Nucleoside Transport in Isolated Human and Rhesus Choroid Plexus Tissue Slices

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Received December 1, 1997; accepted April 6, 1998

KEY WORDS: nucleosides; transport; choroid; plexus; human; HIV.

INTRODUCTION

Nucleoside transport in mammalian cells occur via equilibrative and concentrative mechanisms. At least two equilibrative systems for nucleosides have been identified (1,2): an NBMPR, (6-(4-nitrobenzyl) thio-9-β-d-ribofuranosyl purine)-sensitive, also known as *es* and a NBMPR-insensitive, *ei*, system. The *es* system has a high affinity binding site for NBMPR ($K_d \sim 0.1$ - nM) whereas concentrations up to 1 μM do not affect the *ei* system.

Five concentrative nucleoside transport systems (N1, N2, N3, N4 and N5) have been identified and characterized in a number of tissue and cell types (3–5). These concentrative transporters are Na^+-dependent and have K_m values in the micromolar range. The N1 is selective for purines, N2 is selective for pyrimidines, N3 and N4 transport both purine and pyrimidine nucleosides. However, N3 is more broadly-selective than N4. These concentrative nucleoside transporters are insensitive to NBMPR. However, a concentrative and Na^+-dependent transporter in leukemia cells (N5) have been shown to be sensitive to NBMPR (6).

The microenvironment of the central nervous system is maintained by the blood brain barrier and the choroid plexus (blood-cerebrospinal fluid barrier). For a substance to enter the brain, it must cross one of these barriers. Recent studies from this laboratory have demonstrated the presence of concentrative transporters for both nucleosides and nucleobases in rabbit choroid plexus (5,7–9). These concentrative transporters are Na^+-dependent, but are distinct in substrate selectivity from nucleoside and nucleobase transporters in renal and intestinal epithelia. Namely, the nucleoside and nucleobase transporters in choroid plexus exhibit a broad substrate selectivity, interacting with both naturally-occurring purines and pyrimidines as well as some nucleoside analogs.

Studies have shown that the human choroid plexus may be directly infected with the HIV virus as well as other opportunistic infections (10). The HIV-infected cells were found on the brush border membrane of the choroid plexus as well as in the capillary endothelium of the choroid plexus. AIDS dementia complex is a major neurological complication associated with HIV infection. It is characterized by motor, cognitive and behavioral dysfunctions. An improvement in neurologic function is observed in patients with HIV-associated neurologic disease, when they are administered azidothymidine (11). Some have postulated that the choroid plexus is the site of entry of several antiviral nucleoside analogs including azidothymidine and dideoxyinosine (12,13). However, a more recent study indicates that azidothymidine crosses both the blood brain barrier and choroid plexus via passive diffusion (14).

The overall goal of this study was to determine the mechanisms of nucleoside transport in human choroid plexus. In addition, studies were carried out in choroid plexus from rhesus macaques, a species used as a model to study human viral infections of the central nervous system including AIDS related dementia complex (15). Our data demonstrate the presence of a Na^+-nucleoside transporter in human and rhesus choroid plexus. The transporter in rhesus macaques appears to be broadly-selective for both purine and pyrimidine nucleosides.

MATERIALS AND METHODS

Procurement of Human Choroid Plexus Tissue

Choroid plexus tissue was obtained from subjects undergoing craniotomies. Prior consent was obtained. The choroid plexus tissue was immediately placed upon harvesting into KCl-HEPES buffer of the following composition KCl (120 mM), mannitol (40 mM) and HEPES (4-2(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (25 mM), pH 7.4 (with 1 M Tris) and 2, 4 dinitrophenol (250 μM).

Isolation of Rhesus Choroid Plexus Tissue

In accordance with tissue-sharing policies, male rhesus monkeys were administered a lethal dose of pentobarbital and the skull was opened and the brain was removed. The choroid plexus was immediately harvested and placed in 2,4 dinitrophenol (250 μM) in KCl-HEPES buffer.

ATP-Depletion of Choroid Plexus Tissue Slices

Choroid plexus tissue was cut into 2–3 mm slices and placed into fresh 2,4 dinitrophenol solution (250 μM) and incubated for 20 minutes at 37°C. These conditions, have been shown to be sufficient for the reduction of ATP to less than 10% of control (7). Following this incubation, the choroid plexus slices were stored in buffer at 4°C until uptake experiments were performed.

