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TRANSPORT OF L-[^{125}I]THYROXINE BY IN SITU PERFUSED OVINE CHOROID PLEXUS: INHIBITION BY LEAD EXPOSURE

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Abstract

Lead (Pb) exposure hinders brain development in children by mechanisms that remain unknown. Previous evidence shows that sequestration of Pb in the choroid plexus lowers the production and secretion of transthyretin (TTR), a thyroxine (T_4) transport protein, from the choroid plexus into the cerebrospinal fluid (CSF). This study was undertaken to characterize the uptake kinetics of T_4 by the choroid plexus and to determine if in vivo Pb exposure altered the T_4 uptake in an in situ perfused ovine choroid plexus model. Sheep received ip injections of Pb acetate (20 mg Pb/kg) or Na acetate (as the controls) every 48 h for a period of 16 d. The [^{125}I]T_4 uptake was determined by a paired-tracer perfusion method using 0.5μCi [^{125}I]T_4 and 2 μ Ci [^{14}C]mannitol at various concentrations of unlabeled T_4 (trace to 20 μM). The flux of [^{125}I]T_4 into the choroid plexus followed Michaelis-Menten kinetics with the maximum flux (V_{max}) of 56.6 nmol/min/g and half-saturation constant (K_m) of 10.7 μmol/L, suggesting an evident saturable influx of T_4 into the choroid epithelium. In vivo Pb exposure in these sheep resulted in a significant accumulation of Pb in the choroid plexus and hippocampus. Pb treatment diminished the V_{max} *by 63.7% of control, but did not alter* K_m. *The maximal cellular uptake (*U_{max}*) and net uptake (*U_{net}) in Pb-treated animals were 2.1-fold and 1.9-fold, respectively, lower than those of control. Exposure to Pb, however, did not significantly change the flow rate through the choroid plexus. Data suggest that the choroid plexus may serve as a significant site for T_4 transport into the CSF, and Pb exposure may hinder the influx of T_4 from the blood into the choroid plexus.

The brain requires, but does not synthesize, thyroid hormones for its normal development, maturation, metabolism, and function (Dussault & Ruel, 1987; Legrand, 1984). Although thyroxine (T_4), a major type of thyroid hormone in blood and cerebrospinal fluid (CSF) circulation, is fairly lipophilic, the data from human and animal studies indicate that the transport of thyroxine between blood and the cerebrospinal fluid is restricted and does not follow simple diffusion mechanism responsive to mass balance (Ingenbleek & Young, 1994;

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Kirkegaard & Faber, 1991; Schreiber et al., 1990; Zheng et al., 2001). Thus, it has been suggested that the blood-brain barrier and/or blood-CSF barrier controls thyroxine availability to the cerebral compartment (Kirkegaard & Faber, 1991; van Raaij et al., 1994).

Thyroxine enters the CSF and brain parenchyma by two possible routes. The hormone may cross the blood-brain barrier located at the cerebral capillary endothelium into the brain extracellular fluid (ECF) and then by diffusion enter the CSF. This proposed mechanism is supported by the observation of a wide distribution of radiolabeled $[^{125}I]T_4$ throughout the brain after intravenous injection, but not with intracerebroventricular (icv) injection of free $[^{125}I]T_4$ (Blay et al., 1993), although the possibility of interference from the residual $[^{125}I]T_4$ adhering in the cerebral capillaries was not completely excluded in that study. Dratman et al. (1991) also observed that the brain acquires thyroid hormone mainly through the blood-brain barrier.

Besides the blood-brain barrier route, thyroxine may be taken up in the choroid plexus from the blood via fenestrated capillaries and the loose stroma of choroid plexus into choroidal epithelial cells. Within the choroidal epithelia, thyroxine binds with a high affinity to transthyretin (TTR), a major carrier protein for T_4 in the CSF that is exclusively synthesized and secreted by the choroid plexus into the cerebrospinal fluid. Since CSF TTR (~15 μg/ml) makes up 25% of total CSF protein (Aldred et al., 1995) and conveys about 60–80% of CSF thyroxine (Hagen & Elliott, 1973; Herbert et al., 1986; Larsen & DeLallo, 1989), the uptake of thyroxine into the choroid plexus and the subsequent secretion of T_4 along with TTR into the CSF may constitute an alternative pathway for T_4 transport into the brain (Chanoine et al., 1992; Schreiber et al., 1990; Southwell et al., 1993). However, the quantitative extent to which the blood-brain barrier and blood-CSF barrier contribute to T_4 transport to the brain is poorly understood.

Thyroid hormones have marked effects on the CNS, particularly during the developmental period (Dussault & Ruel, 1987). Deficiency of thyroid hormones during this period produces multiple morphological, biochemical, and electrophysiological alterations of neurons and neuroglia (Dussault & Ruel, 1987; Farsetti et al., 1991; Legrand, 1984; Ruiz-Marcos et al., 1979). In children, deprivation of thyroid hormones produces irreversible mental retardation (Ishaik et al., 2000; Legrand, 1984; Smith et al., 1957). It is known that certain environmental toxicants alter CNS homeostasis of thyroid hormones (Morse et al., 1996; Zoeller & Crofton, 2000).

Previous studies have shown that the choroid plexus sequesters lead (Pb) to a significant degree following Pb exposure (Friedheim et al., 1983; Zheng et al., 1991; Zheng, 2001). Recent evidence further demonstrates that accumulation of Pb in the choroid plexus suppresses TTR production and its secretion into the CNS (Zheng et al., 1996, 1999). Since the endpoint of Pb neurotoxicity resembles that of subtle hypothyroidism in children, namely, a marked reduction in myelination seen in neonatal hypothyroidism (Legrand, 1984) and a notable demyelination in both peripheral and central pathways in Pb-poisoned infants (Khateeb et al., 1988; Wong et al., 1991), it was hypothesized that Pb may alter thyroid hormone status of the CNS by impairing thyroxine transport either into or within the cerebral compartment. This may, in turn, damage brain development and functional maturation and account, in part, for Pb-induced neurotoxicity.

