Primary Cell Culture of the Rabbit Choroid Plexus: An Experimental System to Investigate Membrane Transport

Vikram K. Ramanathan,[1] Andrew C. Hui,[1] Claire M. Brett,[2] and Kathleen M. Giacomini[1,3]

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INTRODUCTION

The choroid plexus, the blood-cerebrospinal fluid (CSF) barrier, is a polarized epithelium which lines the ventricles of the brain and is the site of production of CSF. In addition, the choroid plexus plays an important role in regulating the composition of the CSF, in part, by selectively absorbing and eliminating molecules via specific transporters localized on distinct brush border and basolateral surfaces (1). Traditionally, the blood-brain barrier has been the important focus of studies related to transport of various molecules into and out of the brain (2). However, more recently, studies have begun to define the important role of the choroid plexus in maintaining homeostasis in the brain. For example, transporters for various micronutrients (vitamins, thyroid hormone, amino acids, nucleosides) have been identified in the choroid plexus but are absent in the blood-brain barrier (1,3,4,5). The majority of studies on the transport of small water soluble molecules across the choroid plexus have employed either intact animals (*in situ* perfusion) or freshly isolated and ATP-depleted tissue slices.

Continuous cell culture models for other epithelia (intestinal, renal) have allowed detailed studies of regulation and flux, as well as expression of various transporters (6,7), but no continuous cell line of the choroid plexus is readily available. Primary culture of cerebral endothelium has proved useful for transport studies of the blood-brain barrier (8). Although primary culture of choroid plexus epithelium has been developed (3), this system has not been adapted to study transport, a primary function of the choroid plexus epithelium. We report the development of a primary cell culture of the rabbit choroid plexus grown on Transwell-COL filters and demonstrate its suitability for transport studies.

[1] Department of Biopharmaceutical Sciences, University of California San Francisco, San Francisco, California 94143.

[2] Department of Anesthesiology, University of California San Francisco, San Francisco, California 94143.

[3] To whom correspondence should be addressed.

ABBREVIATIONS: CSF, cerebrospinal fluid; RBC, red blood corpuscles.

METHODS

Cell Culture

Choroid plexus tissue was obtained from male New Zealand White rabbits, and cultured by a modification of a previously described technique (3). The tissue was cut into 2–3 mm pieces, washed with phosphate buffer saline (PBS), and then incubated in 5 ml of trypsin solution (0.25%) for 15 min. The supernatant was withdrawn and saved; fresh trypsin was added to the tissue. This procedure was repeated 8 times. Each time, 1 ml of fetal calf serum (FCS) and 10 ml culture media (see below) were added to the supernatants, which were then placed in petri dishes for 90 min at 37°C to allow RBCs and macrophages to precipitate. After 90 min, the supernatants were collected and spun at 800 g for 8 min; the pellets were then resuspended in 1 ml of culture media and placed in 24-well plates. This cell suspension was further enriched for epithelial cells by allowing these suspensions to stand for an additional 90 min, following which the supernatants were collected and pelleted again. The epithelial cells were resuspended in culture media containing 10% FCS and seeded on laminin treated Transwell-COL filter inserts (6.5 or 12 mm diameter, 0.4 μm pore size) at 10^5 cells/cm^2. Cells were allowed to attach for 2 days. Cell culture media consisted of Ham's F12 and Dulbecco's modified Eagle's media (1:1) supplemented with glutamine (2 mM), penicillin (100 IU/ml), streptomycin (100 μg/ml) and fungizone (0.25 μg/ml). Generally after day 4, serum-free media was used to feed the cells. Serum-free medium selectively retards fibroblast growth in primary epithelial cultures (9). A millicell-ERS resistance meter (Millipore) was used to measure electrical resistance across 5 and 8 day-old monolayers which were grown on 12 mm diameter filter inserts.

Electron Microscopy

Eight days after seeding, electron microscopy was performed. Tissue was fixed overnight in 3% gluteraldehyde in 0.1 M Sorensens buffer at pH 7.4 containing 0.18 M sucrose. Post-fixation was done with 1% Palade buffered osmic acid and dehydration was done in graded ethanol. The Epon Embed 812 kitCell was used for embedding. Thin sections were stained with aqueous saturated uranyl acetate, Reynolds lead citrate, and screened on a JEOL 1200 EX electron microscope at 80 kv.

