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Transferrin Gene Expression and Synthesis by Cultured Choroid Plexus Epithelial Cells

REGULATION BY SEROTONIN AND CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE*

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Primary cultures of rat choroid plexus epithelial cells were established and used to investigate the role of the choroid plexus in the synthesis and secretion of transferrin. Transferrin gene expression was determined by a Northern blot analysis with a transferrin cRNA probe. A single transferrin mRNA species was detected and found to be the same size as the transcripts in the liver and Sertoli cells. Immunoprecipitation of radiolabeled secreted proteins with an antiserum transferrin antibody demonstrated that cultured choroid plexus epithelial cells synthesize and secrete a 70-kDa species of transferrin. Levels of transferrin secretion by rat choroid plexus epithelial cells in culture were measured by radioimmunoassay. Treatment of the choroid plexus epithelial cells in culture with cell-permeable cAMP analogs or serotonin led to time- and concentration-dependent changes in the levels of transferrin in the medium. Dibutyryl-cAMP and 8-bromo-cAMP decreased the levels of transferrin synthesized and secreted by choroid plexus epithelial cells with an EC_{50} value of 30 nM. Serotonin, however, increased the levels of transferrin with an EC_{50} value of 100 nM. A concomitant change in transferrin mRNA concentrations was observed in response to serotonin. These data suggest that the synthesis of transferrin by the choroid plexus is reciprocally regulated by the neurotransmitter serotonin and by regulatory agents coupled to adenylate cyclase. Regulatory agents such as serotonin may have a critical role in modulating the proteins synthesized by the choroid plexus, thereby influencing the composition of the cerebrospinal fluid.

The choroid plexus, an extension of the ependymal layer covering the ventricular surface of the brain, lies in all ventricles. It is a simple structure composed of a monolayer of cuboidal epithelial cells with tight junctions underlined by mesenchymal fibroblasts and a basement membrane bordering capillaries. The apical membrane of the epithelial cell faces the cerebrospinal fluid (CSF),[1] and the basolateral membrane rests on mesenchymal fibroblasts facing the blood. The choroid plexus has at least two major functions. First, it forms the blood-CSF barrier, that is, the blood-brain barrier at the choroid plexus. The tight junctions of the choroid plexus epithelium are responsible for this barrier, inhibiting the diffusion of a wide range of macromolecules and hydrophilic substances from blood into the CSF. Some hydrophilic substances are taken up by the barrier via carrier proteins or specific transport systems, but hydrophobic compounds readily diffuse through the barrier. Second, the choroid plexus is the major site of CSF production, secreting about two-thirds of the total CSF. Until recently, it was generally accepted that CSF proteins are simply an ultrafiltrate of plasma filtered through the choroid plexus epithelium. Evidence now suggests that, in addition to fluid secretion, the choroid plexus also synthesizes and secretes proteins that are found in the CSF. Intact choroid plexus contains messenger RNA (mRNA) for several transport proteins including transferrin, transthyretin, and ceruloplasmin (1-3), carriers for iron, thyroid hormones, and copper, respectively. These findings suggest that the choroid plexus supplies essential carrier proteins necessary for proper function of the central nervous system and, therefore, plays a crucial role in the maintenance of homeostasis of the central nervous system microenvironment.

The function of the epithelial cells of the blood-CSF barrier may be analogous to that of the epithelial cells of the blood-testis barrier. Sertoli cells of the testis, epithelial cells forming the blood-testis barrier, synthesize and secrete various transport proteins such as transferrin, androgen-binding protein, and ceruloplasmin into the seminiferous tubules and reproductive tract (4-6). Furthermore, the production and secretion of transferrin and androgen-binding protein are regulated by various hormones (7). The current study was designed to test the hypothesis that the production and secretion of transferrin in the choroid plexus are regulated by central nervous system hormones and neurotransmitters present in the CSF, analogous to the regulation found in Sertoli cells. This hypothesis was tested in a primary cell culture system established from rat choroid plexus which is enriched in epithelial cells.

MATERIALS AND METHODS

Primary Culture—Primary cell culture of bovine choroid plexus has been described previously (8). Rat choroid plexus primary cell culture was prepared using a modification of this method. Twenty-day-old male Sprague-Dawley rats (Sasco, Omaha, NE) were decapitated, and choroid plexi were rapidly dissected from brains. The tissue was rinsed twice in Ca^{2+}- and Mg^{2+}-free Hanks' buffer (GIBCO) containing 10 μg/ml gentamicin (GIBCO), chopped finely on a Petri dish with a razor blade, and washed once in Hanks' buffer.

