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Biochimica et Biophysica Acta 1191 (1994) 190–196

BBA Biochimica et Biophysica Acta

Further characterization of the sodium-dependent nucleoside transporter (N3) in choroid plexus from rabbit

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(Received 5 November 1993)

Abstract

The Na^+/nucleoside cotransporter in rabbit choroid plexus differs from Na^+/nucleoside cotransporters in other tissues in terms of substrate selectivity and stoichiometry. The overall goal of this study was to further characterize the kinetics of this system (N3). Choroid plexus tissue slices obtained from rabbit brain were depleted of ATP and treated with valinomycin and K^+. Na^+/thymidine uptake at 30 s in the presence of an inside negative potential difference was significantly greater than in the absence of a potential difference. Na^+/thymidine uptake was not significantly affected by replacing chloride with either thiocyanate or sulfate. The K_m of Na^+/guanosine uptake was 149, 85.2 and 30.5 μM in the presence of a 25, 50 and 100 mM Na^+ gradient, respectively, whereas the V_{max} was unaffected, suggesting that Na^+ binds first to the cotransporter, then, the nucleoside. Therapeutically relevant base-modified nucleoside analogs, 5-fluorouridine, 2-chloroadenosine and 5-iododeoxyuridine, significantly inhibited Na^+/thymidine uptake with IC_{50} values (mean ± S.E.) of 12.0 ± 2.3, 21.3 ± 2.2 and 24.4 ± 2.1 μM, respectively, whereas nucleoside analogs structurally modified on the ribose ring, 3'-azidothymidine, dideoxyinosine and dideoxycytidine (100 μM) did not. These studies suggest that Na^+/nucleoside cotransport in the choroid plexus is electrogenic and is not dependent on chloride. This cotransporter, which is present in choroid plexus but not in renal brush-border membrane vesicles from rabbit, may play a role in the disposition of clinically relevant base-modified nucleoside analogs into and out of the brain.

Key words: Nucleoside; Sodium ion/nucleoside cotransport; Choroid plexus

1. Introduction

Flux of nucleosides and some nucleoside analogs across the plasma membrane of mammalian cells involves both equilibrative and secondary active Na^+-cotransport mechanisms [1]. Na^+/nucleoside cotransport systems have been identified in a variety of cell types including intestinal, renal and choroid plexus epithelia, leukemia cells, splenocytes, macrophages, leishmania and lymphoma cells [2–18].

Several subtypes of Na^+/nucleoside cotransport systems have been identified recently in kinetic studies [2,5–7,9,18]. These concentrative nucleoside transport systems, which are insensitive to nitrobenzylthioinosine (NBMPR), differ in terms of their substrate selectivity and stoichiometry. Recently, we described a unique Na^+/nucleoside cotransport system (N3) in rabbit choroid plexus, which is selective for both purine and pyrimidine nucleosides and which has a Na^+ : nucleoside stoichiometric coupling ratio of 2:1 [9]. The Na^+/nucleoside cotransporter in rabbit choroid plexus differs from previously described Na^+/nucleoside transport systems in other tissues which are generally selective for either purine (N1) or pyrimidine (N2) nucleosides and which have Na^+ : nucleoside stoichiometric coupling ratios of 1:1 [2,3,11,12,19]. The system in the choroid plexus may play a role in the transport of nucleosides and some nucleoside analogs into and out of the brain [8–10].

The overall goal of this study was to further characterize the Na^+/nucleoside cotransporter in rabbit choroid plexus in terms of electrogenicity and requirements for specific anions. We determined the kinetic model which characterizes the interaction of Na^+ and nucleosides with the cotransporter. In addition, we

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SSDI 0005-2736(93)E0373-I

examined the substrate selectivity with respect to clinically relevant base-modified nucleoside analogs and compared the specificity of the Na^+/nucleoside transport systems in choroid plexus and renal tissues obtained from the same species. The study demonstrates that the Na^+/nucleoside cotransporter in rabbit choroid plexus is electrogenic and does not have specific anion requirements. Kinetically, Na^+ appears to bind first followed by nucleoside binding. The system in rabbit choroid plexus, N3, is broadly selective for both purine and pyrimidine nucleosides including some nucleoside analogs structurally modified on the base. The system differs from Na^+/nucleoside cotransport systems in rabbit renal cortical brush-border membrane vesicles which involve both N1 and N2 systems.