Transport Experiments

Transport experiments were carried out 8 days after seeding. The following 2 buffers were used (mM): NaCl (128), KCl (4.72), $MgSO_4$ (1.25), $CaCl_2$ (1.25), HEPES (5), pH adjusted to 7.4 with Tris (Na^+-containing buffer), or choline chloride (128), KCl (4.72), $MgSO_4$ (1.25), $CaCl_2$ (1.25), HEPES (5), pH adjusted to 7.4 with Tris (Na^+-free buffer). Transepithelial flux of [^{14}C]-mannitol was determined by adding a tracer amount of [^{14}C]-mannitol in Na^+-containing buffer to the apical compartment and monitoring its appearance in the basolateral compartment. For uptake experiments, monolayers were washed twice with Na^+-containing buffer. Uptake into cells (room temperature) was initiated by the addition of 200 μl of uptake mixture, consisting of 50 μM [^{3}H] proline in buffer (Na^+-containing or Na^+-free), to the apical compartment of each

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insert. To stop uptake, the media was carefully aspirated and the monolayers were then washed immediately 3 times with ice-cold buffer. The filters were cut out and placed in scintillation vials and the cells were solubilized overnight in 500 μl of 0.5% Triton X-100. The total radioactivity was determined by liquid scintillation counting. The protein concentration was determined with the Bio-Rad Protein Assay Kit, with bovine serum albumin as the standard. All experiments were performed on cells obtained from 3–6 wells, unless otherwise indicated in the figure legends.

RESULTS AND DISCUSSION

Other investigators have adapted primary cell culture to investigate a variety of processes in the choroid plexus. Most notably, Southwell et al. used a laminin-coated Transwell-COL system to grow confluent monolayers of choroid plexus epithelial cells from rat to study synthesis and secretion of transthyretin and its role in the transport of thyroxin (3). Because of the large number of rats required to obtain a confluent choroid plexus cell culture, this model may not be practical. Sanders-Bush and co-workers isolated mRNA from rat choroid plexus epithelial cells grown via primary cell culture to study transferrin gene expression and synthesis (10). The technique was adapted to culture epithelial cells from rabbit choroid plexus to study the activity of the Na^+/H^+ antiporter (11). However, neither of these primary cell culture models (10,11) required a confluent monolayer of cells and were prepared on solid support.

The goal of this study was to establish a primary cell culture model that can be used to examine the characteristics of transport in the choroid plexus. Porous filter supports for the culture of epithelia more closely mimic the *in vivo* situation, than do cells grown on plastic support, and may thus provide a physiologically more relevant model (7). Also, a confluent cell culture of choroid plexus epithelium, grown on porous filters, would allow studies of transepithelial flux and the regulation of transporters.

In this study, optical and electron microscopic examination of the cultured choroid plexus cells confirmed the presence of a differentiated monolayer of epithelial cells (Fig 1 and 2). A low magnification (5000 X) electron micrograph revealed well-developed microvilli (Fig 2*a*). At higher magnification (20,000 X), the electron dense areas of cell-cell contact are indicative of regions where tight junctions and desmosomes are characteristically observed in most epithelia. At 15 min, the flux of mannitol was 7 times lower across inserts with cells compared to control (blank filters), indicating that the monolayer acts as a significant barrier to the diffusion of mannitol (Fig 3). The trans-epithelial electrical resistance, corrected for blank filters, was similar in 5 and 8 day-old cultures (43 ± 9 ohm-cm^2 and 42 ± 9 ohm-cm^2 respectively, $n = 4$). These results are consistent are comparable to those obtained (99.4 ± 14.7 ohm-cm^2) in cultured rat choroid plexus (3).

To determine if the primary culture of the choroid plexus is an adequate model in which to study the activity of transporters, the Na^+-stimulated uptake of proline was examined. Previous studies in isolated rabbit (12) and bovine (13) brush border membrane vesicles have shown that proline transport in choroid plexus is Na^+-dependent. Our data demonstrate that rabbit choroid plexus primary cell culture, maintained for up to 8 clays *in vitro,* retains Na^+-coupled proline transport activity in a manner similar to that seen in brush border membrane vesicles (Fig 4 *a*). Similarly, we have shown that another amino acid, taurine, is transported in this primary culture via a Na^+-stimulated mechanism (data not shown).

In contrast, the Na^+-stimulated uptake of [^{3}H]-formycin-B (Fig 4*b*) and [^{3}H]-thymidine (data not shown), which has previously been described in isolated choroid plexus tissue slices (5) was not apparent in this primary cell culture model. The data indicate that it is possible that the cells do not retain all of the characteristics of the intact tissue with respect to its barrier/permeability properties. Alternatively, the relevant nucleoside transporter may be inaccessible to substrate added to the apical compartment.