Uptake Studies

Individual choroid plexus tissue slices were incubated with 140 μl of reaction mixture containing [^{3}H]thymidine (2.5μCi) [^{3}H]guanosine (2 μCi), [^{14}C]mannitol (25.4 μM), 2,4-dinitrophenol (250 μM), in either KCl-HEPES or NaCl-HEPES (120 mM) buffer. [^{14}C]mannitol was used in these experiments to

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0724-8741/98/0700-1145$15.00/0

correct for nonspecific binding of radioactivity to extracellular water.

The uptake was stopped by blotting on laboratory tissue. The choroid plexus tissue slices were then dried and weighed. Tissue slices were dissolved in 3 M KOH. Following tissue dissolution, 3 M HCl was added to neutralize the KOH. [^{3}H] and [^{14}C] were determined in the dissolved tissue samples and the corresponding reaction mixture by dual isotope liquid scintillation counting.

Data Analysis

The radioactive content from each choroid plexus tissue slice was expressed as a volume of distribution (V_d) as previously described (5):

$$V_d = \frac{\text{dpm [}^3\text{H]nucleoside / g choroid plexus}}{\text{dpm [}^3\text{H]nucleoside / g ml media}} - \frac{\text{dpm[}^{14}\text{C]mannitol / g choroid plexus}}{\text{dpm [}^{14}\text{C]mannitol / ml media}}$$

Statistical analysis was carried out by a Student's unpaired *t* test. A probability, P, of less than 0.05 was considered significant. Data points were determined in triplicate for each experiment.

Chemicals

[^{3}H]thymidine (61 Ci/mmol), [^{3}H]guanosine (12 Ci/mmol), and [^{14}C]mannitol (56 mCi/mmol) were purchased from Moravek Biochemicals, Inc., Brea, CA. Thymidine, guanosine, cytidine, and formycin B were purchased from either Sigma or Aldrich. All other chemicals were purchased from either Sigma, Fisher Scientific, or Aldrich. Cytoscint ES scintillation fluid was purchased from ICN Biomedical Inc.

RESULTS

Time Course of Thymidine Uptake

In the absence of a Na^+-gradient, thymidine accumulated in the human tissue slices and reached an equilibrium in approximately five minutes (Figure 1). In the presence of an inwardly-directed Na^+-gradient, thymidine accumulated against a concentration gradient and an "overshoot phenomenon", indicative of secondary active transport was observed.

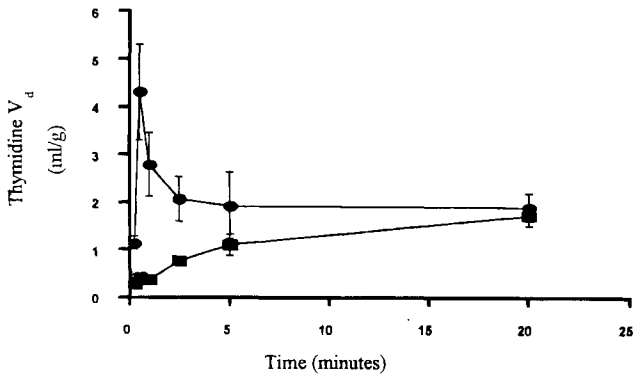

Fig. 1. [^{3}H]-Thymidine uptake into human choroid plexus is Na^+-dependent. The uptake of thymidine (V_d) was examined in the absence (squares) and presence (circles) of an initial inwardly-directed Na^+-gradient. Each data point represents the uptake of thymidine (mean ± S.E.) from four experiments.

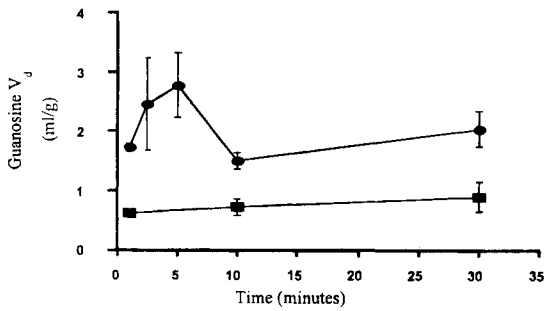

Fig. 2. [^{3}H]-Guanosine uptake into human choroid plexus is Na^+-dependent. The uptake of guanosine (V_d) was examined in the absence (squares) and presence (circles) of an initial inwardly-directed Na^+-gradient. Each data point represents the uptake of guanosine (mean ± S.E.) from a representative experiment.