The current understanding on how brain regulates thyroid homeostasis is incomplete. Two fundamental questions remained unanswered. First, the kinetic aspect of T_4 transport by the blood-CSF barrier has never been characterized, partly owing to technical difficulties in establishing a perfusion model in a rather small tissue. Second, the effect of in vivo exposure to Pb on the transport of T_4 by the blood-CSF barrier has not been documented. Thus, the

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purpose of this study was to (1) characterize the transport properties of T_4 between blood and CSF in isolation from the exchange that occurs across the blood-brain barrier by using in situ perfused choroid plexus of sheep, and (2) determine whether acute exposure of sheep to Pb in vivo altered the uptake of T_4 by the choroid plexus.

MATERIALS AND METHODS

Chemicals

All reagents and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). [^{125}I]Thyroxine (specific activity: 116Ci/mmol) and [^{14}C]mannitol (specific activity: 15–30 Ci/mmol) from Du Pont NEN Life Science Products (London). All reagents were of analytical grade, high-performance liquid chromatography (HPLC) grade, or the best available pharmaceutical grade.

Animals and Pretreatment

Sheep (Clun Forest strain, either gender) weighing 20–25 kg upon arrival were assigned to 2 groups such that the group mean body weights were comparable. The animals were housed in a Royal Veterinary College and allowed to have free access to water and food. Following the quarantine period of 3 d, the animals in Pb-treated group received ip doses of Pb acetate (20 mg Pb/kg) and the control group received Na acetate every 2 d for a period of 16 d. Both control and Pb-treated sheep underwent experimentation at 24 h after the last injection.

Preparation of In Situ Perfused Choroid Plexus Model

Prior to the experiment, each sheep was anesthetized with sodium thiopental (20 mg/kg, iv) and heparinized (10,000 U, iv). CSF samples (about 2–5 ml) were obtained through a butterfly needle (14-gauge) attached to polyethylene tubing. The needle was inserted between the protruberance and the spine of the atlas. CSF samples visibly contaminated with blood were discarded and those free of blood were used for the biochemical analyses. Sheep were then decapitated, brains removed rapidly, and internal carotid arteries cannulated immediately.

The perfusion system was started at a flow rate of 0.5–1.5 ml/min, with the perfusate being directed toward the anterior choroidal arteries by ligation of the other branches on the circle of Willis. The cerebral ventricles were then opened and the exposed choroid plexus in the lateral ventricle was superperfused with artificial CSF (Preston & Segal, 1990). The perfusion fluid draining from the choroid plexuses was collected by a cannula inserted into the great vein of Galen (Deane & Segal, 1985). A simplified diagram of this in situ model is shown in Figure 1. All experiments were performed in compliance with the regulation of the Animals (Scientific Procedures) Act 1986, UK, and approved by Columbia University Internal Review Board.

The perfusate was a modified mammalian Ringer solution (Preston & Segal, 1990) containing 4 g/dl dextran 70 (Gentran), as the colloid osmotic agent, and 0.05 g/dl bovine serum albumin to maintain the integrity of the capillary wall. The artificial CSF was protein and dextran free. Both solutions were saturated with 95% O_2 and 5% CO_2, at pH 7.4, and warmed to 37 °C, the blood perfusate being filtered and debubbled prior to entering the choroid plexuses. The preparation was kept warm with a water jacket and by an external heat source; the temperature and pressure were monitored continuously. The preparation was viable for 2–3 h (Preston & Segal, 1990).

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Paired Tracer Method for Studying Blood to Plexus Transport

A paired-tracer (or single-circulation) method for studying the influx of molecules from the blood side to the choroid plexus epithelium has been described in previous reports (Preston & Segal, 1992; Segal et al., 1990). Briefly, cellular uptake of T_4 by the choroid plexus from the blood was determined from the ratio of the recovery of $[^{125}I]T_4$ to the recovery of $[^{14}C]$mannitol, a nontransported extracellular marker (Deane & Segal, 1985), in choroidal venous outflow. A calibrated “side loop” of the perfusion system was filled with a 100-μl bolus of Ringer solution containing 0.5 μCi $[^{125}I]T_4$ (4.3 fmol), various concentrations of unlabeled T_4, and 2 μCi of $[^{14}C]$mannitol (0.1 nmol). By a system of taps, the flow of perfusate was directed into this side loop and the bolus was driven by the flow of perfusate toward either side of the choroid plexus without a rise in perfusion pressure. Approximately 20 s later, to allow for the bolus to reach the plexus, a “run” of 20 sequential 1-drop samples of venous effluent were collected in about 60 s followed by a continuous 4-min collection. Each brain preparation (both sides of the cannulated choroid plexus) could be used for 10–15 such runs. The final 4-min collection was used to calculate the flow rate. An aliquot (3 ml) of scintillation fluid (Ecoscint, National Diagnostics) was added to each collected drop, 50 μl of final 4-min effluent, and 10 μl of the remaining injection bolus. Radioactivities were then counted using an LKB Rackbeta Spectral 1219 and expressed as disintegrations per minute (dpm) using internal stored quench curves.

The recovered $[^{14}C]$mannitol and $[^{125}I]T_4$ in each venous drop was expressed as a percentage of the $[^{14}C]$mannitol and $[^{125}I]T_4$ injected in the 100-μl bolus (% recovered). For any given drop the recovery of $[^{125}I]T_4$ from the choroid plexus was less than the recovery of $[^{14}C]$mannitol, but peak recovery of both isotopes was simultaneous. The percentage uptake of $[^{125}I]T_4$ relative to mannitol, that is, U%, was calculated for each drop:

$$\mathrm{U\%}\left(\left[^{125}\mathrm{I}\right]\mathrm{T}_4\right)=\frac{\%\left[^{14}\mathrm{C}\right]\mathrm{mannitol\ \ recovered}-\%\left[^{125}\mathrm{I}\right]\mathrm{T4}}{\%\left[^{14}\mathrm{C}\right]\mathrm{mannitol\ \ recovered}}\times 100$$

The U% values for those samples containing the highest levels of recovered radioactivity were averaged to give the maximal cellular uptake (U_{max}) of $[^{125}I]T_4$ for that run.