2. Materials and methods

Preparation of ATP-depleted choroid plexus

Choroid plexuses isolated from the lateral ventricles of the rabbit (New Zealand white rabbit) were cut into 2–3 mm pieces and depleted of ATP by incubating with 2,4-dinitrophenol (250 μM) at 37°C for 20 min or 1 h as described previously [9,20–22]. After the incubation period the tissue slices in the 2,4-dinitrophenol solution were placed on ice until use. Experiments in this laboratory have demonstrated that DNP treatment of choroid plexus tissue slices results in almost total depletion of ATP (ATP concentration reduced to less than 10% of control).

Transport studies in choroid plexus tissue slices

Uptake of nucleosides was studied by incubating each tissue slice in 140 μl of reaction medium containing [^{3}H]guanosine (85 or 170 nM) or [^{3}H]thymidine (17 nM), together with [^{14}C]mannitol (23.2 μM) as a marker of extracellular water space and the respective unlabeled nucleoside (2 μM) as described previously [9]. 2,4-Dinitrophenol (250 μM) was present in the reaction medium to ensure continued depletion of ATP. NBMPR (10 μM) was also included to inhibit passive nucleoside flux via the NBMPR sensitive transporter. Other constituents are specified in the figure legends. All the incubations were carried out at 37°C. After each incubation, tissue slices were blotted on laboratary tissue and dried for 1 h by IR-lamp heater on strips of preweighed aluminum foil as described previously [9]. ^{3}H and ^{14}C in the dried tissue slice and in the corresponding reaction medium were determined by dual isotope liquid scintillation counting on a Beckman Model 1801 liquid scintillation counter as described previously [9]. Counting efficiency of ^{3}H ranged between 45–47% and of ^{14}C ranged between 92 and 94%.

For electrogenicity studies, various media as specified in the figure legends were used to pre-incubate the tissue and in the experiment to generate various conditions. Tissue slices were preincubated with 2,4-dinitrophenol (250 μM) and valinomycin (10 μM) for 1 h in various media as specified in the figure legends. The uptake of thymidine was measured under five different conditions: (1) Both an initial inwardly directed Na^+ gradient and a membrane potential difference (inside negative) were present. (2) No membrane potential difference but an initial inwardly directed Na^+ gradient was present. (3) No Na^+ gradient, but Na^+ was equilibrated across the membrane, and a membrane potential difference (inside negative) was present. (4) No membrane potential difference, but Na^+ was equilibrated across the membrane. (5) Neither a membrane potential difference nor Na^+ was present.

For studies of the effect of anions on Na^+/thymidine cotransport, the uptake of thymidine was measured under voltage-clamped condition. Tissue slices were preincubated with 2,4-dinitrophenol (250 μM) and valinomycin (10 μM) for 1 h in the presence of K^+ (40 mM KCl) and in the absence of Na^+ followed by incubation in various reaction media as specified in the figure legends. K^+ (40 mM) and Na^+ (120 mM) were present in all the reaction media.

For the kinetic studies of Na^+/guanosine cotransport, the uptake of guanosine was measured as a function of Na^+ concentration under voltage-clamped condition. Tissue slices were preincubated with 2,4-dinitrophenol (250 μM) and valinomycin (10 μM) for 1 h in the presence of K^+ (40 mM KCl) and in the absence of Na^+. Afterwards the slices were incubated in the reaction media containing different concentrations of Na^+. K^+ (40 mM KCl) and Na^+ (0–120 mM NaCl) were present in all the reaction media.

For inhibition and IC_{50} studies, tissue slices were preincubated with 2,4-dinitrophenol (250 μM) for 20 min in the medium (120 mM KCl, 80 mM mannitol, 25 mM Hepes-Tris (pH 7.4)), and the uptake of thymidine was measured in the presence of various nucleosides and nucleoside analogs in the same medium in which KCl is replaced by NaCl (120 mM).

Preparation of brush-border membrane vesicles (BBMV)

BBMV were prepared from the outer cortex of rabbit kidney (New Zealand white rabbit) by divalent cation (Mg^{2+}) precipitation [23], as modified in our laboratory [24–27]. Protein concentration was measured using the Bio-Rad Protein Assay Kit™, and bovine serum albumin was used as a protein standard.