The minced tissue was then incubated in Hanks' buffer containing 1 mg/ml (200 units/ml) collagenase (Type I) and 12.5 μg/ml DNase (Type I) for 20 min in a gently shaking water bath at 37 °C. Cell clumps enriched in epithelial cells held together by tight junctions

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[1] The abbreviations used are: CSF, cerebrospinal fluid; 8-Br-cAMP, 8-bromo-cAMP.

were allowed to settle to the bottom of the tube for approximately 5 min, and the supernatant containing primarily non-epithelial dispersed single cells was discarded. The enriched epithelial cell fraction was washed twice more or until the supernatant was clear with Hanks' buffer. The cell clumps were then incubated in Hanks' buffer containing 0.025% trypsin (GIBCO) in the presence of DNase I for an additional 15 min in a shaking water bath at 37 °C. Soybean trypsin inhibitor (3 mg/ml; GIBCO) was added at the end of 15 min, and the cells were pelleted by centrifugation for 2–3 min at 200 × *g*. The supernatant was discarded, and the cells were washed twice with fresh Hanks' buffer.

The final pellet was resuspended in Ham's F-12 medium (GIBCO) supplemented with 10% fetal bovine serum (GIBCO) and plated on 24 multiwell plates (Falcon Plastics, Los Angeles, CA). The cells were allowed to plate for 72 h in a humidified incubator with 5% CO_2 and 95% air atmosphere at 37 °C. Unattached cells were discarded; attached cells were maintained in 0.5 ml of serum-free Ham's F-12 medium for the duration of the experiment. The cells were incubated in serum-free medium for at least 24 h before experimental manipulations were initiated. The medium was changed every 2 days for the duration of culture.

DNA Assay—The amount of DNA/well reflects the degree of plated intact viable cells. The DNA content was determined by the ethidium bromide assay (9) as modified previously (10). Briefly, the cells were sonicated in a buffer containing 10 mM Tris, 5 mM Na_2EDTA, and 20 mM NaCl, pH 7.8. Heparin (2.5 units/ml) and ethidium bromide (2.5 μg/ml) were added, and fluorescence measurements were taken. The wavelength of excitation was 350 nm and that of emission was 585 nm. The sensitivity of the assay was 0.1 μg of DNA. DNA values were used to normalize the data.

Electrophoresis, Fluorography, and Immunoprecipitation—Cells in culture were incubated for 48 h in Met-, Cys-, Gly-free F-12 medium containing 3 μCi/ml each of [^{35}S]Met, [^{35}S]Cys, and [^{3}H]Gly (all purchased from Du Pont-New England Nuclear). Radiolabeled proteins in the conditioned medium were collected and electrophoretically separated on 5–15% gradient polyacrylamide-sodium dodecyl sulfate gels under reducing conditions using the Laemmli procedure (11). Gels were then fluorographed using 2,5-diphenyloxazole in acetic acid (12). Briefly, the gel was rinsed in glacial acetic acid for 5 min, soaked for 2 h in 20% 2,5-diphenyloxazole in glacial acetic acid, followed by a 30-min rinse in water. The gel was dried and exposed to preflashed x-ray film for 4–7 days at −70 °C.

Transferrin immunoprecipitations were performed with a double-antibody precipitation (4) procedure or with *Staphylococcus aureus* (Pansorbin, Calbiochem) fixed with formalin (10%, w/v) as described previously (13). 25 μl of rabbit anti-rat serum transferrin antiserum (Cappell Laboratories) or nonimmune rabbit serum and 200 μl of immunoprecipitation buffer (50 mM Tris, 0.15 M NaCl, 0.1% (v/v) Triton X-100, 1% (w/v) deoxycholate, pH 7.5) were added to 200 μl of culture medium containing radiolabeled proteins. After incubating for 24 h at 4 °C, 25 μl of rabbit anti-goat IgG or Pansorbin was added, and the mixture was incubated for an additional 24 h at 4 °C. Pansorbin was first incubated with nonlabeled choroid plexus-conditioned medium for 6 h at 4 °C in order to minimize nonspecific binding. The samples were then centrifuged at 12,000 × *g* for 15 min (for secondary antibody) or 4,000 × *g* for 5 min (for Pansorbin) at 4 °C, and the pellet was washed five times with 200 μl of immunoprecipitation buffer. The pellet was heated for 30 min in sodium dodecyl sulfate sample buffer (30% glycerol, 3% sodium dodecyl sulfate, 3% β-mercaptoethanol, 0.01% bromphenol blue, and 0.1 M Tris, pH 6.8) with occasional mixing prior to centrifugation and analysis by gel electrophoresis.