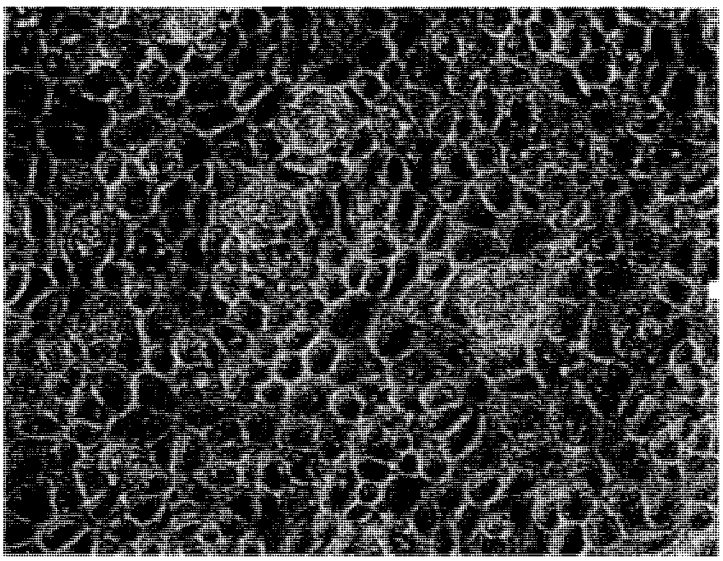

Fig. 1. Light micrograph of a confluent monolayer of 8 day-old rabbit choroid plexus cells culture grown on laminin-treated Transwell-COL filters.

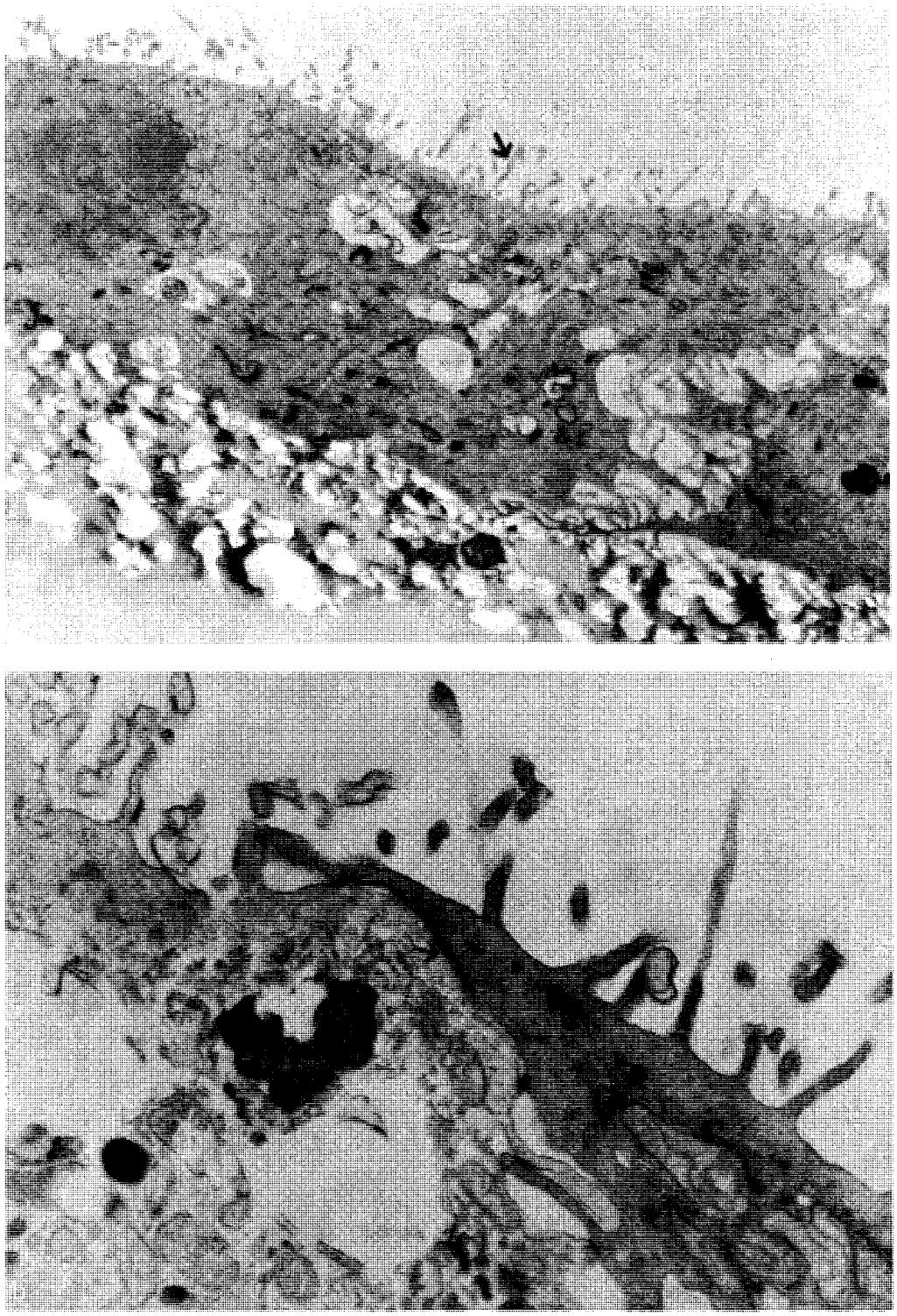

Fig. 2. Electron micrographs 8 day-old rabbit choroid plexus cells cultured on laminin-treated Transwell-COL filters at *(a)* 5000 × (microvilli indicated with arrow) and *(b)* 20,000 × magnification (tight junction is indicated with arrow).