Transport studies in BBMV

The uptake of thymidine and guanosine as a function of the concentration of various nucleoside analogs was measured at 22°C by a fast filtration technique as described previously [28]. Briefly, an aliquot (10 μl) of BBMV suspension (10–15 mg protein/ml) was added

to 40 μl of reaction medium containing [^{3}H]thymidine (2 μM) or [^{3}H]guanosine (2 μM) in buffer (10 mM Hepes, 150 mM KCl or NaCl (pH 7.4) with 1 M KOH or 1 M NaOH) and incubated for 5 s. At the end of each incubation, the uptake was stopped by adding 3 ml of ice-cold buffer and filtering the suspension under vacuum through a membrane filter (0.3 μm, pH type, Millipore, Bedford, MA). After washing the filter 3 times with the ice-cold buffer, the filter was placed into 5 ml of scintillant. Radioactivity associated with the filter was measured by liquid scintillation counting. The radioactivity measured after filtering the reaction medium without any membrane vesicles (blank value) is subtracted from radioactivity associated with the filtered BBMV solution to obtain the uptake values.

Data analysis

Uptake of nucleoside in each choroid plexus tissue slice was expressed as a volume of distribution (V_d), and was corrected for extracellular water space using the V_d of mannitol as previously described [9,22,29]. Usually, the percentage of nonspecific uptake (extracellular water space) ranged between 28 and 86% depending upon the uptake time and experimental conditions.

$$V_d = \frac{\text{dpm of [}^2\text{H]nucleoside/g of tissue}}{\text{dpm of [}^2\text{H]nucleoside/ml of medium}} - \frac{\text{dpm of [}^{14}\text{C]mannitol/g of tissue}}{\text{dpm of [}^{14}\text{C]mannitol/ml of medium}}$$

In each experiment the V_d was determined in triplicate to generate each data point.

Uptake of nucleosides in BBMV was expressed as pmol/mg protein. In each experiment the uptake of nucleoside in BBMV was determined in five measurements to generate each point.

For the kinetic study of Na^+/guanosine cotransport, the initial transport rate (v), determined at 30 s, was expressed as nmol/g tissue per s and fit to the Michaelis-Menten equation. Fits were carried out using the nonlinear least square regression program, FIT FUNCTION, on the National Institutes of Health computer system, PROPHET.

$$v = V_{max} \cdot C/(K_m + C)$$

where V_{max} is the maximum transport rate; K_m is the concentration of guanosine when the initial rate is at one-half of the maximum, and C is the concentration of guanosine in the reaction medium.

The IC_{50} of the various nucleosides and nucleoside analogs in inhibiting Na^+/guanosine or Na^+/thymidine cotransport was obtained by fitting the data to the following sigmoidal inhibition model (29):

$$V = V_0/\left(1 + (C/IC_{50})^n\right)$$

where V is the uptake of guanosine or thymidine in the presence of inhibitor, V_0 is the uptake of guanosine or thymidine in the absence of inhibitor, C is the concentration of inhibitor, and n is the Hill coefficient. All the fits were carried out on a Macintosh SE computer using a nonlinear fitting routine of the software, Kaleidagraph. Statistical analysis was carried out by unpaired Student's t-test. A probability, P, of less than 0.05 was considered significant.

Chemicals

[^{3}H]Guanosine (15 Ci/mmol), [^{3}H]thymidine (74 Ci/mmol) and [^{14}C]mannitol (54 mCi/mmol) were obtained from Moravek Biochemicals, Brea, CA. Nucleosides and nucleoside analogs including guanosine, inosine, cytidine, thymidine uridine, formycin B, 2-chloroadenosine, 5-fluorouridine, 5-iododeoxyuridine and 8-azidoadenosine, were purchased from Sigma. All other chemicals were obtained from either Sigma, Fisher Scientific, or Aldrich. Cytoscint ES scintillation fluid was obtained from ICN Biomedical, Irvine, CA. Bio-Rad protein Assay Kit™ was purchased from Bio-Rad, Richmond, CA.

3. Results

The effect of a transmembrane potential difference on Na^+-driven thymidine uptake in isolated choroid plexus tissue slices was examined using K^+-diffusion potentials (Fig. 1). The uptake of thymidine (mean ± S.E.) at 30 s and 1.5 min, was 0.9 ± 0.16 and 1.23 ± 0.19 ml/g, respectively, in the presence of Na^+ equilibrated across the membrane and an initial inside negative membrane potential difference ($[K^+]_{in} > [K^+]_{out}$). These values are significantly greater than the values of 0.33 ± 0.07 and 0.77 ± 0.08 ml/g, respectively, obtained in the absence of both a membrane potential difference and Na^+ ($P < 0.05$). The value at 30 s is also significantly greater than the value of 0.46 ± 0.10 ml/g obtained in the absence of a membrane potential difference, but with Na^+ equilibrated across the membrane ($P < 0.05$). In the presence of an initial inwardly directed Na^+ gradient, but in the absence of a membrane potential difference, an 'overshoot phenomenon' was observed, indicating that a Na^+ gradient independently can support the transport of thymidine against its concentration gradient. However, in the presence of both a membrane potential difference and a Na^+-gradient, both the initial rate of Na^+-driven thymidine uptake and the magnitude of the transient overshoot were significantly higher than the respective values obtained in the presence of a Na^+ gradient alone ($P < 0.01$). These results suggest that Na^+/thymidine cotransport is an electrogenic process.