Transferrin Radioimmunoassay—Rat serum transferrin was radioiodinated using chloramine T (7). With this method, approximately 5 μCi/μg iodinated transferrin was obtained. Choroid plexus-conditioned medium was collected, and 200 μl/sample was assayed for transferrin as described previously (7). CSF was collected from the cisterna magna of the rat and also analyzed for transferrin content. Approximately 250 μl of CSF was obtained per rat. CSF samples were clear and colorless with no apparent blood contamination. Rat transferrin antibody (100 μl of fresh 1/3,000 dilution of a 1 mg/ml solution of IgG) was added to the unknowns and standards. Iodinated transferrin containing approximately 30,000 cpm was added, and samples were incubated for 1 h at 37 °C. Then, 100 μl of 1/16 titer of goat anti-rabbit IgG was added and incubation continued for another hour at 37 °C. 1 ml of polyethylene glycol buffer (132 mg/ml polyethylene glycol 3,600, 50 mM Tris, pH 7.5) was added; after a 15-min incubation at room temperature, the samples were centrifuged at 3,000 × *g* for 2 h at 4 °C. Transferrin levels were determined by counting the precipitated pellet with a Beckman 4000 γ counter using a Beckman DP 5000 data reduction system. The sensitivity of the assay was approximately 5 ng of transferrin as described previously (7). Nonspecific binding was less than 2.4% of the total radioactivity added.

RNA Isolation and Northern Blot Analysis—Total RNA was isolated from choroid plexus cells using guanidine isothiocyanate extraction. The cells were lysed in a guanidine isothiocyanate lysis buffer containing 5 M guanidine isothiocyanate, 10 mM EDTA, 50 mM Tris, and 8% β-mercaptoethanol. Nucleic acids were precipitated with 4 M LiCl for 24 h at 4 °C and then centrifuged at 12,000 × *g* for 60 min. The pellet was resuspended in lysis buffer (10 mM Tris-HCl, 0.1 M NaCl, 2 mM EDTA, and 1% sodium dodecyl sulfate) containing 50 μg of proteinase K and incubated for 2 h at 37 °C. Samples were extracted with Tris-saturated phenol and CH_3Cl. RNA was then precipitated by the addition of ethanol and 7.5 M NH_4OAc. Total RNA was separated electrophoretically on 1.2% formaldehyde-agarose gels and subsequently transferred to Magna Nylon 66 (Micron Separations, Inc.). The RNA blot was hybridized as described previously (14) with a ^{32}P-labeled transferrin cRNA (15) overnight at 60 °C and then visualized by autoradiography. Autoradiographs were quantitated using an LKB 2202 Ultroscan laser densitometer.

RESULTS

Primary Cell Culture—Dissociated choroid plexus cells were plated at approximately 2 × 10^5 cells/well or 3 μg DNA/well in Ham's F-12 medium supplemented with 10% fetal bovine serum. When the medium was supplemented with 10% calf serum instead of fetal bovine serum or not supplemented with any serum, cells did not attach. The plated cells maintained polygonal epithelial-like morphology, and minimal fibroblast-like cells were observed morphologically (Fig. 1). Contamination by fibroblast-like cells did not appear to increase during culture in the presence or absence of fetal bovine serum.

Alterations in cell number were slight for at least 9 days in culture as evidenced by DNA assay. Approximately 2.7 μg of DNA/well was found on day 4 of culture. A gradual and slight decrease was observed with approximately 80% of the cells or 2.1 μg of DNA remaining on day 9 of culture. These data suggest that the cells remain viable for a minimum of 9 days in culture.

Characterization of Transferrin Expression—A typical profile of the radiolabeled proteins synthesized and secreted by the cells on day 5 of culture is shown in Fig. 2, *lane 1*. Several major proteins were secreted including a protein with an approximate molecular mass of 70 kDa. On the basis of its molecular mass, this protein was postulated to be transferrin.

