at later times. For example, TTR concentration in medium at 3 h following the chase was 7.5-fold lower in Pb groups than in the controls. As depicted by autoradiography in Fig. 4B, the densities of TTR bands in Pb-treated groups were accordingly lower than those in the corresponding controls.

It is noteworthy that Pb treatment at this concentration did not significantly alter the cell viability, nor did it induce any visible morphological alterations in cultured cells (data not shown). In addition, we found that the same Pb treatment did not significantly inhibit the uptake of [35 S]methionine by the cultured choroidal epithelial cells ($p > 0.1$, $n = 3$).

**Alteration by Pb of T4 Transepithelial Transport at the Blood-CSF Barrier**

We further established a two-chamber system to study the transport kinetics of [125 I]T4 across the epithelial barrier by continuously monitoring the radioactivity in both chambers. Our earlier studies (Zheng et al., 1998) have shown that in the absence of cultured cells, addition of [125 I]T4 to the outer chamber results in a concentration-driven diffusion of [125 I]T4 between two chambers. The radioactivity eventually reaches...
the same level in both chambers, indicating an equilibrium status. In the presence of cells, when \([^{125}\text{I}]T_4\) was added to the outer chamber (contacting the basement of epithelial cells), the radioactivity migrates from the outer to inner chamber (contacting apical surface of epithelial cells) to a higher concentration in the inner chamber. The current work was carried out under similar experimental conditions to our previous study.

As shown in Fig. 5A, after addition of \([^{125}\text{I}]T_4\) into the outer chamber, the radioactivity in the inner chamber of the control group rose steadily at the early stage. There was a linear increase in radioactivity in the inner chamber during the first 6 h. This was followed by a slower ascending phase until the plateau was reached (Fig. 5A). In the Pb-treated group, the steady-state concentration \(C_{ss}\) of radiolabels in the inner chamber was 27% lower as compared to the control (Table 2). In parallel with the changes of radioactivity in the inner chamber, \([^{125}\text{I}]T_4\) in the outer chamber declined in the initial phase, indicating an uptake of \([^{125}\text{I}]T_4\) by epithelial cells (Fig. 5B). The initial uptake rate constant \(k_u\) of \([^{125}\text{I}]T_4\) in the control group approximated 0.15 h\(^{-1}\), while in the Pb-treated group, the rate constant was reduced to 0.10 h\(^{-1}\), about 33% decrease (Table 2). The steady state concentration of radiolabels in the outer chamber of the Pb-treated group was 41% higher than that of the controls. These data are consistent with the notion that exposure of the cells to Pb in the culture medium repressed the transport of \([^{125}\text{I}]T_4\) radioactivity from the outer to the inner chamber.

### TABLE 2

<table>
<thead>
<tr>
<th>Pb Exposure on Transport of ([^{125}\text{I}]T_4) at the Cultured Choroidal Epithelial Layer</th>
<th>Blank (no cells)</th>
<th>Control (with cells)</th>
<th>Pb (30 (\mu\text{M}) with cells)</th>
</tr>
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<tbody>
<tr>
<td><strong>Inner chamber</strong></td>
<td></td>
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<tr>
<td>(C_{max}) (dpm/5 (\mu\text{l})) &amp; 838 ± 16.4 &amp; 1368 ± 61.1 &amp; 992 ± 12.8*</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(k_i) (h(^{-1})) &amp; 0.25 &amp; 0.21</td>
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<tr>
<td><strong>Outer chamber</strong></td>
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<tr>
<td>(C_{max}) (dpm/5 (\mu\text{l})) &amp; 739 ± 2.6 &amp; 462 ± 21.5 &amp; 650 ± 39.1*</td>
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</tr>
<tr>
<td>(k_u) (h(^{-1})) &amp; 0.15 &amp; 0.10</td>
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</table>

*Note. Data present means ± SD, \(n = 3\) separate assays. *\(p < 0.05\) as compared to the control group. \(k_i\), initial release rate constant; \(k_u\), initial uptake rate constant.*
DISCUSSION

Our previous studies by RT-PCR analysis (reverse-transcriptase polymerase chain reaction) and immunocytochemistry have demonstrated that the cultured choroidal epithelial cells established in this laboratory possess specific TTR mRNA and synthesize and secrete TTR proteins (Zheng et al., 1998). The pulse-chase studies presented in this report further corroborate that these cultured epithelial cells dynamically produce and secrete TTR (Fig. 1). Our observations support the view that the choroid plexus epithelia serve as an important site of TTR production in the CNS (Aldred et al., 1995; Herbert et al., 1986; Schreiber et al., 1995).