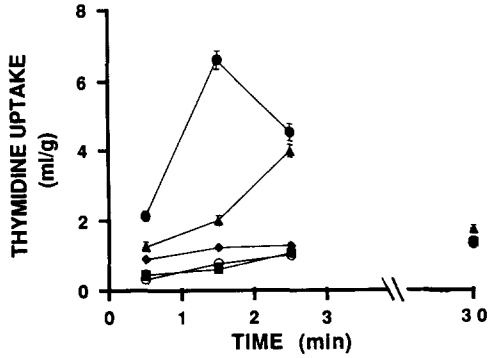

Fig. 1. Effect of valinomycin-induced K^+-diffusion potential on Na^+-driven thymidine uptake in ATP-depleted choroid plexus tissue slices from rabbit. The solid circles represent data obtained in the presence of an inside-negative potential difference and a Na^+-gradient. The triangles represent data obtained in the presence of a Na^+-gradient, but in the absence of a potential difference. The diamonds represent data obtained in the presence of Na^+ equilibrated across the cells and a potential difference. The squares represent data obtained in the absence of a potential difference, but with Na^+ equilibrated across the membrane, and the open circles represent data obtained in the absence of both Na^+ and a potential difference. Tissue slices were preincubated with 10 μM of valinomycin and 250 μM of 2,4-dinitrophenol for 1 h in media containing *N*-methylglutamine (NMG, 120 mM) and KCl (40 mM) (open circles, triangles), NaCl (120 mM) and KCl (40 mM) (squares), KCl (120 mM) and NaCl (60 mM) (diamonds) or KCl (120 mM) (circles). The uptake was carried out in the presence of NMG (120 mM) and KCl (40 mM) (solid circles), NaCl (120 mM) and KCl (40 mM) (squares, triangles), NMG (120 mM) and NaCl (60 mM) (diamond) or NaCl (120 mM) (solid circles). All the media contained 15 mM of Hepes-Tris (pH 7.4). The osmolarity was maintained with mannitol. Points represent the mean (±S.E.) of data obtained in three separate experiments.

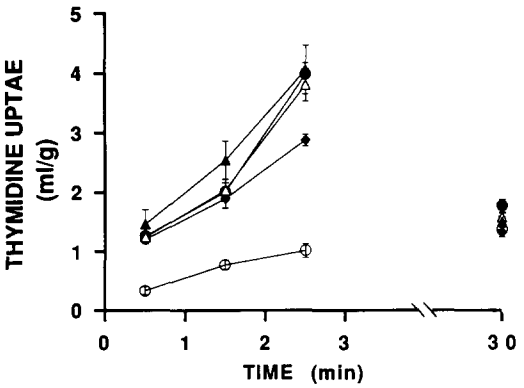

Fig. 2. Effect of anions on Na^+-driven thymidine uptake in ATP-depleted choroid plexus tissue slices from rabbit. The uptake was determined in the presence of 120 mM of NaCl (solid circles), NaBr (diamonds), NaSCN (open triangles), 60 mM of Na_2SO_4 (solid triangles) and 120 mM of NMG (open circles). The respective potassium salt (40 mM) was present in all the reaction media. Tissue slices were preincubated with 10 μM of valinomycin and 250 μM of 2,4-dinitrophenol for 1 h in medium containing NMG (120 mM), KCl (40 mM), Hepes-Tris (15 mM) (pH 7.4). Points represent the mean (±S.E.) of data obtained in three separate experiments.

In the presence of an initial inwardly directed Na^+-gradient and either chloride, thiocyanate or sulfate, overshoot phenomena of similar magnitudes were observed (Fig. 2). At each time point, thymidine uptake was not significantly affected by any of these anions. However, in the presence of bromide, Na^+/thymidine uptake at 2.5 min was significantly lower in comparison to its uptake in the presence of other anions ($P < 0.05$).