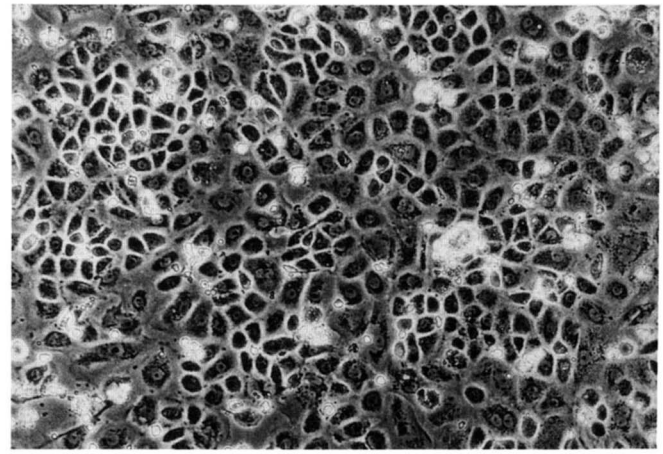

FIG. 1. **Phase contrast photomicrograph of rat choroid plexus primary cultures enriched in epithelial cells.** The cells were prepared and plated as described under "Materials and Methods." This photograph was taken on day 5 of culture. Magnification: × 250.

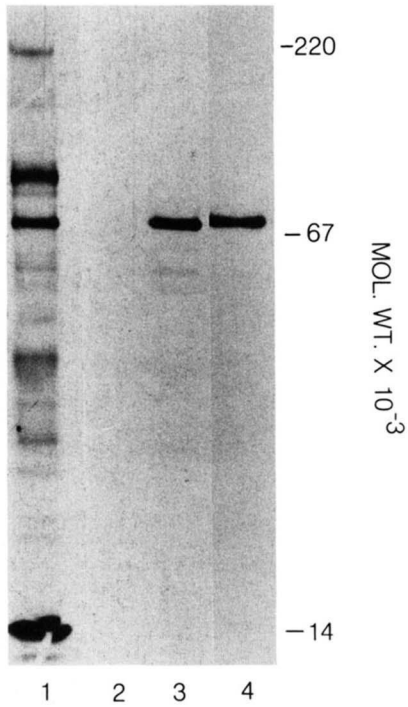

FIG. 2. **Fluorograph of radiolabeled proteins synthesized and secreted by choroid plexus epithelial cells in culture.** *Lane 1,* total proteins secreted into the medium. *Lane 2,* immunoprecipitation with nonimmune rabbit serum. *Lane 3,* immunoprecipitation of transferrin using a double-antibody method. *Lane 4,* immunoprecipitation of transferrin using rabbit anti-rat serum transferrin and Pansorbin. This fluorograph is representative of two experiments.

In order to evaluate this further, immunoprecipitation with rat serum transferrin antibody was performed. The 70-kDa protein immunoprecipitated with anti-rat serum transferrin using either secondary antibody (Fig. 2, *lane 3*) or Pansorbin (Fig. 2, *lane 4*). The electrophoretic protein profile of the supernatant after the immunoprecipitation lacked the 70-kDa protein band (data not shown), indicating that most, if not all, of the 70-kDa protein was immunoreactive with the serum transferrin antibody.

To evaluate transferrin gene expression by choroid plexus epithelial cells, total RNA was isolated from the cells and separated electrophoretically on a formaldehyde-agarose gel. Northern blot analysis was performed using a ^{32}P-labeled transferrin cRNA probe to detect the presence of transferrin mRNA. Hybridization was detected with a single RNA species in choroid plexus (Fig. 3, *lane A*). As found previously (15), Sertoli cells (Fig. 3, *lane B*) and liver (data not shown) also contained a single RNA species of 2.6 kilobases, which hybridized to the probe. These results demonstrate the presence of transferrin mRNA and confirm that the transferrin gene is expressed in choroid plexus epithelial cells.

Regulation of Transferrin—To confirm the use of the radioimmunoassay and to compare immunological characteristics of choroid plexus-secreted transferrin, CSF transferrin, and serum transferrin, an antibody displacement experiment was performed (Fig. 4). The parallel displacement curves indicate that the proteins are immunologically similar. The amount of transferrin secreted by cultured cells varied among preparations with an average of 66 ng/μg DNA/24 h on day 5 of culture, decreasing to 53 ng of transferrin/μg DNA/24 h on day 9 of culture. The level of intracellular transferrin was nondetectable, indicating that transferrin is not sequestered in the cells. The level of transferrin in CSF was found to be 25 ± 3 μg/ml.