In addition, the net uptake (U_{net}) over the whole run and final 4-min sample was calculated:

$$U_{net}\left(\left[^{125}\mathrm{I}\right]\mathrm{T}_4\right)=\frac{\Sigma\left[^{14}\mathrm{C}\right]\mathrm{mannitol\ \ recovered}-\Sigma\left[^{125}\mathrm{I}\right]\mathrm{T4}}{\Sigma\left[^{14}\mathrm{C}\right]\mathrm{mannitol\ \ recovered}}\times 100$$

where the sums are of tracer recoveries for the whole run plus the final 4-min sample.

The kinetic study of $[^{125}I]T_4$ uptake was performed under conditions in which the plexuses were perfused with different concentrations of unlabeled T_4. A stock solution of unlabeled T_4 (1 m*M*) was made fresh daily by dissolving the T_4 powder in 0.5 ml of 0.1 m*M* NaOH followed by dilution in total 20 ml saline. The stock solution was further diluted to appropriate concentrations with saline immediately before experimentation so that only an aliquot (2–5 μl) of the diluents was added into the perfusate. The concentration of unlabeled T_4 in perfusate varied from 0.015 to 20 μ*M* in a 100-μl bolus. Under these conditions the 100-μl injected bolus contained both labeled and unlabeled T_4 and tracer amounts of $[^{14}C]$mannitol. The bolus underwent mixing with the main perfusion fluid before reaching the plexuses, so the final concentration of T_4 was estimated by calculating a dilution factor (usually 5–7) based on the passage of $[^{14}C]$mannitol through the plexuses, as described by

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Segal et al. (1990). For example, a 100-μl injected bolus containing 0.15 μ*M* T_4 would reach the plexuses at the actual concentration of 0.023 μ*M* with an expected dilution factor of 6.5.

The unidirectional flux of $[^{125}I]T_4$ (μmol/min/g) was calculated, using the U_{max} values, from the following equation:

$$\text{Flux}=-F\cdot ln\left(1-U_{max}\right)\cdot S$$

where *F* is the perfusate flow rate (ml/min/g) and *S* the unlabeled T_4 concentration (μ*M*). The average weight of sheep choroid plexus is 0.195±SD 0.08 g (*n*=100), which was taken from previous studies (Segal et al., 1990).

To calculate the V_{max} and K_m, the flux values in each run of all experiments were plotted against the actual concentration of T_4 in each corresponding run. The data sets were evaluated by a kinetic analysis software package, PKAnalyst (MicroMath, Inc., Salt Lake City, UT). A concentration-effect math model that simulates Michaelis-Menten relationship was used to fit the observed data from which to estimate V_{max} and K_m:

$$E=E_{min}+\frac{\left(E_{max}-E_{min}\right)\times Conc^{p}}{C_{50}^{p}+Conc^{p}}$$

where *E* represents the flux; E_{min}, the minimum flux; E_{max}, the maximum flux (i.e., V_{max}) that is asymptotically reached at high doses; C_{50}, the concentration that elicits 50% of the maximum flux (i.e., K_m); and *P* (power), the parameter that determines the shape of the curve (PKAnalyst Users Manual).

Atomic Absorption Spectrophotometry Analysis

Pb concentrations in the blood, CSF, choroid plexus, frontal cortex, hippocampus, and cerebellum were determined by a flameless graphite furnace atomic absorption spectrophotometry (AAS). Tissues were combusted in acid-washed crucibles at 800 °C for 3–4 h. The crucibles were then rinsed with 200 μl of 0.5% nitric acid (HNO_3)/0.2% ammonium phosphate ($NH_4H_2PO_4$) and transferred to autosampler vials. The CSF samples were diluted 40-fold with 0.5% nitric acid/0.2% ammonium phosphate solution in autosampler vials immediately before AAS to avoid contamination. A Perkin-Elmer model 3030 Zeeman AAS, equipped with an HGA-600 graphite furnace, was used for quantification. The detection limit for this method was 1.35 ng Pb/ml assay solution. Blood Pb concentrations (BPb) were determined using the procedure of Fernandez and Hilligoss (1982) as reported in our previous studies (Zheng et al., 1996, 1999, 2001).

Statistics

Statistical analysis for comparison of two means was performed using Student's *t*-test. In all cases, a probability level of $p < .05$ was considered as the criterion of significance.

RESULTS

Uptake of $[^{125}I]T_4$ from Blood to the Choroid Plexus

Using the paired-tracer method, the steady state extraction of $[^{125}I]T_4$ relative to $[^{14}C]$mannitol at the basolateral face of the perfused choroid plexus was examined. A typical example of the $[^{125}I]T_4$ fractional extraction measured during one run (a single drop per sample collected for 20 samples) is shown in Figure 2. On entering the choroidal vessels, the

recovery of [^{14}C]mannitol arose steadfastly to reach the maximum values, suggesting that mannitol diffused across the fenestrated capillaries into the extracellular space of the choroid plexus. The remainder diffused back into the circulation and was recovered in the collected drops (Figure 2A). [^{125}I]T_4 injected in the same bolus that accompanied the mannitol was also recovered in the collected drops, but the percentage of recovery of T_4 was smaller than extracellular marker mannitol, although T_4 had access to the same compartments as mannitol. This extra loss of T_4 represents the cellular uptake of T_4 by the choroidal epithelial cells. Figure 2B shows the uptake of [^{125}I]T_4 into the choroid plexus expressed as the percent of recovery of [^{125}I]T_4 relative to that of mannitol; this corrects for nonspecific loss of T_4 via the extracellular distribution. The peak values of the uptake, that is, the region where the greatest recovery occurred, were then averaged (as shown by points joined by a line in Figure 2B) to give rise to the value of U_{max}.