To ascertain the mechanism by which Na^+ affects nucleoside transport, we carried out kinetic studies of Na^+-driven guanosine uptake in the presence of various Na^+ gradients under voltage-clamped conditions (Fig. 3). The K_m (mean ± S.D.) of guanosine was 149 ± 23.6, 85.2 ± 8.6 and 30.5 ± 4.2 μM in the presence of a 25, 50 and 100 mM Na^+ gradient, respectively, whereas the V_{max} (mean ± S.D.) of Na^+-driven guanosine uptake (5.0 ± 0.4, 5.1 ± 0.2 and 4.4 ± 0.2 nmol/g per s, respectively) was unaffected.

The effect of nucleosides on the Na^+-driven transport of guanosine and thymidine in both choroid plexus tissue slices and renal BBMV was examined. In the choroid plexus, both the purine nucleosides, formycin B and inosine, and the pyrimidine nucleosides, cytidine, thymidine and uridine, were potent inhibitors of Na^+-driven guanosine uptake as shown by their IC_{50} values (Table 1). However, in the renal BBMV, purine nucleosides selectively inhibited Na^+/guanosine uptake at lower concentrations and pyrimidine nucleosides selectively inhibited Na^+/thymidine uptake. Uridine inhibited both Na^+/thymidine and Na^+/guanosine uptake.

The interaction of several nucleoside analogs with the Na^+/nucleoside cotransporter in the choroid plexus was investigated (Table 2). The base-modified nucleoside analogs (100 μM), 5-fluorouridine (5-FUrd), 2-

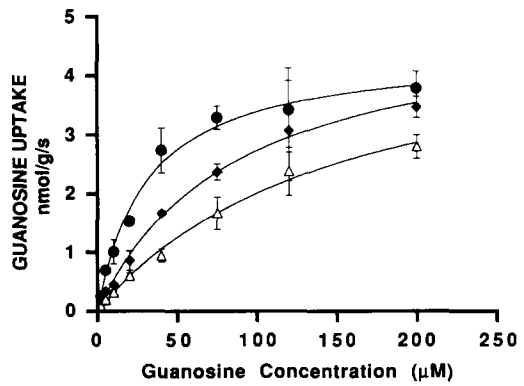

Fig. 3. Kinetics of Na^+-driven guanosine uptake in ATP-depleted choroid plexus tissue slices from rabbit. The uptake was determined at 30 s in the presence of 25 (triangles), 50 (diamonds) and 100 mM (circles) of NaCl, respectively. Tissue slices were preincubated with 10 μM of valinomycin and 250 μM of 2,4-dinitrophenol for 1 h in the medium containing choline chloride (100 mM), KCl (40 mM), Hepes-Tris (15 mM) (pH 7.4). Points represent the mean (±S.D.) of triplicates of a representative experiment.

Table 1
Inhibition of Na^+-driven guanosine and thymidine transport by other nucleosides

Inhibitor	Choroid plexus		Kidney	
	thymidine [a]	guanosine	thymidine	guanosine
Purines				
Formycin B	22.2±3.1	42.4±2.3	>150	85.7±12.6
Guanosine	7.9±0.7	30.5 [b]	>150	–
Inosine	9.2±0.6	10.3±1.0	>150	20.7± 3.1
Pyrimidines				
Cytidine	4.6±0.4	13.5±6.4	16.7± 7.5	>150
Thymidine	13.0±2.3	15.6±1.3	–	>150
Uridine	9.5±2.0	8.2±1.6	51.4±27.6	50.5± 5.2

The uptake of guanosine or thymidine was determined at 20 s (choroid plexus) or 5 s (BBMV) in the presence of an initial inwardly directed Na^+ gradient and varying concentrations of other nucleosides. The concentration of nucleoside producing 50% inhibition of Na^+-driven guanosine or thymidine transport (IC_{50}) was derived from individual inhibition curves. The values represent mean±S.E. of data obtained from two to three separate experiments. Results are given in μM.
[a] Data from Wu et al., 1992.
[b] Derived from a Michaelis-Menten study of Na^+/guanosine transport.

Table 2
Effect of nucleoside analogs on Na^+-driven thymidine transport

Inhibitor	Uptake (ml/g)
Control	2.35±0.31
ddI	2.32±0.17
ddC	2.31±0.48
AZT	1.95±0.28
5-FUrd	0.5 ±0.01 *
2-ClAdo	0.68±0.26 *
5-IdUrd	1.00±0.07 *

The uptake (at 30 s) was determined in the presence of an intial inwardly directed Na^+ gradient and 100 μM of various nucleoside analogs. The values represent the mean±S.E. of data obtained from three separate experiments. Values with * mean significantly different from the control ($P < 0.05$).