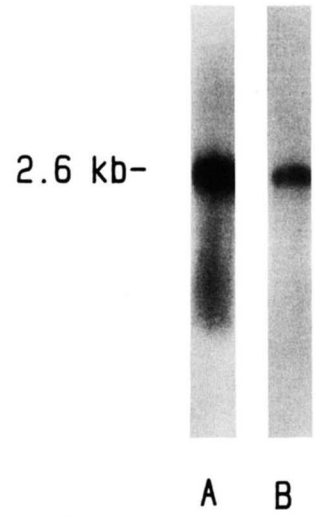

FIG. 3. **Northern blot analysis of choroid plexus epithelial cells.** *Lane A,* RNA (25 μg) isolated from dissociated rat choroid plexus epithelial cells. *Lane B,* RNA (10 μg) isolated from cultured rat Sertoli cells, prepared as described previously (10). Total RNA was electrophoretically separated on formaldehyde-agarose gel, blotted onto Magna Nylon 66, and hybridized with a ^{32}P-transferrin cRNA as described under "Materials and Methods." The autoradiogram is representative of three experiments.

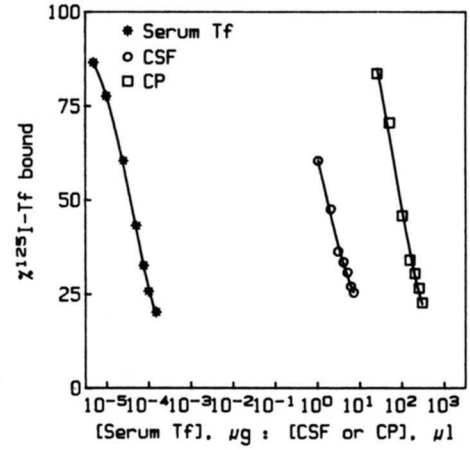

FIG. 4. **Displacement of the binding of ^{125}I-rat serum transferrin from an antibody to rat serum transferrin.** The bound ^{125}I-rat serum transferrin was displaced by increasing amounts of unlabeled rat serum transferrin (*Tf*), choroid plexus-secreted transferrin (*CP*), or CSF transferrin (*CSF*). Data are expressed as the percentage of ^{125}I-transferrin bound. Each point is the mean of two experiments in triplicate.

The effects of cyclic AMP (cAMP), serotonin, and insulin on the secretion of transferrin by cultured cells were investigated. Cells were maintained in culture with or without regulatory agents for up to 6 days starting on day 4 of culture. The medium was collected every 48 h and analyzed for transferrin content and normalized to DNA. Both dibutyryl-cAMP and 8-bromo-cAMP (8-Br-cAMP) significantly reduced transferrin levels. The inhibitory effect of these cell-permeable analogs of cAMP was time- and concentration-dependent. Maximum inhibition of transferrin secretion by the cAMP analogs was obtained on day 4 of treatment (Fig. 5). The levels of secreted transferrin remained reduced for at least 6 days. The effect of 8-Br-cAMP on day 4 was concentration-dependent with a maximum inhibition to 55% of control (Fig. 6). The approximate EC_{50} value for 8-Br-cAMP was 30 nM. The effect of serotonin on transferrin secretion by cultured cells was also time- and concentration-dependent but opposite

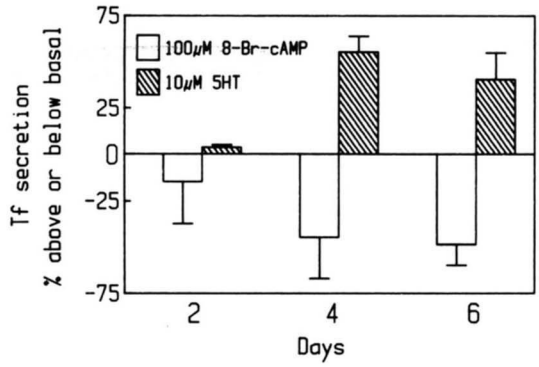

FIG. 5. **Time-dependent changes in the effects of 10 μM serotonin (*5HT*) and 100 μM 8-Br-cAMP on transferrin secretion by choroid plexus epithelial cells.** The cells were treated with regulatory agent for 2, 4, or 6 days, starting on day 4 of culture. The medium was collected every 48 h and analyzed by transferrin radioimmunoassay. Data are expressed as the percentage above or below basal transferrin secretion (150 ng/μg DNA/48 h). Each bar is the mean of three experiments in triplicate. The *error bars* represent S. E. *Tf*, transferrin.