Our initial studies in rats suggest that the sequestration of Pb in the choroid plexus following chronic Pb exposure accompanied a diminished CSF TTR (Zheng et al., 1996). The present studies extend our previous observations and further show that Pb exposure significantly inhibits the total TTR (cells + media) production by cultured choroidal epithelial cells (Fig. 2), while the concentrations of total protein in both cultured cells and media were not significantly altered by Pb exposure (Table 1). The cytotoxicity of Pb has been associated with the suppression of biosynthesis of a number of cellular proteins, largely owing to the strong binding of the metal to cellular sulfhydryl groups (Goering, 1993). As we did not examine the expression of TTR mRNA before and after Pb treatment, the questions as to how and at what stage Pb influences TTR biosynthesis, as well as possible questions regarding gene regulation processes in the cultured cells, remain unanswered.

Pb exposure significantly inhibits the rate and amount of newly synthesized [35S]TTR secreted into the cultured media (Fig. 4). The effect of Pb on TTR secretion could be due to a direct interaction of Pb ions on TTR secretion processes. The decline of intracellular TTR concentration could occur as the results of a continuous outflow of the newly synthesized [35S]TTR to the culture medium, coupled with intracellular enzymatic or nonenzymatic degradation of TTR. Accordingly, a blockage of TTR secretion should bring about the retention of TTR within the cells. This, indeed, appears to be the case in our studies. While Pb treatment decreased the secretion rate of new [35S]TTR molecules into the extracellular space (Fig. 4A), the intracellular TTR following Pb exposure remained at a constant level (Fig. 3A), which reflects a retarded outflow for newly synthesized [35S]TTR to exit to the culture media.

Whereas the internalization of TTR is reportedly mediated by TTR receptors on the cell surface (Divino and Schussler, 1990), the processes that govern TTR secretion from the plexus to the CSF are as yet unknown. Accordingly, the mechanism(s) whereby Pb interacts with TTR secretion remains a subject for speculation. We recently observed that Pb potently inhibited the activity of mitochondrial complex-I (NADH-ubiquinone reductase), the enzyme that is critical to the energy production in mitochondrial respiratory chain (unpublished data). Others have reported that Pb inhibits cellular ATPases (Chanez et al., 1986; Rajanna et al., 1991). In addition, we have demonstrated that Pb exposure promotes the translocation of protein kinase C (PKC) from the cytosol to membrane in the choroid plexus (Zhao et al., 1998). We do not know whether the alterations in energy production or PKC activity by Pb underlie, either directly or indirectly, the inhibitory action of Pb on TTR secretion. It is noteworthy, however, that PKC plays a crucial role in a variety of cellular functions such as in transducing cellular signals, in regulating membrane ion channels, and in controlling phosphorylation of key enzymes and proteins (Nishizuka, 1986; Bressler et al., 1996).

The reduction in [35S]TTR secretion could also be attributable to an arrested de novo biosynthesis or an increased rate of intracellular degradation of TTR in Pb-exposed cells. The lack of sufficient intracellular TTR would then lead to less TTR molecules available for secretion. As shown in Fig. 2, Pb treatment inhibited the total TTR, particularly at later time. Interestingly, this delayed onset of Pb effect on TTR concentration (Fig. 2) coincided with a greater suppression of TTR secretion observed at later times of the chase phase (Fig. 4A). Thus, the inhibitory effect of Pb on TTR synthesis might contribute, at least in part, to the diminished secretion of TTR. In addition, the possibility that Pb may directly bind to TTR molecules needs to be considered and substantiated in future studies.