Pb in Blood, CSF, and Selected Brain Regions

Following Pb exposure, the mean blood Pb concentration (BPb) was significantly increased from 2 μg/dl in the control group to 51 μg/dl in the Pb-treated group, an increase of 24-fold. Pb concentrations in the CSF were also significantly increased (Figure 3). Sheep under this treatment regimen showed a significant accumulation of Pb in the choroid plexus (1.5-fold higher than controls) and hippocampus (2.5-fold higher than controls). Pb concentrations in other brain regions did not seem to increase significantly (Table 1), possibly due to small numbers of samples in each group.

Inhibition of T_4 Transport Following In Vivo Pb Exposure

The uptake of T_4 at the blood face of the tissue was measured at various concentrations of the unlabeled hormone. Figure 4 delineates the kinetics of [^{125}I]T_4 uptake by the choroid plexus where the unidirectional flux of [^{125}I]T_4 in each run was plotted against the actual T_4 concentration in that run. The model-fit kinetics, as judged by the best correlation coefficient (r) among various models, are presented as the line curves in Figure 4 and the corresponding parameters are summarized in Table 2.

In general, the uptake of [^{125}I]T_4 by the choroid plexus followed Michaelis-Menten type kinetics, indicating the presence of a saturable uptake mechanism for T_4. Pb exposure substantially suppressed the maximum flux of T_4 to the choroid plexus (V_{max}) by 2.8-fold (Table 2). However, the half-saturation constant of the flux (K_m) was not affected by Pb treatment.

At a perfusate concentration of 20 nM, which is close to normal T_4 concentrations in serum (Morse et al., 1996), Pb treatment significantly inhibited U_{max} by 2.1-fold. Similarly, the net uptake (U_{net}) of [^{124}I]T_4 in Pb-treated animals was reduced by 1.9-fold as compared to controls (Figure 5). These results demonstrate that in vivo Pb exposure inhibited the flux of T_4 from the blood to the choroid plexus.

Effect of Pb on the Effluent Flow from the Choroid Plexus

The blood flow rate to the choroid plexus is critical to the delivery of materials to the basolateral side of the choroidal epithelia. A decreased flow, due to edema or declined viability of choroidal capillary function during the experiment, could impede T_4 from reaching the choroidal epithelia, the factors that possibly contribute to the inhibitory effect of Pb on T_4 transport by the choroid plexus. Thus, the rate of steady state effluent flows was examined in the venous outflow by averaging the effluent volumes during the last 4-min collection at the end of each run while maintaining the perfusion inflow at a constant rate. The flow rate was further normalized by the average weight of the sheep plexus tissue

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(0.195g ± 0.08 SD, Segal et al., 1990), and expressed as R_{EF} (ml/min/g). The R_{EF} was not statistically significantly different between Pb and control groups (Figure 6).

DISCUSSION

The in situ perfused sheep choroid plexus model offers several advantages as an ideal tool for studying transport of molecules from the blood to the CSF. Unlike the in vivo CSF sampling method, where the CSF concentrations of substances reflect the dynamic balance of transport between the blood-brain barrier and blood-CSF barrier, the in situ perfused choroid plexus excludes the inference from the blood-brain barrier and enables the characterization of the kinetic behavior of given molecules at the blood-CSF barrier. Since the tissue under investigation remains intact in the brain, and the molecule investigated enters the plexus directly via the blood interface, this preparation provides a better similarity to real-life choroid plexus than in vitro incubation of dissected plexus tissues or using cultured choroidal epithelial cells. In addition, introducing the dual-tracer technique corrects the nonspecific loss of T_4, making it possible to calculate the unique uptake (or removal) kinetics of T_4 by the choroid plexus. The current study with toxic metal Pb further indicates that this model may well serve the purpose to investigate the functional alterations of materials transported by the choroid plexus following in vivo exposure to toxicants.

The data presented in this report demonstrate uptake of $[^{125}I]T_4$ from the perfusate to the choroid plexus. The uptake of T_4 relative to mannitol is a measure of uptake primarily by the cells of the choroidal epithelium, since the capillaries of the choroid plexus are fenestrated, allowing movement of the relatively small molecules out of the vascular system. From the kinetics point of view, the uptake of T_4 by the choroidal epithelium appeared to follow a saturable process; the flux of T_4 increased as T_4 concentrations in the perfusate rose and eventually reached a plateau. The high uptake values of both U_{max} and U_{net} at physiological levels suggest a ubiquitous influx of T_4 into the choroidal epithelium. These observations support the view that the choroid plexus epithelia may serve as an important site for T_4 transport to the CNS (Aldred et al., 1995; Herbert et al., 1986).

While a saturable T_4 uptake by the plexus appeared evident, the mechanism(s) whereby the choroidal epithelium takes up T_4 from the bloodstream remain uncertain. The studies with $[^{125}I]T_3$ in the similar perfused sheep plexus model have shown an energy-independent, carrier-mediated transport of T_3 at the choroid plexus. T_3 transport also has some cross-competition with large neutral amino acids (Preston & Segal, 1992). However, earlier studies by Dickson et al. (1987) using the in vitro incubated choroid plexus failed to demonstrate a carrier-mediated uptake of T_4 at the choroid plexus. Schreiber et al. suggested that the choroid plexus synthesizes thyroid hormone carrying protein transthyretin (TTR), which binds to T_4 and is only secreted into the CSF (Schreiber et al., 1990; Southwell et al., 1993). As T_4 is fairly lipid soluble with a high oil-water partition coefficient (Schreiber et al., 2001), T_4 molecules may cross the blood side of the plexus by a concentration gradient and enter the cytoplasm of the cells, where the molecules are bound to newly synthesized TTR. The unidirectional secretion of TTR toward the CSF then relocates T_4 in the CSF compartment. Thus, it seems possible that the production of TTR by the choroid plexus may serve as the rate-limiting step in transport of T_4 from the plexus cytoplasm to the CSF.