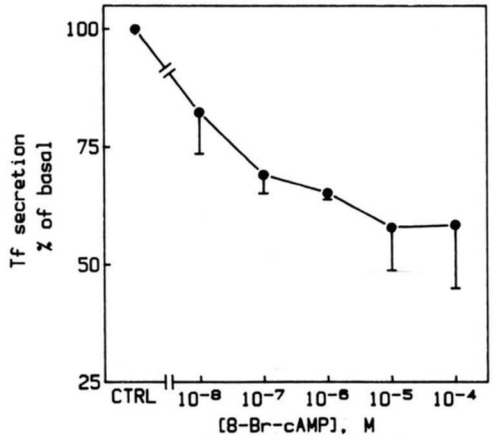

FIG. 6. **Effect of increasing concentrations of 8-Br-cAMP on transferrin secretion by choroid plexus epithelial cells in culture after 4 days of treatment.** Data are expressed as the percentage of basal transferrin secretion. Each point is the mean of three experiments in triplicate. The *error bars* represent S. E. *Tf*, transferrin.

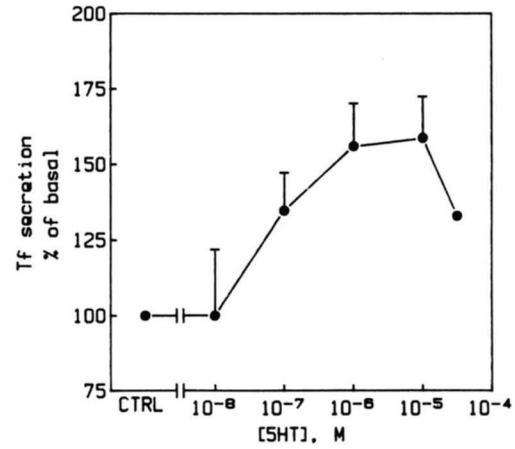

FIG. 7. **Effect of increasing concentrations of serotonin (*5HT*) on transferrin secretion by choroid plexus epithelial cells in culture after 4 days of treatment.** Data are expressed as the percentage of basal transferrin secretion. Each point is the mean of five experiments in triplicate. The *error bars* represent S. E. No bar indicates that the S. E. was less than the size of the symbol. *Tf*, transferrin.

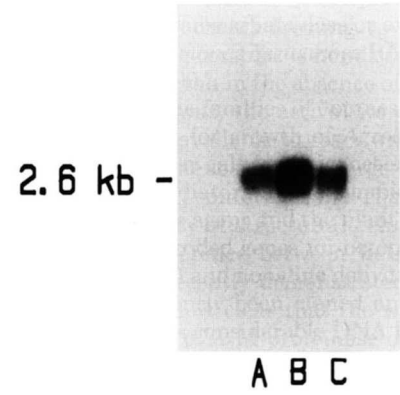

FIG. 8. **Effects of serotonin and 8-Br-cAMP on transferrin gene expression.** Cells were treated with 10 μM serotonin, 100 μM 8-Br-cAMP, or vehicle for 4 days. Total RNA was isolated and Northern blot analysis was performed as described under "Materials and Methods." *Lane A*, control (2.7 μg of RNA); *lane B*, 10 μM serotonin (2.3 μg of RNA); and *lane C*, 100 μM 8-Br-cAMP (2.00 μg of RNA). The ratio of μg of RNA/μg DNA was 0.04 for all treatments. This gel is representative of three separate experiments.

to that of the cAMP analogs. On day 2, the presence of 10 μM serotonin did not alter transferrin secretion (Fig. 5). Transferrin secretion was increased by 1.6-fold on day 4 of treatment, and this effect remained for at least 6 days. The serotonin-induced effect was concentration-dependent (Fig. 7) with an EC_{50} value of approximately 100 nM. Insulin had negligible effects on transferrin synthesis and secretion (data not shown). The level of intracellular transferrin was below the limit of detection (5 ng) for all of the treatment conditions.

Northern Blot Analysis of Steady State Transferrin mRNA—In order to determine if the changes in protein synthesis reflect alterations in transferrin gene expression, the effects of serotonin (10 μM) and 8-Br-cAMP (100 μM) on transferrin mRNA levels were determined. Cells were treated with these agents as described in the previous section. After 4 days, the cells were collected, and RNA was isolated. Hybridization observed with a Northern analysis was quantitated with a densitometer and normalized to total RNA loaded onto the gel. The treatments did not change the ratio of total RNA/μg DNA (0.04 for all treatments) indicating no significant changes in total RNA levels. As shown in Fig. 8, serotonin treatment increased the apparent level of transferrin mRNA in choroid plexus epithelial cells. Densitometer scanning confirmed that serotonin increased transferrin mRNA to 247% of control, and 8-Br-cAMP treatment led to 129% of control. This experiment was repeated twice. The mean value for transferrin mRNA after serotonin treatment was 245 ± 80% of control ($n = 3$) and 89 ± 42% of control ($n = 3$) after 8-Br-cAMP treatment.