Table 3
Inhibition of Na^+-driven thymidine transport by nucleoside analogs modified on the base

Inhibitor	IC_{50} (μM)
5-Fluorouridine	12.0±2.3 (n [a] = 1.13±0.16)
2-Chloroadenosine	21.3±2.2 (n = 1.71±0.14)
5-Iododeoxyuridine	24.4±2.1 (n = 0.94±0.14)

The uptake of thymidine was determined at 20 s in the presence and absence of an initial inwardly directed Na^+ gradient (120 mM), repectively, and varying concentrations of nucleoside analogs. The value of IC_{50} was derived from the individual inhibition curves (see Materials and methods). The values represent mean±S.E. of data obtained from three separate experiments.
[a] n is a slope factor.

chloroadenosine (2-ClAdo) and 5-iododeoxyuridine (5-IdUrd), significantly inhibited Na^+-driven thymidine uptake at 30 s ($P < 0.01$), whereas the nucleoside analogs structually modified on the ribose ring, dideoxyinosine (ddI), dideoxycytidine (ddC) and 3′-azidothymidine (AZT) did not significantly inhibit Na^+-driven thymidine transport. We further examined the effect of 5-FUrd, 2-ClAdo and 5-IdUrd on the Na^+-driven thymidine transport. These three compounds were potent inhibitors of the Na^+-driven thymidine transport as indicated by their IC_{50} values (Table 3). The inhibition curves were monophasic for these three compounds. For 5-FUrd and 5-IdUrd, but not 2-ClAdo, the slope factor, n, did not differ significantly from 1 (Table 3), suggesting a competitive interaction for these two compounds.

4. Discussion

Na^+/nucleoside cotransporters have been identified in a number of species including mouse, rat, rabbit, cow and human [2–18]. The cotransporters have been classified into two major classes based primarily upon substrate specificity, namely, purine nucleosides are transported primarily by the N1 cotransporter, whereas, pyrimidine nucleosides are transported primarily by the N2 cotransporter with the exception of adenosine and uridine which are transported by both cotransporters. Recently, we identified two Na^+/nucleoside cotransporters which differ from the N1 and N2 systems. One, found on the human renal cortical brush-border membrane, appears to be similar to the N2 cotransporter except that it also transports guanosine [19]. This system is termed N4. The other (N3), in rabbit choroid plexus epithelium, appears to transport both purine and pyrimidine ribo- and deoxyribonucleosides [9]. The goal of this study was to further characterize this cotransporter (N3).

First, we determined whether Na^+/nucleoside transport in the choroid plexus was an electrogenic process. The data shown in Fig. 1 suggest that a membrane potential can drive Na^+/nucleoside cotransport in the choroid plexus and are consistent with previous studies which have shown that Na^+/nucleoside cotransport in bovine, rabbit, rat, and human renal brush-border membrane vesicles [7,19,28,31] is electrogenic.

Many secondary active Na^+ cotransporters in the central nervous system as well as some epithelial transporters require chloride [32,33]. Our data (Fig. 2) demonstrate that specific anions including chloride are not necessary for Na^+/nucleoside cotransport in the choroid plexus. Substituting thiocyanate or sulfate for chloride resulted in no appreciable reduction in Na^+-driven thymidine uptake. The reason why bromide decreased Na^+/thymidine uptake is not clear.

The mechanism by which Na^+ affects the Na^+/nucleoside cotransporter was examined in kinetic stud-

ies (Fig. 3). The data, indicating that the affinity of the cotransporter for nucleosides is dependent on Na^+ concentration, whereas the maximum velocity of the cotransporter is not, are consistent with a K kinetics model in which the cosubstrate, Na^+, binds first to the cotransporter, then, the substrate, nucleoside [34]. Previous studies from other laboratories have shown that the affinity of the Na^+/glucose cotransporter in intestinal BBMV [35] and Na^+/succinate cotransporter in renal BBMV [36] is also affected by Na^+ in a similar fashion. No consistent effect of Na^+ on the V_{max} of Na^+-cotransporters has been observed [37].