Following in vivo Pb exposure, the sheep had an average blood Pb concentration of 51 μg/dl. In agreement with previous reports (Friedheim et al., 1983; Manton et al., 1984; Zheng et al., 1991), Pb exposure led to a significant accumulation of this metal in the choroid plexus. In addition, control animals had Pb nearly three to four times higher in the choroid plexus than in other brain regions examined. Moreover, these results confirm the observation in

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humans and rats that upon exposure Pb mainly accumulated in the hippocampus, an area that coordinates learning and memory (Campbell et al., 1982; Scheuhammer & Cherian, 1982).

Pb exposure reduced the uptake of $[^{125}I]T_4$ by the choroid plexus from the perfusate. The U_{max}, the measure of maximal cellular uptake, was reduced about twofold in Pb-treated animals. The overall net uptake (U_{net}), which takes into account the backflux of T_4 during the entire perfusion period, was also markedly reduced. Kinetic analyses further reveal that while Pb exposure reduced V_{max}, it did not affect Michaelis-Menten constant, K_m, suggesting that Pb did not directly compete with T_4 for uptake. Several factors may contribute to Pb-induced reduction of T_4 transport by the choroid plexus. Previous in vivo results in rats suggest that the sequestration of Pb in the choroid plexus following chronic Pb exposure is associated with a diminished concentrations of TTR in the CSF (Zheng et al., 1996). A more recent human study also indicates an inverse association between TTR concentrations and Pb levels in the CSF (Zheng et al., 2001). In cultured primary choroidal epithelial cells using a pulse-chase technique, it was further shown that Pb exposure significantly inhibits de novo biosynthesis of $[^{35}S]$TTR by the choroidal epithelia and impedes the rate of secretion of $[^{35}S]$TTR into the CSF (Zheng et al., 1999). In the current study, TTR concentrations in the CSF were not directly determined. Thus, a direct correlation between the steady state concentrations of TTR and Pb in the CSF cannot be established. Nonetheless, it appears possible that Pb exposure may affect the plexus's ability in producing TTR. The lack of sufficient intracellular TTR would then lead to lower amounts of TTR molecules available for T_4 transport toward the CSF.

The influx of materials into the choroid plexus is also dependent upon the flow rate that proceeds through the plexus vasculature. A reduced flow rate to the choroid plexus, either due to the direct damage of the vascular structure by in vivo chemical exposure, or due to a cumulative injury to the choroidal capillary during a persistent in situ perfusion, could greatly affect the influx of T_4 into the choroid plexus. In the current study, the average effluent perfusion rate was not affected by Pb treatment. Thus, it seems unlikely that a reduction in T_4 influx is due to the effect of Pb on the flow rate through the choroid plexus. It is remotely possible that Pb may interact with other unknown molecular and cellular processes involving T_4 uptake by choroidal epithelium.

In conclusion, the current results show that the choroid plexus readily took up T_4 from the blood compartment, lending itself to be a significant site for T_4 transport to the CNS. In vivo Pb exposure greatly reduced the uptake of T_4 from the blood into the choroid plexus. This suggests that the toxic effects of Pb on TTR production and secretion may contribute to the reduced transport of $[^{125}I]T_4$ by the blood-CSF barrier.

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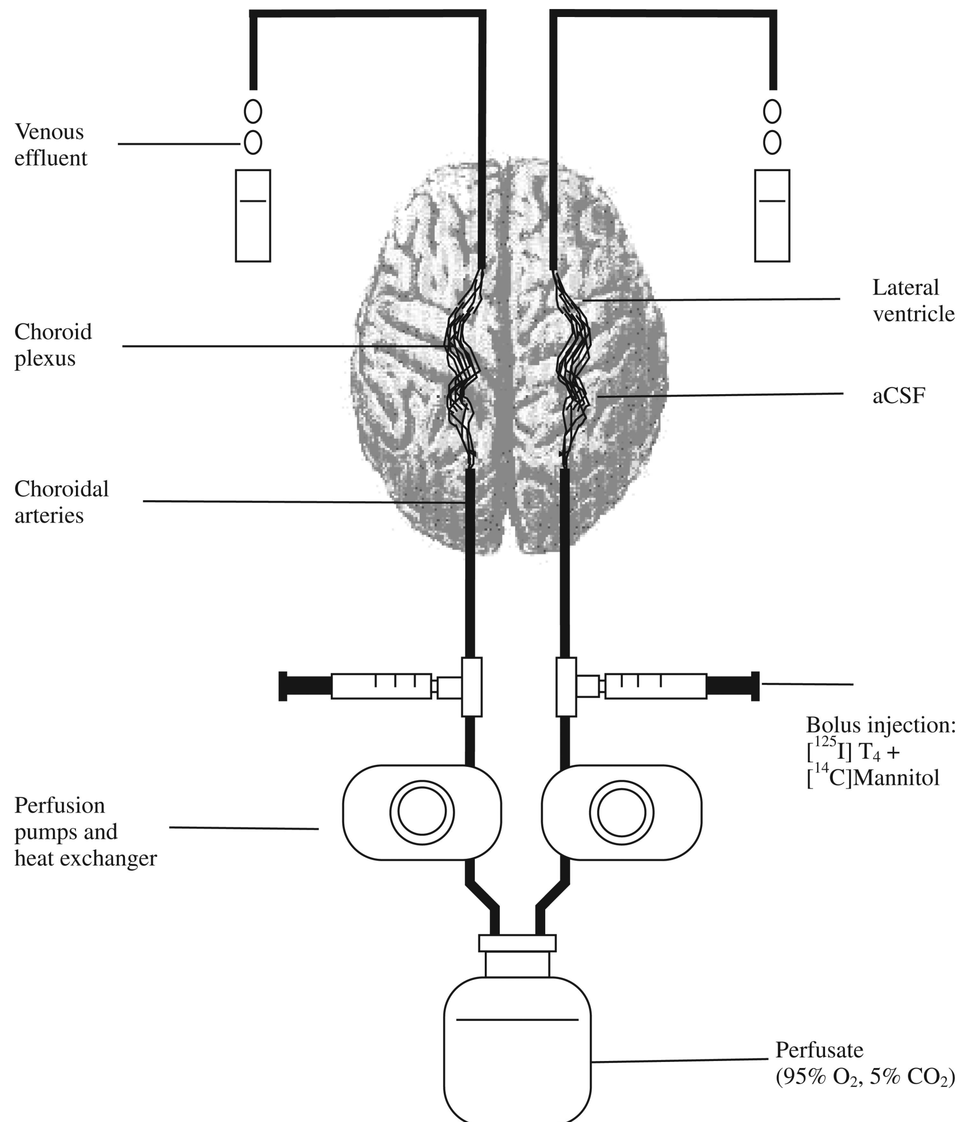