DISCUSSION

The choroid plexus forms the blood-CSF barrier and secretes a major portion of the total CSF volume. These functions have been studied extensively; however, the role of choroid plexus in determining CSF content has not been thoroughly investigated. A primary cell culture system was established to study this function. The cultured cells from rat choroid plexus were predominantly polygonal, consistent with the morphology of epithelial cells in a bovine choroid plexus primary cell culture system characterized previously (8). Only a few of the attached cells had apparent fibroblast-like morphology, suggesting an enrichment of epithelial cells. In con-

trast to the findings in bovine culture, proliferation of fibroblast-like cells was minimal in the current study, possibly due to the differences in the methods of cell preparation. Several lines of evidence indicate that transferrin, an iron carrier protein, is synthesized and secreted by cultured choroid plexus cells. One of the major proteins in the medium has an apparent molecular mass of 70 kDa and co-migrates with rat serum transferrin. This protein can be immunoprecipitated with an anti-rat serum transferrin antibody. The displacement of radioiodinated transferrin from serum transferrin antibody by the choroid plexus-secreted protein parallels that of serum transferrin. Finally, the epithelial cells contain mRNA for transferrin, demonstrating that the transferrin gene is expressed in these cells. These results are consistent with recent findings in intact tissue (1) and indicate that rat choroid plexus-secreted transferrin is similar to rat serum transferrin. Comparison of the transferrin gene copy number and structural organization in chick liver and oviduct has demonstrated that transferrin produced by these tissues is the product of the same gene, although its expression is regulated differently (16). It is likely that transferrin produced by liver, Sertoli cells, and choroid plexus is the product of the same gene.

Transferrin is the major constituent of the β-globulin fraction of CSF (17). Because the tight junctions of the choroid plexus epithelial cells form a barrier to macromolecules such as serum proteins, it is likely that the majority of transferrin in CSF is synthesized within the choroid plexus. Transferrin plays an essential role in the transport of iron, which is required by all living cells including neurons and other cells of the central nervous system. Based on analogy with the transferrin cycle in other cells (18), a working model of transferrin function in the choroid plexus has been developed. A basal cell surface transferrin receptor binds serum diferric transferrin and internalizes the complex into vesicles by receptor-mediated endocytosis. Iron is released in a pH-dependent fashion in an acidic vesicle, and the receptor-transferrin complex is recycled back to the basal membrane surface. Transferrin synthesized in the epithelial cells binds the iron and is subsequently secreted into the CSF. Although the transferrin receptor and serum transferrin presumably return to the basal membrane of the polarized epithelial cell, transferrin synthesized by the choroid plexus epithelial cell is postulated to be released at the apical membrane into the CSF compartment. This model is similar to that proposed for the transport of iron across the blood-testis barrier via Sertoli cell-derived transferrin (4, 19). Consistent with this model, transferrin found in the CSF is immunologically similar to that found in the conditioned medium of cultured rat choroid plexus cells. Local synthesis of as much as 70% of transferrin in the central nervous system has been suggested (17), and our studies in culture demonstrate that the choroid plexus epithelial cells contribute to this local synthesis. The synthesis and secretion of transferrin by the choroid plexus may explain the apparent enrichment (20) of transferrin in CSF compared to total protein. Earlier reports suggested that transferrin in CSF exists in two principal components that are immunologically similar (21). One type contains 4 sialic acid residues/molecule similar to serum transferrin, but the other contains no sialic acid. Whether the choroid plexus selectively synthesizes one of these forms of transferrin or if some type of protein modification takes place in the central nervous system is not known.

Transferrin was used as a functional marker protein for studies of neurohumoral regulation of the choroid plexus. Other transport proteins such as transthyretin are also enriched in the CSF in comparison to albumin (22). Immunoprecipitation of the proteins in the medium of cultured choroid plexus cells with anti-human transthyretin antibody suggests that transthyretin is another major protein synthesized and secreted by the choroidal epithelium (data not shown). This observation confirms studies that show that intact choroid plexus synthesizes and secretes transthyretin (2). It has been reported that as much as 20% of newly synthesized and 50% of newly secreted proteins is transthyretin (2). High levels of ceruloplasmin mRNA and transferrin mRNA are also expressed in the choroid plexus (3, 23). Thus, the choroid plexus is a unique tissue specializing in the synthesis of carrier proteins necessary for proper central nervous system function. Insulin-like growth factor II gene transcripts (24) have also been localized in the choroid plexus. The CSF to plasma ratio of insulin-like growth factor II is higher than that expected if insulin-like growth factor II were transported into the CSF via nonspecific routes (25), again supporting that choroid plexus epithelial cells play a major role in the formation and secretion of proteins into the CSF.