Time Course of Guanosine Uptake

In the absence of a Na^+-gradient, guanosine accumulated in the tissue slices and reached an equilibrium in approximately ten minutes (Figure 2). However, in the presence of an inwardly-directed Na^+-gradient, guanosine accumulated against a concentration gradient. An "overshoot phenomenon", indicative of secondary active transport was observed.

Inhibition Studies

The effect of various nucleosides on Na^+-stimulated thymidine uptake in the rhesus monkey choroid plexus was examined (Figure 3). At concentrations of 100 μM, cytidine, formycin B and guanosine significantly inhibited Na^+-dependent thymidine uptake (V_d) at 30 s ($p < 0.05$). These data suggest that there is a Na^+-nucleoside transporter in rhesus choroid plexus that is broadly-selective.

DISCUSSION

Previous studies demonstrated that thymidine and guanosine actively accumulate in rabbit choroid plexus tissue slices

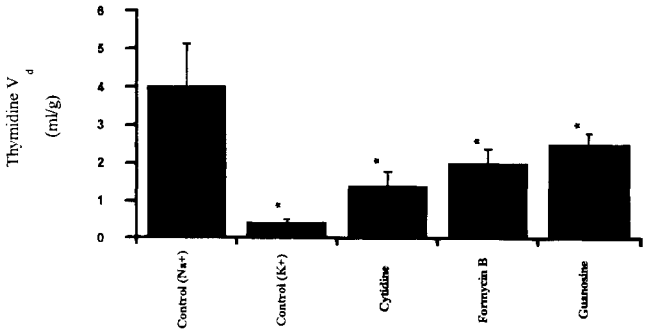

Fig. 3. The effect of nucleosides (100 μM) on thymidine (6 μM) uptake (at 30 s) in ATP-depleted rhesus choroid plexus tissue slices. Cytidine, formycin B and guanosine significantly ($p < 0.05$) inhibited Na^+-dependent thymidine uptake. Bars represent the mean (± S.D.) of data obtained in one experiment.

in the presence of a Na^+-gradient (5). In this study we obtained limited quantities of choroid plexus from humans to determine whether similar Na^+-stimulated nucleoside transport occurs in humans. By using ATP-depleted tissue, we experimentally-imposed an initial inwardly-directed Na^+-gradient in human choroid plexus slices and directly demonstrated (Figures 1 and 2) that thymidine and guanosine transport are coupled to a Na^+-gradient. These data provide the first demonstration of Na^+-dependent nucleoside transport in human choroid plexus. Because of limited availability of human choroid plexus, further characterization studies could not be performed.

The substrate selectivity of the Na^+-stimulated thymidine transporter from limited data in rhesus choroid plexus appears to be similar to that of Na^+-stimulated thymidine transport in rabbit choroid plexus. The rabbit choroid plexus Na^+-nucleoside transporter is broadly-selective for purine and pyrimidine nucleosides. In rhesus choroid plexus, the purine and pyrimidine nucleosides cytidine, formycin B and guanosine were potent inhibitors of Na^+-dependent thymidine uptake (Figure 3).

At least five Na^+-dependent systems for nucleoside transport have been identified. To date, these membrane proteins have not been purified. However, recent functional expression of these transporters in Xenopus laevis oocytes has led to cloning of several of the Na^+-driven nucleoside transporters. For example, an N2 subtype (rCNT1) was cloned from rat jejunum (16) and an N1 subtype (SPNT) from rat liver (17). More recently, the human homolog of both rCNT1 (hCNT1) (18) and SPNT (hSPNT1] (19) were cloned from kidney via homology techniques. Since these transporters share similar functions, it is not surprising that much homology exists among the Na^+-nucleoside transporters. The molecular structure is similar in their mid-regions but differs greatly at the N and C termini regions. The tissue specific roles of these transporters is yet to be completely defined.

In conclusion, this study demonstrates that both thymidine and guanosine are transported in human choroid plexus via Na^+-dependent mechanisms. Our data also suggest that the nucleoside transporter in rhesus macaques is similar to N3, the nucleoside transporter in rabbit choroid plexus which is broadly selective for both purine and pyrimidine nucleosides. The study provides the first evidence of secondary active Na^+-dependent transporter(s) for nucleosides in choroid plexus from human and rhesus macaques. These transporter(s) may play a role in nucleoside homeostasis in the brain and in the targeting of selected nucleoside analogs to the central nervous system.

ACKNOWLEDGMENTS

This work was supported in part by NIH Grant GM42230, and GM 26691.

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