Because the Na^+/nucleoside cotransporter in the rabbit choroid plexus differs from previously described Na^+/nucleoside cotransporters in terms of its substrate selectivity, we addressed the question of whether this cotransporter reflects a species difference in nucleoside transporters, i.e., does the Na^+/nucleoside cotransporter in rabbit tissues exhibit broad substrate selectivity? Previously, Jarvis and his colleagues [31] identified a Na^+/nucleoside cotransporter in rabbit BBMV by using uridine as a substrate. Because uridine can be transported by both N1 and N2 transporters, it was not possible to determine the subtype of Na^+/nucleoside transporters. Our comparative studies in rabbit renal brush-border membrane vesicles demonstrated that both N1 and N2 Na^+/nucleoside cotransporters are present (Table 1); namely, purine nucleosides selectively inhibited Na^+/guanosine uptake, whereas pyrimidine nucleosides selectively inhibited Na^+/thymidine uptake. Uridine, a substrate of both N1 and N2 transporters, was an exception. In contrast, in choroid plexus tissue slices, both purine and pyrimidine nucleosides were potent inhibitors of both Na^+/thymidine [9] and Na^+/guanosine uptake. These data support the notion that the unique broadly selective Na^+/nucleoside cotransporter in rabbit choroid plexus is not species dependent. Further studies are needed to determine whether this cotransporter is present in choroid plexus of other mammalian species.

Many nucleoside analogs are marketed or being developed as antiviral and antineoplastic agents. For example, AZT (zidovudine) is currently the drug of choice for AIDS. 2-Chlorodeoxyadenosine is effective in the treatment of hairy cell leukemia [38]. 5-Iodo-deoxyuridine is used clinically as a radiosensitizer in the treatment of sarcomas [39]. In addition, thymidine has been used to modulate the cytotoxic effect of fluorouracil in the treatment of a variety of malignant neoplasms [40]. Data in the literature have suggested that the blood-brain barrier is not the site of entry of some nucleosides, including thymidine, and nucleoside analogs into the brain [41,42]; therefore, the choroid plexus may play a role in the transport of nucleosides and nucleoside analogs into and out of the brain [8–10,41–43]. To improve our understanding of the mechanisms by which nucleoside analogs are transported in the choroid plexus, we investigated the interaction of several clinically relevant nucleoside analogs with the choroid plexus Na^+/nucleoside cotransporter, N3. Nucleoside analogs structurally modified on the ribose ring, ddI, ddC and AZT did not inhibit Na^+/thymidine uptake (Table 2), consistent with our previous studies [9]. However, the nucleoside analogs modified on the base, 5-FUrd, 2-ClAdo and 5-IdUrd, interact with this cotransporter with IC_{50} values in the range of the K_m values of Na^+/thymidine [9] and Na^+/guanosine cotransport in the choroid plexus. Previously, we demonstrated that these base-modified nucleoside analogs interact with the Na^+/nucleoside cotransporter in human renal BBMV [19]. The data obtained in the current study suggest that the Na^+/nucleoside cotransporter, N3, may play a role in the transport of nucleosides and clinically relevant base-modified nucleoside analogs into and out of the cerebrospinal fluid and ultimately the brain.

5. Acknowledgements

This work was supported by National Institute of Health Grants GM 42230, GM 36780 and GM 26691. Xiaochun Wu was supported by Cheng Scholars Program at University of California, San Francisco.

6. References

[1] Plagemann, P.G.W., Wohlhueter, R.M. and Woffendin, C. (1988) Biochim. Biophys. Acta 947, 405–443.
[2] Vijayalakshmi, D. and Belt, J.A. (1988) J. Biol. Chem. 263, 19419–19423.
[3] Jakobs, E.S. and Paterson, A.R.P. (1986) Biochim. Biophys. Res. Commun. 140, 1028–1035.
[4] Le Hir, M. and Dubach, U.C. (1985) Pfluegers Arch. 404, 238–243.
[5] Le Hir, M. (1990) Renal Physiol. Biochem. 13, 154–161.
[6] Lee, C.W., Cheeseman, C.I. and Jarvis, S.M. (1990) Am. J. Physiol. 258, F1203–F1210.
[7] Williams, T.C. and Jarvis, S.M. (1991) Biochem. J. 274, 27–33.
[8] Spector, R. (1980) Arch. Biochem. Biophys. 205, 85–93.
[9] Wu, X., Yuan, G., Brett, C.M., Hui, A.C. and Giacomini, K.M. (1992) J. Biol. Chem. 267, 8813–8818.
[10] Wu, X., Hui, A.C. and Giacomini, K.M. (1993) Pharm. Res. 10, 611–615.
[11] Cradford, C.R., Ng, C.Y.C., Noel, L.D. and Belt, J.A. (1990) J. Biol. Chem. 265, 9732–9736.
[12] Dagnino, L., Lee Bennett, L. and Paterson, A.R.P. (1991) J. Biol. Chem. 266, 6312–6317.
[13] Plagemann, P.G.W. and Woffendin, C. (1989) Biochim. Biophys. Acta 981, 315–325.
[14] Plagemann, P.G.W. (1991) Biochem. Pharmacol. 42, 247–252.
[15] Holstege, A., Gengenbacher, H.-M., Jehle, L. and Hoppmann, J. (1991) Hepatology 14, 373–380.
[16] Ogbunude, P.O.J., Al-Jaser, M.H. and Baer, H.P. (1991) Exp. Parasitol. 73, 369–375.