Results in the present manuscript show for the first time that transferrin synthesis and secretion by rat choroid plexus is regulated by physiological agents. Cell-permeable analogs of cAMP decrease transferrin synthesis by the epithelial cells of the choroid plexus. Our preliminary data suggest that the cAMP-induced decrease in protein secretion is not restricted to transferrin, but that total protein synthesis is reduced (data not shown). These findings suggest that cAMP-induced changes in gene expression by choroid plexus epithelial cells are nonselective, as also found in Sertoli cells of the testis (7, 13). In Sertoli cells of the testis, however, cAMP stimulates protein synthesis and secretion (7, 13), suggesting a differential tissue-specific gene regulation.

Serotonin, via the serotonin$_{1c}$ receptor, enhances phosphoinositide hydrolysis in the choroid plexus, increasing the formation of second messengers, inositol 1,4,5-trisphosphate and diacylglycerol (26). It was therefore of interest to examine the effects of serotonin on transferrin synthesis. Unlike cAMP, serotonin increases transferrin synthesized by choroid plexus cells. The EC_{50} value of serotonin is approximately 100 nM in agreement with its potency at the serotonin$_{1c}$ receptor (27) and its nanomolar concentrations in the CSF (28, 29). Although the physiological significance of transferrin regulation by serotonin and cAMP is not understood, the high potency of these agents suggests that the observed effects are physiologically relevant. Regulation of transferrin synthesis by a neurotransmitter such as serotonin provides evidence for central control of protein synthesis by the choroid plexus independent of the liver, the major source of systemic transferrin. Consistent with this interpretation, serotonin receptors on choroid plexus epithelia are activated only by serotonin found in the CSF and not that found in the blood (30). A similar independent regulation of transthyretin synthesis by the choroid plexus and the liver has been reported (2).

Cyclic AMP and serotonin also reciprocally modulate CSF production. Cholera toxin, an agent known to stimulate adenylate cyclase thereby raising the intracellular levels of cAMP, stimulates CSF production (31), but serotonin reduces CSF production (32, 33). The inhibitory effect of serotonin may be at least partially serotonin$_{1c}$ receptor-mediated because it is blocked by ketanserin, a serotonin$_{1c}$ receptor antagonist (27). Several receptors, including the β-adrenergic receptor, are positively coupled to adenylate cyclase in the choroid plexus (34). The reciprocal regulation by cAMP analogs and serotonin on transferrin secretion and CSF formation may indicate reciprocal roles for second messengers of the adenylate

cyclase and phosphoinositide hydrolysis pathways in the functions of the choroid plexus.

The site of regulation of transferrin production is not clear, but it is not likely that it is the secretory process of the cell. Most of the transferrin synthesized by choroid plexus epithelial cells in culture is secreted into the medium rather than sequestered within the cell. Thus, transferrin secretion is apparently not rate-limiting and would, therefore, be relatively unsusceptible to regulation. Consistent with this interpretation, the regulation of transferrin synthesis and secretion in oviduct (16), Sertoli cells (15), and liver (35) have all been attributed to changes in the level of transferrin gene expression. Furthermore, analyses of the steady state levels of mRNA in cultured choroid plexus cells using Northern blots suggest that serotonin increases transferrin mRNA, consistent with regulation by transcription or mRNA stability. Northern blots of transferrin mRNA after 8-Br-cAMP treatment failed to show a significant decrease. However, we cannot rule out cAMP regulation at the level of gene expression by Northern analysis alone.

Emerging evidence shows striking similarities between the choroid plexus epithelial and Sertoli cells of the testis that may exemplify general functions of other barrier epithelia. They both form a barrier between blood and extracellular fluid of target cells that are exquisitely sensitive to changes in their specialized environment. The choroid plexus epithelial and Sertoli cells regulate the composition of secreted fluids, maintaining the homeostasis of the microenvironment of the central nervous system and the germ cells, respectively. We have developed a culture system that serves as a useful model to study such functions in the choroid plexus, a tissue specializing in the synthesis of carrier proteins such as transferrin. By using transferrin as a marker protein in our studies, we have demonstrated that the choroid plexus cells in culture maintain the appropriate machinery necessary for regulation of protein synthesis by neurotransmitters such as serotonin. Furthermore, studies suggest that regulation of choroid plexus transferrin synthesis by cAMP differs from that found in Sertoli cells, suggesting differential tissue-specific regulation which may have physiological significance.

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