The intracellular [35S]TTR present in Pb-treated cells, however, did not seem to decline greatly, but rather stayed in a relatively constant level (Fig. 3). This was expected. If Pb had only suppressed the secretion process without influencing the TTR production, the cellular TTR would have continuously built up and led to a time-related increase in intracellular [35S]TTR. Based on the results in Fig. 3, a constant level of cellular TTR in the Pb-treated group may reflect a compromised effect of Pb on the inhibition of TTR secretion (resulting in cellular TTR increases) and the repression of TTR production (resulting in cellular TTR decreases). Taken in conjunction, these studies establish that the abnormal dynamics in TTR secretion and/or production in the choroid plexus occurs as a consequence of Pb sequestration in this blood–CSF barrier.

The bulk of evidence from the past two decades suggests that TTR is importantly involved in the transport of thyroxine from the blood to the cerebral compartment (Chanoine et al., 1992; Ingenbleek and Young, 1994; Nilsson et al., 1992; Schreiber et al., 1990, 1995). Chanoine et al. (1992) have shown that in wild-type rats, the injection with a competitive inhibitor for T4-TTR binding results in a significant reduction in the percentage of injected [125I]T4 in the choroid plexus, CSF, cerebral cortex, and cerebellum. Nilsson et al. (1992) reported that rats administered with cycloheximide (a protein synthesis inhibitor) displayed an altered pattern of T4 distribution in the choroid plexus and other brain regions. As the choroid plexus expresses TTR genes early in the fetal development (Thomas et al., 1989; Cavallaro et al., 1993; Schreiber...
et al., 1995), expression of TTR in this tissue may help to control thyroid hormone homeostasis in the CNS from early in fetal development throughout life and play a fundamental role in brain development. This possibility is argued against by the works of Palha et al. (1994), which indicate that TTR-deficient mice are euthyroid and “phenotypically normal.” However, it is possible that these congenitally TTR-deficient mice may not serve as an appropriate model for study of thyroid hormone delivery to the brain, since expression of the thyroid-binding globulin gene may seem to be turned on at early stage of embryo development to compensate for the loss of TTR expression. Whether this process in TTR knock-out mice would also occur in wild-type rats born with developed thyroid regulatory system but under excess Pb exposure is unknown. It is also possible that other compensatory mechanisms might give rise to the “normal” phenotype of these mice. Notably, whether this mutant strain of mice are “phenotypically normal” with regard to the behavioral/neurologic parameters remains uninvestigated. It is not clear whether the absence of TTR in mice has more subtle and still unexplored effects on brain thyroid hormone economy similar to those observed in rats by Chanoine et al. (1992) and Nilsson et al. (1992). Such subtle differences in brain thyroid hormone economy may underlie the cognitive and/or behavioral deficits not being investigated in the TTR-deficient mice by Palha et al. (1994).

The data from our chamber study, which is a reproduction of that of Southwell et al. (1993), showed that the presence of Pb in the culture medium markedly inhibited the transport of $^{125}$I$T_4$ across the choroidal epithelial barrier (Fig. 5 and Table 2). The reduction of TTR production/secretion caused by Pb treatment (Figs. 2–4) could explain the effect of Pb on $T_4$ transport at this barrier. Hence, these studies are consistent with the hypothesis that normal synthesis and secretion of TTR by the choroid plexus epithelia are needed to maintain normal $T_4$ transport at the blood-CSF barrier. If this in vitro observation can be extended to the in vivo situation, then distortion of TTR production/secretion in the choroid plexus by Pb exposure would be expected to impair the transport of thyroid hormones from the blood to the cerebral compartment. Such an impairment could account for the known loss of cognitive abilities observed in Pb-poisoned children. This notion, however, will require further experimental proof before it can be accepted.

In conclusion, the results of current pulse-chase studies revealed that in vitro Pb exposure reduced the production and secretion of TTR by cultured choroid plexus epithelial cells. Experiments using a two-chamber polarized cell culture model of the blood-CSF barrier further demonstrated that this reduced production and secretion of TTR upon Pb exposure is coincident with reduced transport of $^{125}$I$T_4$ across the model choroidal epithelial barrier. This association between lessened synthesis and secretion of TTR and lessened $T_4$ transport at the blood-CSF barrier, along with the question of whether the possible impaired transport of $T_4$ via TTR ultimately affects whole brain economy of thyroid hormones which these observations raise, deserves further investigation.

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REFERENCES