[17] Plagemann, P.G.W. (1991) J. Cell. Biochem. 46, 54–59.
[18] Gutierrez, M.M., Brett, C.M., Ott, R.J., Hui, A.C. and Giacomini, K.M. (1992) Biochim. Biophys. Acta 1105, 1–9.
[19] Gutierrez, M.M. and Giacomini, K.M. (1993) Biochim. Biophys. Acta 1149, 202–208.
[20] Carter-Su, C. and Kimmich, G.A. (1979) Am. J. Physiol. 237 (1), C67–C74.
[21] Suzuki, H., Sawada, Y., Sugiyama, Y., Iga, T. and Hanano, M. (1987) J. Pharmacol. Exp. Ther. 243, 1147–1152.
[22] Whittico, M.T., Yuan, G. and Giacomini, K.M. (1990) J. Pharmacol. Exp. Ther. 255, 615–623.
[23] Booth, A.G. and Kenny, A.J. (1974) Biochem. J. 142, 575–581.
[24] Hsyu, P.-H. and Giacomini, K.M. (1987) J. Biol. Chem. 262, 3964–3968.
[25] Hsyu, P.-H., Gisclon, L.G., Hui, A.C. and Giacomini, K.M. (1988) Am. J. Physiol. 254, F56–F61.
[26] Hsyu, P.-H. and Giacomini, K.M. (1987) Am. J. Physiol. 252, F1065–F1072.
[27] Ott, R.J., Hui, A.C., Yuan, G. and Giacomini, K.M. (1991) Am. J. Physiol. 261, F443–F451.
[28] Lee, C.W., Cheeseman, C.I. and Jarvis, S.M. (1988) Biochim. Biophys. Acta 942, 139–149.
[29] Murphy, V.A. and Johanson, C.E. (1989) Biochim. Biophys. Acta 979, 187–192.
[30] De Lean, A., Munson, P.J. and Rodbard, D. (1978) Am. J. Physiol. 235, E97–E102.
[31] Williams, T.C., Doherty, A.J., Griffith, D.A. and Jarvis, S.M. (1989) Biochem. J. 264, 223–231.
[32] Moyer, M.S., Insler, N. and Dumaswala, R. (1992) Biochim. Biophys. Acta 1109, 74–80.
[33] Amara, S.G. (1992) Nature 360, 420–421.
[34] Stein, W.D. (1990) Channels, Carriers, and Pumps: An Introduction to Membrane Transport, pp. 195–199, Academic Press, New York.
[35] Kaunitz, J.D. and Wright, E.M. (1984) J. Membr. Biol. 79, 41–51.
[36] Wright, S.H., Hirayama, B., Kaunitz, J.D., Kippen, I. and Wright, E.M. (1983) J. Biol. Chem. 258, 5456–5462.
[37] Wright, E.M., Turk, E., Zabel, B., Mundlos, S. and Dyer, J. (1991) J. Clin. Invest. 88, 1435–1440.
[38] Estey, E.H., Kurzrock, R., Kantarjian, H.M., O'Brien, S.M., McCredie, K.B., Beran, M., Koller, C., Keating, M.J., Ginsberg, C.H., Huh, Y.O., Stass, S. and Freireich, E.J. (1992) Blood 79, 882–887.
[39] Goffman, T., Tochner, Z. and Glatstein, E. (1991) Cancer 67, 572–576.
[40] Weckbecker, G. (1991) Pharmac. Ther. 50, 367–424.
[41] Cornford, E.M. and Oldendorf, W.H. (1975) Biochim. Biophy. Acta 394, 211–219.
[42] Terasaki, T. and Pardridge, W.M. (1988) J.Inf. Dis. 158, 630–632.
[43] Sawchuk, R.J. and Hedaya, M.A. (1990) Pharm. Res. 7, 332–338.