FIGURE 1.

Illustration of in situ sheep choroid plexus perfusion model. The perfusion system was maintained at a flow rate of 0.5–1.5 ml/min at 37 °C, with the perfusate being directed toward the anterior choroidal arteries. The cerebral ventricles were exposed and the choroid plexus in the lateral ventricle was superperfused with an artificial CSF. The perfusion fluid draining from choroid plexuses was collected by a cannula inserted into the great vein of Galen.

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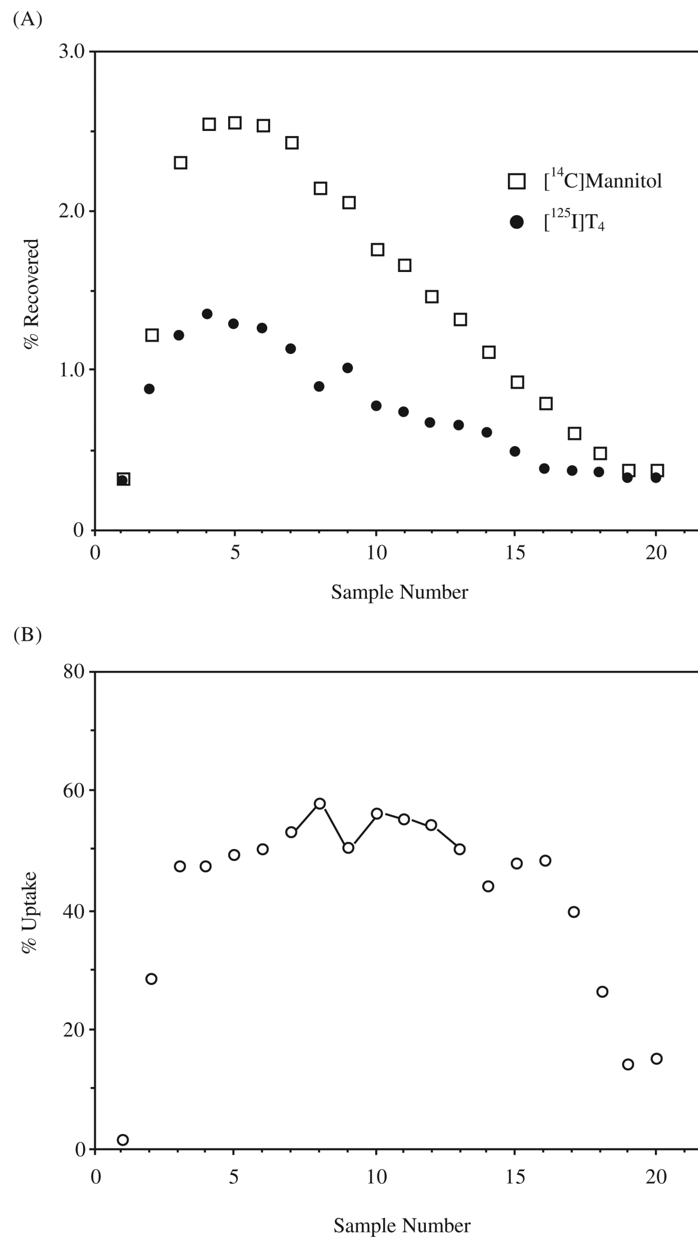

FIGURE 2.

A typical run of paired-tracer study of T_4 transport by sheep choroid plexus. (A) Recovery of [^{14}C]mannitol and [^{125}I]T_4 in 1 run of 20 venous samples (as % of radioactivity injected). The low recovery of [^{125}I]T_4 in comparison to [^{14}C]mannitol indicates T_4 uptake at the basolateral face of the choroid plexus. (B) Uptake (%) of [^{125}I]T_4 in each venous sample relative to [^{14}C]mannitol. Samples that contained the higher recovery of isotopes are joined by a line, and these values are averaged to estimate U_{max} (%). Data from control sheep 3. T_4 concentration: 1.2 μ*M* in the perfusate.