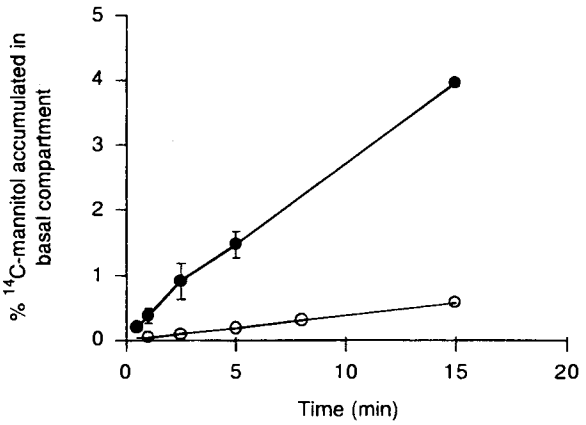

Fig. 3. Flux of [^{14}C]-mannitol across 8 day-old cultured rabbit choroid plexus cells cultured on laminin-treated Transwell-COL filters (open circles, n = 1) and across blank filters (closed circles, mean ± SD, n = 2). Data are expressed as a percentage of the [^{14}C]-mannitol concentration present initially in the apical compartment. If standard deviation bars are not present, standard deviations are encompassed within the point.

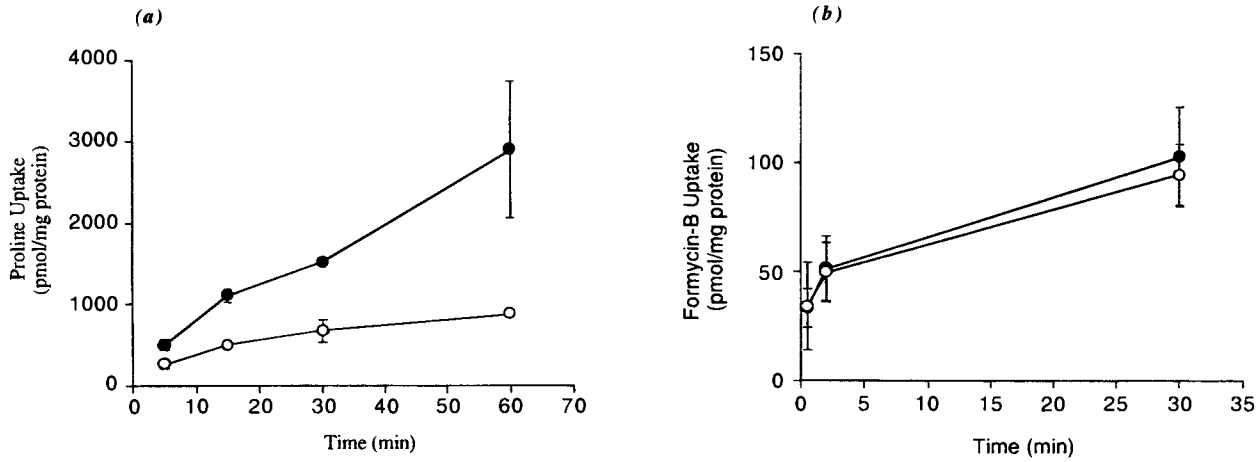

Fig. 4. Time course of *(a)* proline (mean ± SD, n = 4) and *(b)* formycin-B (mean ± SD, n = 3) uptake into rabbit choroid plexus cell culture on laminin-treated Transwell-COL filters, in the presence (closed circles) and absence (open circles) of sodium.

In summary, this study demonstrates that a primary culture of rabbit choroid plexus epithelium can be maintained for 8 days on porous filter support. The cells show differentiated morphological characteristics similar to those of intact choroid plexus epithelium. The cells were used to study the Na^+-dependent uptake of proline, which to the best of our knowledge, is the first demonstration of a Na^+-stimulated cotransporter in choroid plexus cells grown in primary culture. These data suggest that this cell culture model can be used in studying the regulation of these, and possibly other plasma membrane transporters. Our resistance measurements and mannitol flux data, suggest that the model may be useful in studying the transepithelial flux of drugs and endogenous substances.

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