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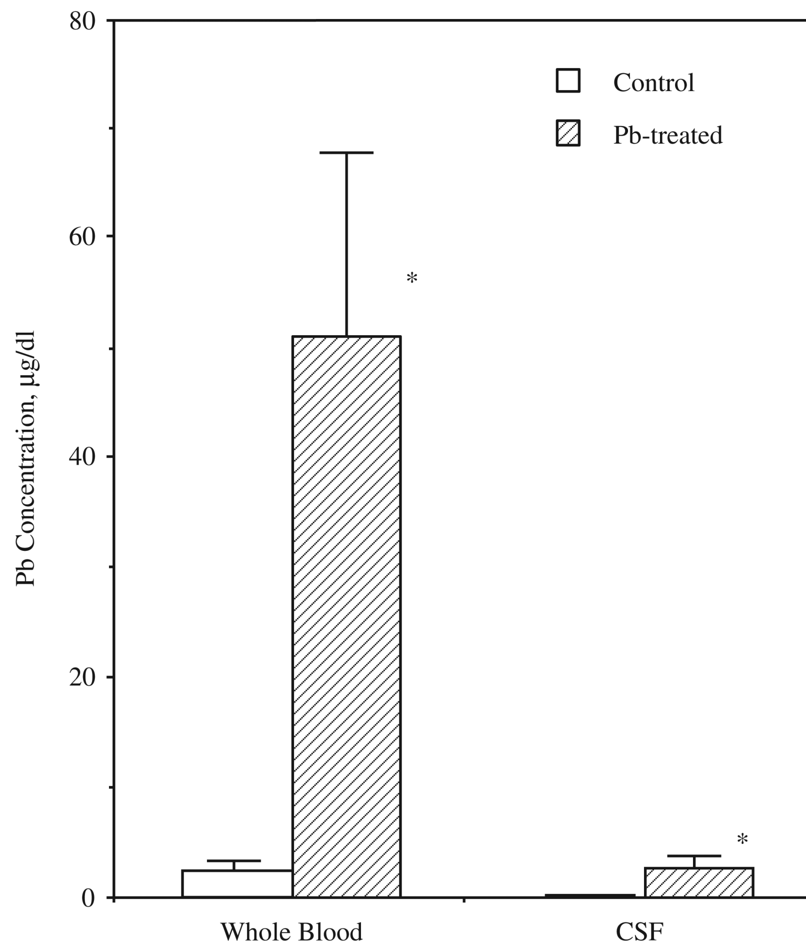

FIGURE 3.
Blood and CSF concentrations of Pb. Sheep received ip injections of Pb acetate (20 mg Pb/kg) or Na acetate (equivalent acetate molar concentration) every 48 h for a period of 16 d. CSF samples were obtained by cistern magna punctuation and blood samples by iv collection. All samples were analyzed for Pb by AAS. Data represent mean ± SE, n = 4–5. Asterisk indicates significant difference from control ($p < .05$).

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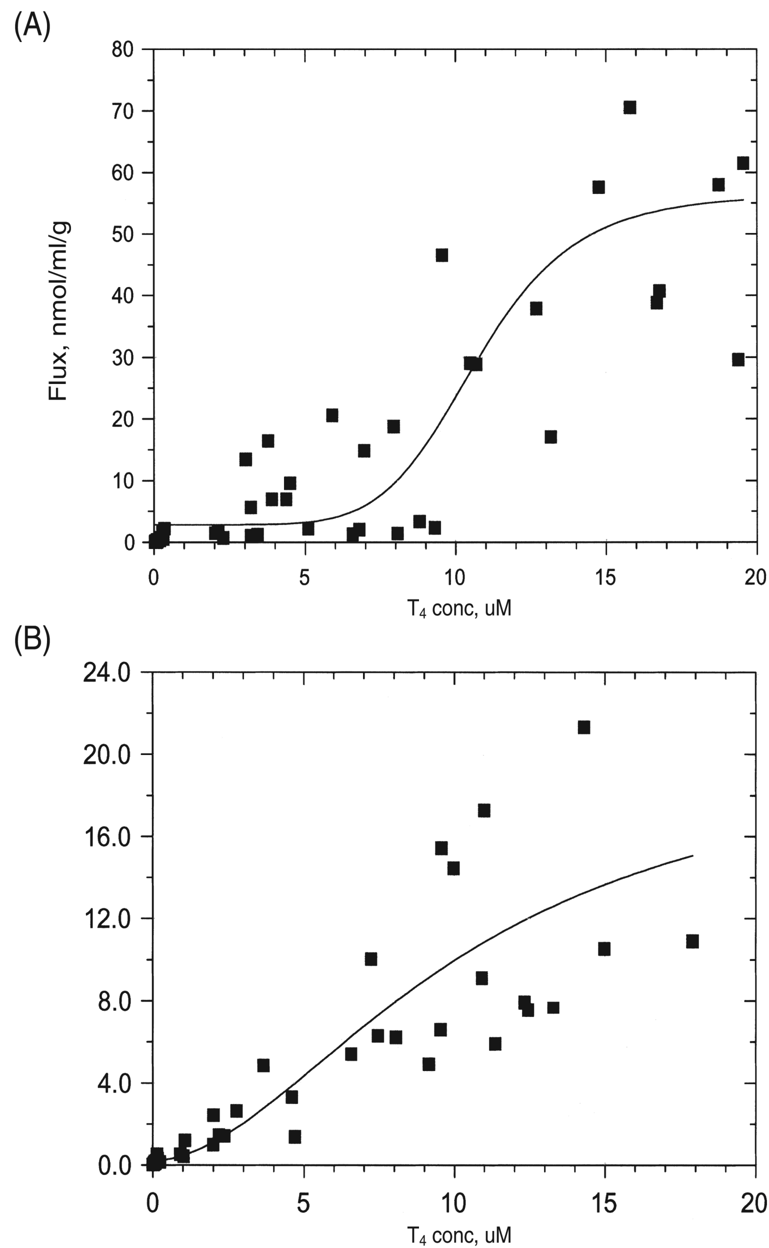

FIGURE 4.

Uptake kinetics of T_4 by the choroid plexus. The flux values in each run of all experiments were plotted against the concentrations of T_4 in each corresponding run. The data sets were evaluated by the kinetic models that best simulated the observed data. The lines indicate the best fit of uptake kinetics of T_4 in perfused sheep choroid plexuses. (A) Control group: Data were collected from 4 sheep with a total of 54 runs. (B) Pb-treated group: There were 5 sheep with 58 runs.

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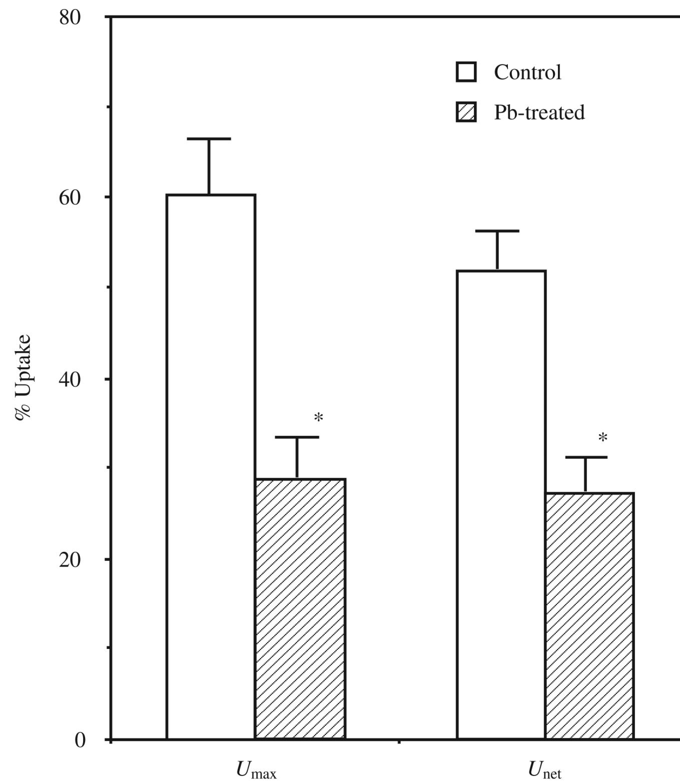

FIGURE 5.

Inhibition by Pb of the uptake (U_{max}, U_{net}) of $[^{125}I]T_4$ by the choroid plexus. The choroid plexuses were perfused in the presence of T_4 (0.02 μ*M*). Data represent mean ± SE. Asterisk indicates significant difference from control ($p < .05$).

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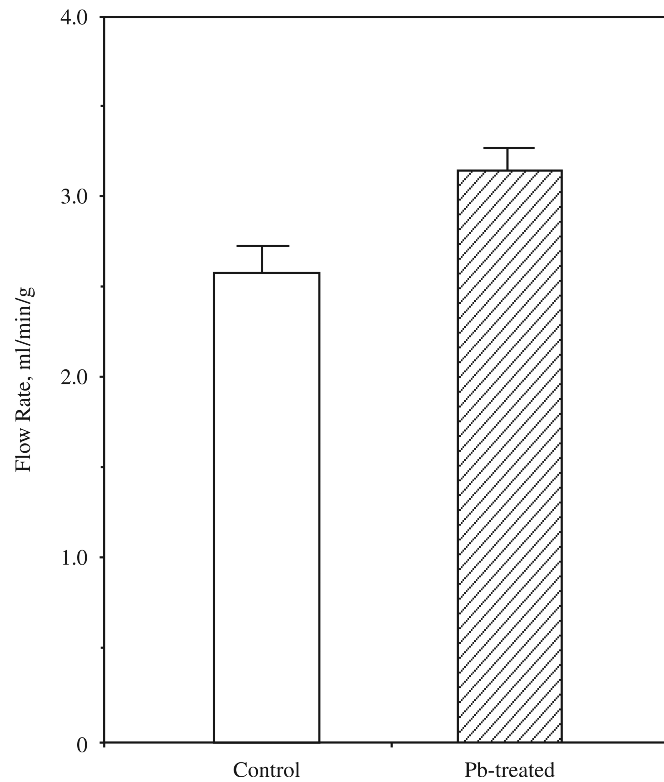

FIGURE 6.
Effect of Pb on the plexus effluent flow rate (R_{EF}). The rate of effluent flow in the venous outflow was determined by averaging the effluent volumes during the last 4-min collection at the end of each run. Data represent mean ± SE, n = 4 for control sheep and n = 5 for Pb-treated sheep.

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TABLE 1

Concentrations of Pb in Sheep Blood, CSF, Choroid Plexus, and Selected Brain Regions

Tissues	Control	Pb-treated
BPb (μg/dl)	2.14 ± 0.96	51.1 ± 16.6[a]
CSF Pb (μg/dl)	<D.L.	2.67 ± 0.86
Choroid plexus (mg/g)	0.96 ± 0.10	1.54 ± 0.37[a]
Frontal cortex (mg/g)	0.23 ± 0.03	0.55 ± 0.06
Hippocampus (mg/g)	0.26 ± 0.04	0.87 ± 0.12[a]
Cerebellum (mg/g)	0.28 ± 0.06	0.56 ± 0.19

Note. Sheep were injected ip with 20 mg Pb/kg as Pb acetate once every 2 d for 16 d. Data are given as mean ± SE, $n = 4$ for controls and $n = 5$ for Pb-treated animals. D.L., detection limit.

[a]Significant at $p < .05$ as compared to the control group.

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TABLE 2

Kinetic Parameters of $[^{125}I]T_4$ Uptake by the Perfused Choroid Plexus Following In Vivo Exposure to Pb

Parameters	Control	Pb-treated	Percent inhibition
V_{max} (nmol/min/g)	56.61 ± 7.173	20.57 ± 11.95[a]	63.7
K_m (μ*M*)	10.74 ± 0.949	10.45 ± 7.130	2.70
Correlation	.8595	.8625	

Note. Data represent mean ± SD, *n* = 4 for the control and *n* = 5 for the Pb-treated animals. Parameters were estimated from the model fit equations described in the main text.

[a]Significant at $p < .05$ as compared to the control group.